Regulation of axonal regeneration following the central nervous system injury in adult mammalian

Ran LIU¹, Xi-Ping CHEN^{1,2}, Lu-Yang TAO^{1,2}

¹Forensic Sciences Department, ²Aging and Nervous Diseases Laboratory, Soochow University, Suzhou 215123, China

Abstract: It has been well established that the recovery ability of central nervous system (CNS) is very poor in adult mammals. As a result, CNS trauma generally leads to severe and persistent functional deficits. Thus, the investigation in this field becomes a “hot spot”. Up to date, accumulating evidence supports the hypothesis that the failure of CNS neurons to regenerate is not due to their intrinsic inability to grow new axons, but due to their growth state and due to lack of a permissive growth environment. Therefore, any successful approaches to facilitate the regeneration of injured CNS axons will likely include multiple steps: keeping neurons alive in a certain growth-state, preventing the formation of a glial scar, overcoming inhibitory molecules present in the myelin debris, and giving direction to the growing axons. This brief review focused on the recent progress in the neuron regeneration of CNS in adult mammals.

Keywords: central nervous system; adult; regeneration; regulation; brain injury

1 Background

Central nervous system (CNS) injury generally leads to severe and persistent functional deficits, and it has been proposed for a long time that neurons in adult mammalian CNS fail to regenerate after injury^[1]. It is now known that the failure of adult axons to regenerate is not exclusively due to neurons' intrinsic and irreversible lack of regenerative ability, but is largely attributable to the presence of an inhibitory cellular environment^[2]. Thus, adult neurons are not only able to sprout new axonal or dendritic branches in response to various changes of their environment, but also have a genuine endogenous ability to regenerate after lesion formation. A recent review summarized certain conditions under which axonal regeneration does occur. These conditions consist of:

(a) alteration of environment favorable to regeneration, such as providing peripheral nerve bridges, and transplantation of Schwann cells or olfactory ensheathing glia to the

injury region;

(b) administration of neurotrophic factors in cell body and axon tips;

(c) blockage of growth-inhibitory molecules such as Nogo-A, myelin-associated glycoprotein, and oligodendrocyte-myelin glycoprotein;

(d) prevention of chondroitin-sulfate-proteoglycans-related scar tissue formation at the injury site using chondroitinase ABC; and

(e) elevation of intrinsic growth potential of injured neurons via enhancing intracellular cyclic adenosine monophosphate level^[3].

Here, we will mainly evaluate the new information emerging in this area.

2 Intrinsic regenerative ability of mature CNS

A prevailing view in neuroscience is that mature CNS has relatively little capacity to respond adaptively to injury. The evidence of injury-induced neurogenesis in adult hippocampus suggests the existence of an endogenous repair mechanism of cognitive dysfunction following traumatic brain injury (TBI)^[4].

Accumulating studies have documented that the CNS

Corresponding author: Xi-Ping CHEN
Tel: 86-512-62185006
E-mail: xiping.chen@163.com
Article ID: 1673-7067(2008)-06-0395-06
CLC number: Q42
Document code: A
Received date: 2008-02-18

has the ability to compensate for deafferentation by initiating a plasticity response capable of restoring lost synaptic contacts. Scheff SW *et al.* showed that the hippocampus ipsilateral to the cortical contusion had a significant plasticity response, though synapse replacement in this area did not necessarily result in remarkable improvement in spatial learning^[5]. Some evidences indicate that the postnatal subventricular zone (SVZ) is a niche for continuous neurogenesis in the adult brain and likely plays a fundamental role in self-repair responses under neurodegenerative conditions^[6]. However, another research shows that adult neurogenesis only occurs in two privileged regions of the brain. The first region is the interconnected system of the lateral ventricle wall and olfactory bulb, and the second region is the dentate gyrus of the hippocampal formation, where newly produced neurons arise from proliferating cells at the border between the hilus and the granule cell layer^[7].

