Review Article

Biotargets in neural regeneration

Qingqing Fang[1], Tongtong Xu[2], Changyue Wu[2], Songlin Zhou[1], Hualin Sun[1]

[1]Jiangsu Key Laboratory of Neuroregeneration, Co-Innovation Center of Neuroregeneration, [2]School of Medicine, Nantong University, Nantong 226001, China

Contributions: (I) Conception and design: H Sun; (II) Administrative support: H Sun; (III) Provision of study materials or patients: S Zhou; (IV) Collection and assembly of data: Q Fang, T Xu, C Wu; (V) Data analysis and interpretation: Q Fang, T Xu, C Wu; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Dr. Hualin Sun. Jiangsu Key Laboratory of Neuroregeneration, Nantong University, 19 Qixiu Road, Nantong 226001, China. Email: sunhl@ntu.edu.cn.

Abstract: The peripheral nervous system (PNS) exhibits a much larger capacity for regeneration than the central nervous system (CNS). The main reason is that the neurons in PNS still have certain intrinsic capacity for regeneration and the Schwann cells in PNS provide a suitable regeneration microenvironment. Factors that enhance the intrinsic growth potential of adult neurons are key players in the successful repair and regeneration of neurons following injury. Therefore, the full understanding of the mechanism of peripheral nerve regeneration will help us to solve the problems encountered in CNS regeneration. In this review, a number of intrinsic regeneration-promoting mechanisms have been described, including the role of transcription factors [signal transducers and activators of transcription 3 (STAT3), activating transcription factor 3 (ATF3), c-Jun, cyclic AMP response element binding protein (CREB) and Sox] in neuronal regeneration, the regulating factors [the phosphatase and tensin homolog (PTEN)-mTOR, PI3K-glycogen synthesis kinase 3 (GSK3), suppressor of cytokine signaling 3 (SOCS 3), histone deacetylases 5 (HDAC5) and reactive oxygen species (ROS)] for neuronal regeneration, the regulation of non-coding RNA [microRNAs (miRNAs), long-chain non-coding RNAs (lncRNAs) and circular RNAs (circRNAs)] in neuronal regeneration, the regulation of regeneration pathway in neural regeneration and the exosomes in neural regeneration. Finally, the prospect of neural regeneration was discussed, which contributes to greatly broaden the way to study the ability of nerve regeneration, identify more key biotarget molecules in neuronal regeneration, and provide innovative strategies to trigger and enhance the nerve regeneration.

Keywords: Neural regeneration; transcription factors; regulating factors; non-coding RNA; exosomes

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Introduction

Neurons prolongate their axons through great distances to contact with their targets during development by means of many factors, signaling pathways and a growth-favoring microenvironment. The intrinsic regeneration capacity of neuron is significantly decreased, especially in the central nervous system (CNS), once the contacts are established (1). In general, following an injury, neurons need to be changed into a regenerative state that characterizes the developmental stage. The neurons in the mammalian peripheral nervous system (PNS) are easy to revert to a regenerative state after injury, while the neurons in the CNS are hard to return to the regenerative state (2). Therefore, the full understanding of the mechanism of peripheral nerve regeneration will help us to solve the problems encountered in the regeneration of the CNS. Here, we highlight current progress in the intrinsic capacity for neuronal regeneration, including the role of transcription factors [signal transducers and activators of transcription 3 (STAT3), activating transcription factor 3 (ATF3), c-Jun, cyclic AMP response element binding protein (CREB) and

Sox], the regulating factors [the phosphatase and tensin homolog (PTEN)-mTOR, PI3K-glycogen synthesis kinase 3 (GSK3), suppressor of cytokine signaling 3 (SOCS 3), histone deacetylases 5 (HDAC5) and reactive oxygen species (ROS)], the regulation of non-coding RNA [microRNAs (miRNAs), long-chain non-coding RNAs (lncRNAs) and circular RNAs (circRNAs)], the regulation of regeneration pathway and the exosomes in neural regeneration. Finally, the current strategies aimed at neural regeneration was discussed, which contributes to greatly broaden the way to study the ability of nerve regeneration, identify more key biotarget molecules in neuronal regeneration, and provide innovative strategies to trigger and enhance the nerve regeneration.