3 The factors promoting CNS regeneration after injury

Adult axons in the mammalian CNS do not elicit spontaneous regeneration after injury, although many affected neurons have survived after the neurotrauma. However, considerable studies have pointed out that axonal regeneration does occur under certain conditions^[3]. Many factors promote neuroregeneration, such as neurotrophins, molecular guidance cues, and so on.

3.1 Neurotrophins Neurotrophins are a group of structurally relevant polypeptide growth factors that contribute to the development, maintenance, and function of the peripheral nervous system and CNS^[8]. In Parkinson's disease, brain dopamine neurons degenerate most prominently in the substantia nigra. Neurotrophic factors promote the survival, differentiation and maintenance of neurons in developing and adult vertebrate nervous systems. Especially, the glial cell line-derived neurotrophic factor (GDNF) is the most potent neurotrophic factor for dopamine neurons so far^[9]. Additionally, a recent study proposed that the GDNF receptor GFR α 1 functioned as a ligand-induced cell adhesion molecule (LICAM) to establish precise synaptic contacts and to induce presynaptic differentiation. They found that GFR α 1 was enriched at pre- and postsynaptic compartments in hippocampal neurons, and GDNF triggered the trans-homophilic binding between GFR α 1 molecules and the cell adhesion

between GFR α 1-expressing cells. This represents the first example of a cell-cell interaction mediated by LICAM. Presynaptic differentiation induced by GDNF is markedly reduced in neurons lacking GFR α 1, which indicates a role of GDNF signaling in the hippocampal synaptogenesis *in vivo*^[10]. Lindholm P *et al.* used injection of conserved dopamine neurotrophic factor (CDNF) into the striatum to evaluate the role of CDNF as a trophic factor in dopamine neurons. They found that single injection of CDNF before delivery of 6-OHDA into the striatum significantly reduced amphetamine-induced ipsilateral turning behavior and almost completely rescued dopaminergic tyrosine-hydroxylase-positive cells in the substantia nigra. When administered four weeks after 6-OHDA delivery, intrastriatal injection of CDNF was able to restore the dopaminergic function and prevent the degeneration of dopaminergic neurons in the substantia nigra. This result suggests that CDNF might be helpful for treatment of Parkinson's disease^[9].

Systemic administration with neurotrophins was generally proven ineffective. The accompanying reviews by Sahenk and Blesch highly emphasized application of localized neurotrophin for treatment of neuropathies and neurodegeneration^[11]. However, a recent review underlined the overlapping mechanisms that the neurotrophins was used to support survival, growth, and synaptic plasticity in different neuronal populations^[8]. There is still much to be discovered about the mechanisms and actions of neurotrophins.

3.2 Molecular guidance cues During development, multiple guidance cues direct the formation of appropriate synaptic connections. There are four major classes of axon guidance molecules: ephrins, netrins, semaphorins, and slits, which signal through one or more specific receptors^[12].

Netrins are a family of secreted molecules. They have critical functions in axon guidance and cell migration during neuronal development. In addition to its role as a chemotropic molecule, netrin-1 also acts as a survival factor. Both uncoordinated 5 (UNC5, including UNC5A, UNC5B, UNC5C and UNC5D) and deleted in colorectal cancer (DCC) are transmembrane receptors for netrin-1. A study shows that netrin-1 induces interaction of UNC5B with the brain-specific GTPase PIKE-L. This interaction triggers the activation of PtdIns-3-OH kinase signaling, prevents UNC5B's proapoptotic activity, and enhances neuronal survival^[13]. In contrast, another study suggests that Netrin-1, Slits, and

their receptors may contribute to the regenerative failure of axons in adult CNS by inhibiting axon outgrowth or by participating in the formation of CNS scar. Wehrle R and Camand E examined the expressions of netrin-1 and netrin receptors after sagittal myelotomy in adult rats to evaluate the potential role of netrin-1 after spinal cord injury. They found that the dominant expression of UNC5H on axons and neurons in injured spinal cord and the persistent expression of netrin-1 on oligodendrocytes surrounding lesions were consistent with the hypothesis that netrin-1 is a myelin-associated inhibitor of axonal regeneration after spinal cord injury^[14]. All these results suggest that netrin-1 is a bifunctional axonal guidance cue, and capable of attracting or repelling developing axons via activation of receptors of the DCC or NC5 family.

3.3 S-100B and other factors S100B is a neurotrophic/mitogenic protein generated by astrocytes. Kleindienst A *et al.* utilized infusion of S100B into the lateral ventricle to assess cell proliferation by injecting the mitotic marker bromodeoxyuridine (BrdU) on day 2 post-injury. Quantification of BrdU-immunoreactive cells in the dentate gyrus revealed an S100B-enhanced proliferation as assessed on day 5 post-injury, persisting up to 5 weeks. This result suggests that S100B may offer a beneficial effect to raise significant innate repair mechanisms of the brain, and facilitate memory consolidation after TBI. Thus, the increase of neurogenesis induced by S100B was correlated with the improvement of cognitive performance^[4].

It is well known that the growth hormone (GH) / insulin-like growth factor-1 (IGF-I) axis is involved in the regulation of brain growth, development and myelination. Moreover, GH and particularly IGF-I have been attributed to have neuroprotective effects in different *in vitro* and *in vivo* experimental models, and IGF-I could increase progenitor cell proliferation and new neurons, oligodendrocytes, and blood vessels in the dentate gyrus of hippocampus^[15]. A new study, by using new approaches of CSMN purification and culture, as well as *in vivo* analyses, reported that IGF-I specifically enhanced the extent and rate of axonal outgrowth in murine corticospinal motor neurons (CSMN) via the IGF-I receptor and downstream signaling pathways^[16].

4 The factors inhibiting CNS regeneration after injury

Hunt D *et al.*^[17] reviewed that axon regeneration could be prevented in injured CNS by axon growth-inhibitory ligands expressed in the oligodendrocytes, NG2-glia and reactive astrocytes in the lesion and degenerating tracts and by fibroblasts in the scar tissues. Growth cone receptors (Rc) could bind inhibitory ligands to activate a Rho-family GTPase intracellular signaling pathway, which then will disrupt the growth cone collapse induced by actin cytoskeleton.

4.1 Myelin-associated regeneration inhibitors Neurons in adult mammalian CNS are unable to regenerate their axons *in vivo* after trauma. Numerous factors contribute to the absence of this spontaneous regeneration. For instance, injured neurons fail to re-express some growth-associated proteins that expressed earlier in development; several molecules synthesized by oligodendrocytes and astrocytes, such as the myelin-associated neurite outgrowth inhibitors chondroitin sulfate proteoglycans, myelin-associated glycoprotein (MAG), oligodendrocyte myelin glycoprotein (OMgp) and Nogo, have been shown to inhibit axonal outgrowth in the CNS, and some of them are upregulated upon injury^[18]. MAG is the first myelin protein characterized as an inhibitor of axon outgrowth. Its inhibitory effect *in vitro* is restricted to adult neurons^[19]. It is a Schwann cell-based glycoprotein and has been implicated as a mediator of an outside-in signaling cascade influencing the cytoskeletal integrity of axons^[20]. MAG plays two generally known pathophysiological roles in the CNS: maintenance of myelin integrity, and inhibition of CNS axonal regeneration. Recent data has shown the relationship between low MAG antibody titer and the improvement in neuropathic symptoms^[21].

4.2 Astrocytes, the extracellular matrix molecules, and scar formation It is known that glial scars form after the CNS injuries and act as a barrier for neuron regeneration. Chondroitin sulfate proteoglycans (CSPGs), Tenascin and Semaphorin3A are the molecules of extracellular matrix. They are widely expressed in the developing and adult CNS and have potent inhibitory effects on the regeneration of CNS. CSPGs are the principal inhibitory component of glial scars, which form following the damage to the adult CNS and act as an obstacle for regenerating axonal regeneration^[22]. The increase of CSPGs could halt regeneration of axons and induce the formation of dystrophic growth cones. Semaphorin3A (Sema3A) is expressed by meningeal fibroblasts and binds to neuropilin1, which in turn is upregulated by the neurons