Role of transcription factors in nerve regeneration

STAT3

STAT3 is a member of the STATs family in mammals. The STATs contains six regular domains—an amino terminal for STAT3 dimerization, DNA binding domain for recognition of gene promoters, α-helical linker domain for transcriptional activation, a coiled-coil domain, a classical SH2 docking domain, and a transcriptional activation domain (3). The inactivated STAT is located in the cytoplasm. After it is activated by JAKs, Src, and G-protein coupled receptors, it can form dimer or tetramer and be transported to the nucleus, where they interact with to consensus sequences to induce target gene expression (3). In the nervous system, STAT3 has a wide range of expression and is most deeply studied. In normal neurons, STAT3 are normally quiescent, when the neurons are damaged, STAT3 phosphorylation will rapidly increase (4). After sciatic nerve injury, STAT3 was phosphorylated and increased from 6 h and last for one month in axons at the lesion site. Recent studies have shown that STAT3 selectively regulates the initiation of neurite outgrowth rather than the later perpetuation of neurite outgrowth in both PNS and CNS (5). Altogether, these studies show an early and persistent expression of phosphorylated STAT3 during the regenerative process. Once the regeneration was completed, the expression levels of phosphorylated STAT3 decreased remarkably, which suggested that phosphorylated STAT3 played an essential role in the regenerative process (3). The use of JAK inhibitor AG-490 in the lesion site for 4 weeks not only reduced the phosphorylation of STAT3, but also significantly inhibited the expression of growth associated protein-43 (GAP-43), an axonal membrane protein (6), which suggested the JAK/STAT3 pathway played an important role in the regulation of GAP-43 expression and neurite outgrowth and regeneration.

ATF3

ATF3 is a member of ATF/CREB family of transcription factors. ATF/CREB proteins can form heterodimers with each other to influence transcription regulation of genes. In most types of cells, ATF3 normally displayed a low level expression, but nerve damage can cause rapid activation of ATF3 (7). Several signaling pathways have been involved in the activation of ATF3 following stress, such as p53-dependent and JNK/SAPK-dependent mechanisms (5). In addition, the activation of ATF3 could also be induced by many extracellular signals such as serum, fibroblast growth factor (FGF), cytokines and forskolin (8). ATF3 can bind c-Jun forming through their leucine zipper structures to control the transcriptional activation of various regeneration-associated genes and neurite outgrowth. So far, in neurons, Hsp27 is the only one who was confirmed as downstream target gene of ATF3 (9). Peripheral nerve injury can enhance the expression of Hsp27 in dorsal root ganglion (DRG), posterior horn and motor neurons (10). Hsp27 promotes neurite outgrowth of DRG neurons, possibly via phosphorylation-dependent interactions of Hsp27 with the cytoskeleton (5). At the same time, ATF3 also have a role in promoting the survival of neurons by avoiding JNK-mediated neuronal death. In PC12 cells, ATF3 can directly bind to the promoter of Hsp27 to activate Hsp27 and promote its expression (11). The expression of Hsp27 was also upregulated in DRGs of ATF3 transgenic mice, suggesting that Hsp27 is also the target gene for ATF3 *in vivo* (12).

c-Jun

C-Jun is a major component of the heterodimer transcription factor activator protein-1 (AP-1). The growth factors, cytokines, injury, and stress pressures related to signaling pathways can lead to c-Jun activation (13). The activation of c-Jun is controlled by JNKs, which phosphorylates the N-terminus of c-Jun (14). JNK is a phosphokinase of c-Jun, which activates c-Jun by phosphorylating the c-Jun Ser site (15). Nerve damage increases the phosphorylation of c-Jun induced by JNK, whereas JNK inhibitors can reduce c-Jun phosphorylation,

ATF3 expression, and nerve growth without affecting cell viability (16). The c-Jun was activated rapidly after nerve injury and continued until the nerve injury was repaired completely (17). However, knockout c-Jun not only reduces the rate of nerve regeneration which not only leads to the reduction of target muscle innervation and the delay of functional recovery, but also reduces the death of damaged neurons (17). This suggests that c-Jun can promote the regeneration of damaged neurons and its apoptosis. However, overexpression of c-Jun in Purkinje neurons does not promote its regeneration, suggesting that c-Jun promoting nerve regeneration is highly dependent on the cellular environment (18).

CREB

CREB is a member of the family of ATF/CREB transcription factors containing bZIP domain, which is a transcriptional factor of cyclic adenosine monophosphate (cAMP) signaling pathway (19). CREB can be activated through phosphorylation by many kinases, including PKA, MAPK, and CaMKIV, and it is inactivated through dephosphorylating by phosphatases such as PP1 and PP2A (20). The activation of CREB is closely related to the intracellular cAMP concentration. The cAMP as a second messenger can directly activate PKA, which can further phosphorylate CREB and make it enter the nucleus, thereby promoting the establishment of cytoskeleton and inducing axonal extension. The activation of CREB can also alleviate the inhibitory effect of myelin, thereby promoting nerve regeneration (21).

CREB plays an essential role in the growth and regeneration of axons. The growth of peripheral nerve and central nerve system will be blocked, after the CREB knocked out (22). In vitro cultured DRG and superior cervical ganglia (SCG) neurons from CREB null mice, the length of axon growth of DRG and SCG neurons was shorter than that in control group. Important targets of CREB include arginase I and BDNF. Arginase I can promote the regeneration of axons by promoting the synthesis of polyamines (23). Inhibition of CREB will result in inhibition of arginase I expression, and overexpression of CREB will significantly increase the expression of arginase I, but it is not yet clear that whether CREB directly regulates arginase I (21).

Sox

The *Sox* transcription factor family consists of 20 members in the human genome. Each plays many diverse roles (24). *Sox* family (*Sox4*, *Sox11*, *and Sox12*) genes display a novel expression pattern after retinal and optic nerve injury, which suggests that they play an important role in retinal and optic nerve regeneration (25). *Sox*11 is common to a many types of regenerating neurons. Wang et al found that DRG neurons and corticospinal tract (CST) neurons fail to upregulate *Sox*11 after spinal axon injury in adult mice. Furthermore, *Sox11* overexpression decreases DRG axonal death, and enhances CST sprouting and regenerative axon growth in injury (26). *Sox11* significantly induced an improvement of motor recovery after spinal cord injury. At the same time, which nestin/doublecortin displayed an up-regulation in spinal cord (27). The capacity of *Sox11* enhancing nerve regeneration *in vivo* is mainly dependent on its transcriptional activation of the regeneration-associated gene, such as small proline rich protein 1a (*Sprr1a*) (28). These data show that *Sox11*, a key transcription factor, can confer an enhanced intrinsic regenerative capacity to CNS neurons (26).

Regulating factors for neuronal regeneration

PTEN-mTOR

The PTEN protein is a phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase. PTEN is comprised of a tensin-like domain and a catalytic domain which is similar to that of the dual specificity protein tyrosine phosphatases (29). The deletion of PTEN can not only reduce cell apoptosis, but also can promote axonal regeneration (30). The suppressed PTEN can promote the accumulation of PIP3, whereas PIP3, as a second messenger, can cause a series of downstream reactions such as activation of phosphatidylinositol-dependent protein kinase (PDK), protein kinase B (Akt or PKB) GSK and so on (31). Rapamycin, an inhibitor of mTOR, inhibits regeneration due to deletion of PTEN (30), which indicates that PTEN deletion-induced axonal regeneration is dependent on the mTOR signaling pathway. However, PTEN deletion-induced axonal regeneration is not only dependent on mTOR pathway. Park *et al.* found that GSK3 is also involved in this process. At the end of the axon, GSK3 regulates the recombination of the cytoskeleton by adjusting the assembly of the microtubules, which is important for the regeneration of axons (32). In addition, GSK3 can also be involved in the transcriptional regulation of genes through regulating the activation of many

transcription factors, and thus further involved in nerve injury and repair (33). However, these pathways involved in axonal regeneration without relying on mTOR still need further investigation.

Neuronal regeneration caused by the absence of PTEN occurs not only in retinal ganglion cells (RGCs). In the CST, the activity of mTOR is reduced not only with the growth and development of cortical neurons, but also with the damage of cortical neurons. If the PTEN in the cortical neurons is deleted to maintain the activity of mTOR, it will greatly promote the axonal regeneration of CST (34). In addition, the absence of PTEN or TSC2 in DRG also promotes axonal growth and regeneration (35). These indicate that PTEN/mTOR plays an essential role in the axonal regeneration and is independent of the activation of the ribosomal S6 protein kinase pathway (36).