projecting to the site of injury, to induce growth cone collapse. *Sema3A* is also expressed on the surface of neuronal cells to interact with proteoglycans, which contributes to the inhibitory activity of extracellular matrix, and may be involved in controlling the invasion of Schwann cells into the CNS. *Tenascin-R* is a cell-adhesion protein that is upregulated in the adult CNS after spinal cord injury and might act as a barrier for regeneration. It has been shown recently that *tenascin-R*-deficient mice display enhanced functional recovery after spinal cord injury^[22].

5 Overcoming the inhibitors of regeneration

During the last few years, there has been an explosion in the number of experiments aimed at overcoming the inhibitors of neuroregeneration. Investigation in this field is beginning to blossom. Some researchers blocked *Nogo-A* by *Lingo-1* (IN-1) neutralizing antibody to allow axonal sprouting and new synaptic contact formation in intact neurons, and found that axons of injured cortical neurons could regrow after treatment with IN-1.

Similarly, *MAG* has been targeted to antagonize the known receptors and to inhibit the intracellular signal transduction of these inhibitory molecules^[2]. In addition, a recent study indicated that some key proteins, such as *Rho* and *PKC*, were implicated in *NgR*-mediated signaling cascades, and elucidated the implications of *cAMP* and *Arginase I* in overcoming the growth-inhibitory influence of myelin. This suggests that their association might be a potent way to enable the functional-relevant regeneration in adult mammalian CNS^[2].

A latest review summarized some substances that can promote repair and regeneration of the injured spinal cord, such as *monosialoganglioside* (*GM-1*), *4-aminopyridine* (*4-AP*), *brain-derived neurotrophic factor* (*BDNF*), *GDNF*, *Nogo* and *MAG*-inhibitors. Those transplanted tissues and cells, such as blood macrophages, bone marrow transplant with *GM-CSF*, *olfactory ensheathing cells*, fetal tissues, stem or progenitor cells, have been reported to produce neurological improvements^[23]. Taken together, these proteins provide new moleculars handled for designing therapeutic interventions for CNS injuries.

It is noteworthy that the reactive astrocytes, or the functional activation of astrocytes, abundantly occur in the brain of animal models exposed to the insults including trauma,

ischemia, and *6-OHDA* or *MPTP*-treated Parkinson's disease. Although they were traditionally assumed to impede neuronal regeneration by forming glial scars, growing evidence has indicated that they do offer crucial benefits in the functional recovery of brain injuries. The reactive astrocytes can produce various neurotrophic factors for neuron survival, synthesize extracellular substrates for axonal outgrowth and synaptogenesis, act as scavengers for free radicals and excess glutamate, and promote neurogenesis of neural progenitor cells in the adult brains^[24]. It is also shown that reactive astrocytes may play important roles in protection of nigral dopamine neurons or transplanted dopamine cells through their neurotrophic functions and active interactions with dopamine neurons or neural progenitor cells. Future approaches ought to target the neurotrophic functions of reactive astrocytes in the basal ganglia and the interventions to facilitate dopamine neurons' survival and axonal regeneration or dopamine progenitor cells' differentiation. Novel pharmaceutical and cell replacement strategies will be developed hopefully for prevention and treatment of Parkinson's disease by potential manipulation of reactive astrocytes in the basal ganglia^[25]. Clinical and laboratory studies have suggested that amphetamine treatment, when paired with rehabilitation, can improve the functional recovery after stroke or TBI. Researches showed that animals with sensorimotor cortical lesions had significant improvement after stroke and TBI when they received amphetamine treatment linked with rehabilitation^[25].

In a model of conditioning lesion of adult CNS neurons, the level of *cAMP* was elevated by either administration of *cAMP* analogues or priming with neurotrophic factors to promote axonal regeneration. It suggests that artificially increasing *cAMP* level in older neurons can alter their growth-state and can induce axonal growth in the presence of myelin-associated inhibitors, which further expands the range of possible therapeutic strategies for treatment of spinal cord injury^[1]. Numerous obstacles impeding successful regeneration have made it clear that the combined therapies will be necessary to achieve the axonal regeneration after CNS injury. It is noteworthy that recent works have combined stimulating the regenerative ability of adult neurons with administration of neurotrophins, growth factors, or other reagents to make glial scar more permissive. A conclusion could be made that removing inhibitory factors within the scar, providing a growth-permissive bridge across the scar and increasing the

intrinsic growth capacity of regenerating neurons will be essential for long-term functional restoration in the CNS^[22].

6 Conclusion and perspective

Besides the approaches mentioned above, there are some other strategies to improve neuronal regeneration from injury. Recent evidence indicates that a combination approach involving different repair strategies will be required to obtain long-term axonal regeneration within injured adult CNS. However, a therapeutic breakthrough still needs to wait.

Acknowledgements: This work was supported by the National Natural Science Foundation of China (No.30571909, No.30872666), the Youth Teacher Foundation of Jiangsu Province (No.BU134701), China, and the Medical Development Foundation of Soochow University (No. EE134615). We thank Tom QIN for polishing English language of our manuscript.

References:

- [1] Domeniconi M, Filbin MT. Overcoming inhibitors in myelin to promote axonal regeneration. *J Neurol Sci* 2005, 233: 43-47.
- [2] Schimchowitsch S, Cassel JC. Polyamine and aminoguanidine treatments to promote structural and functional recovery in the adult mammalian brain after injury: a brief literature review and preliminary data about their combined administration. *J Physiol Paris* 2006, 99: 221-223.
- [3] Cui Q. Actions of neurotrophic factors and their signaling pathways in neuronal survival and axonal regeneration. *Mol Neurobiol* 2006, 33: 155-179.
- [4] Kleindienst A, McGinn MJ, Harvey HB, Colello RJ, Hamm RJ, Bullock MR. Enhanced hippocampal neurogenesis by intraventricular S100B infusion is associated with improved cognitive recovery after traumatic brain injury. *J Neurotrauma* 2005, 22: 645-655.
- [5] Scheff SW, Price DA, Hicks RR, Baldwin SA, Robinson S, Brackney C. Synaptogenesis in the hippocampal CA1 field following traumatic brain injury. *J Neurotrauma* 2005, 22: 719-732.
- [6] Givogri MI, de Planell M, Galbiati F, Superchi D, Gritti A, Vescovi A, *et al.* Notch signaling in astrocytes and neuroblasts of the adult subventricular zone in health and after cortical injury. *Dev Neurosci* 2006, 28: 81-91.
- [7] Cooper-Kuhn CM, Winkler J, Kuhn HG. Decreased neurogenesis after cholinergic forebrain lesion in the adult rat. *J Neurosci Res* 2004, 77: 155-165.
- [8] Twiss JL, Chang JH, Schanen NC. Pathophysiological mechanisms for actions of the neurotrophins. *Brain Pathol* 2006, 16: 320-332.
- [9] Lindholm P, Voutilainen MH, Laurén J, Peränen J, Leppänen VM, Andressoo JO, *et al.* Novel neurotrophic factor CDNF protects and rescues midbrain dopamine neurons *in vivo*. *Nature* 2007, 448: 73-77.
- [10] Ledda F, Paratcha G, Sandoval-Guzman T, Ibanez CF. GDNF and GFR α 1 promote formation of neuronal synapses by ligand-induced cell adhesion. *Nat Neurosci* 2007, 10: 293-300.
- [11] Blesch A. Neurotrophic factors in neurodegeneration. *Brain Pathol* 2006, 16: 295-303.
- [12] Howell DM, Morgan WJ, Jarjour AA, Spirou GA, Berrebi AS, Kennedy TE, *et al.* Molecular guidance cues necessary for axon pathfinding from the ventral cochlear nucleus. *J Comp Neurol* 2007, 504: 533-549.
- [13] Tang X, Jang SW, Okada M, Chan CB, Feng Y, Liu Y, *et al.* Netrin-1 mediates neuronal survival through PIKE-L interaction with the dependence receptor UNC5B. *Nat Cell Biol* 2008, 10: 698-706.
- [14] Wehrle R, Camand E, Chedotal A, Sotelo C, Dusart I. Expression of netrin-1, slit-1 and slit-3 but not of slit-2 after cerebellar and spinal cord lesions. *Eur J Neurosci* 2005, 22: 2134-2144.
- [15] Isgaard J, Aberg D, Nilsson M. Protective and regenerative effects of the GH/IGF-I axis on the brain. *Minerva Endocrinol* 2007, 32: 103-113.
- [16] Ozdinler PH, Macklis JD. IGF-I specifically enhances axon outgrowth of corticospinal motor neurons. *Nat Neurosci* 2006, 9: 1371-1381.
- [17] Hunt D, Coffin RS, Anderson PN. The Nogo receptor, its ligands and axonal regeneration in the spinal cord. *J Neurocytol* 2002, 31: 93-120.
- [18] Decourt B, Bouleau Y, Dulon D, Hafidi A. Expression analysis of neuroleukin, calmodulin, cortactin, and Rho7/Rnd2 in the intact and injured mouse brain. *Brain Res Dev Brain Res* 2005, 159: 36-54.
- [19] Chen Y, Aulia S, Tang BL. Myelin-associated glycoprotein-mediated signaling in central nervous system pathophysiology. *Mol Neurobiol* 2006, 34: 81-91.
- [20] Steck AJ, Stalder AK, Renaud S. Anti-myelin-associated glycoprotein neuropathy. *Curr Opin Neurol* 2006, 19: 458-463.
- [21] Renaud S, Fuhr P, Gregor M. High-dose rituximab and anti-MAG-associated polyneuropathy. *Neurology* 2006, 66: 742-744.
- [22] Busch SA, Silver J. The role of extracellular matrix in CNS regeneration. *Curr Opin Neurobiol* 2007, 17: 120-127.
- [23] Apostolova I, Irintchev A, Schachner M. Tenascin-R restricts posttraumatic remodeling of motoneuron innervation and functional recovery after spinal cord injury in adult mice. *J Neurosci* 2006, 26: 7849-7859.
- [24] Lim PA, Tow AM. Recovery and regeneration after spinal cord injury: a review and summary of recent literature. *Ann Acad Med Singapore* 2007, 36: 49-57.
- [25] Chen LW, Yung KL, Chan YS. Reactive astrocytes as potential manipulation targets in novel cell replacement therapy of Parkinson's disease. *Curr Drug Targets* 2005, 6: 821-833.
- [26] Ramic M, Emerick AJ, Bollnow MR, O'Brien TE, Tsai SY, Kartje GL. Axonal plasticity is associated with motor recovery following amphetamine treatment combined with rehabilitation after brain injury in the adult rat. *Brain Res* 2006, 1111: 176-186.

成年哺乳动物中枢神经系统损伤后神经元轴突再生的调节

刘冉¹, 陈溪萍^{1,2}, 陶陆阳^{1,2}

苏州大学¹法医学系, ²衰老与神经疾病实验室, 苏州 200125

摘要: 成年哺乳动物中枢神经系统损伤后修复十分困难, 常导致严重的持续性神经功能障碍, 因此中枢神经系统损伤修复的研究成为当今热点。最新研究证明, 中枢神经系统神经元轴突再生障碍不是因为其内在的再生能力不足, 而是与受伤神经元所处的状态及生长环境有关。调节损伤神经元轴突再生至少应该包括如下步骤: 维持神经元存活并处于一种生长状态, 防止胶质瘢痕形成, 清除存在于髓鞘碎片间的神经再生阻滞因子及指引轴突再生方向。本文对近年来有关成年哺乳动物中枢神经系统神经元轴突再生及其调节的研究成果进行综述。

关键词: 中枢神经系统; 成年; 神经元轴突再生; 调节; 脑损伤