PI3K-GSK3

Phosphorylation of GSK3 has been indicated to regulate axon growth during development and regeneration. Zhang *et al.* demonstrated that GSK3 was not sensitive to Akt-mediated inactivation and sensory axon regeneration was normal after peripheral axotomy in GSK3 mutant mice. The activity of GSK3 is negatively regulated by PI3K signaling after peripheral nerve injury, and the PI3K-GSK3 pathway is functionally prerequisite for sensory axon regeneration (37). Saijilafu *et al.* found that PI3K signal is activated after peripheral nerve injury and that PI3K pathway is necessary for sensory axon regeneration. Moreover, GSK3, rather than mTOR, mediates PI3K-dependent augmentation of the axon growth potential after peripheral nerve injury. Furthermore, PI3K-GSK3 signal may be induced by a transcription factor Smad1, which has been shown to promote sensory axon regeneration. Together, these results suggested that PI3K-GSK3-Smad1 signaling was essential for promoting sensory axon regeneration in the mammalian nervous system (2).

SOCS3

The SOCS family consists of eight members that play a role in inhibiting the cytokine signaling in the cell. SOCS protein inhibits signal transduction and transcriptional activation mainly by binding to tyrosine residues of JAK or cytokine receptors. SOCS protein is also involved in the regulation of many physiological functions of cells, including inflammatory response, immune response, endocrine and carcinogenic reactions (38). SOCS3 is widely expressed in the nervous system, especially in neurons of the hippocampus, basal ganglia, thalamus, and cerebellar granule cell layers. The up-regulation of SOCS3 by IL-6 can specifically inhibit the activation of JAK/STAT3 in peripheral nerves and the anti-inflammatory effects (39). The study found that knockout of SOCS3 in RGCs will also enhance neuronal survival and axonal regeneration after peripheral axotomy. In SOCS3 and gp130 double knockout mice, axonal regeneration can be significantly inhibited, suggesting that deletion of SOCS3-induced axonal regeneration is largely dependent on the gp130 signal. In addition, after adenovirus transfection of RGCs to overexpress SOCS3, axonal regeneration was almost completely inhibited (40). The role of recombinant human ciliary neurotrophic factor in intravitreal injection is also inhibited by adenovirus-mediated overexpression of SOCS3, which suggests that overexpression of SOCS3 can inhibit cell viability (41).

HDAC5

Histone acetyl transferases (HATs) are conjugate enzymes which transfer the acetyl group of their cofactor-acetyl coenzyme A to lysine residues of histone proteins. The study of acetylation modification is mainly focused on the influence of cell chromosome structure and the activation of transcriptional regulators and the most common one is the histone acetylation. HDACs function to deacetylate the lysines on histone tails causing condensation and subsequent repression of chromatin. HATs and HDACs are antagonistic enzymes, which regulate gene expression through acetylation and deacetylation of histone proteins.

Epigenetic regulation of neuronal signaling through histone acetylation suggests that transcription govern neuronal memory, plasticity and learning (42). HDACs regulate many key neuronal processes such as the differentiation of neural stem/progenitor cells, neuronal death and degeneration, learning and memory (43). It was found that HDAC5 nuclear export is an important step in the chain reaction of peripheral nerve regeneration. HDAC5 nuclear export induced by injury improves histone acetylation to activate the expression of pro *regenerative-associated* genes and HDAC5 nuclear export is required for axon regeneration (44). HDAC5 nuclear export can open a series of genes that promote axonal regeneration, at the same time it can also move to the injured site interaction with the actin binding protein filamin A to

help the formation of microtubules, thereby promoting the regeneration of axons (45,46). HDAC5 nuclear export does not exist in the nerve cells of brain and spinal cord, which can explain in part why axonal regeneration is very difficult in the CNS (44). Rivieccio *et al.* have demonstrated that HDAC6 inhibition can promote neuron survival and regeneration (47).

ROS

Cells have evolved intrinsic antioxidant capacity to maintain a precise homeostatic regulation of the ROS and reactive nitrogen species (RNS) in physiological conditions and to detoxicate ROS and RNS excessive accumulation in pathological conditions (44). ROS are involved in a large number of degenerative conditions, such as aging, neurodegenerative diseases and neurological disorders (45). Increased generation of ROS is an important pathological characteristic in the brains of patients with Alzheimer's disease (AD) (43). There are a number of antioxidants that have been investigated therapeutically for promoting peripheral nerve regeneration. Pyrroloquinoline quinone (PQQ) is an antioxidant that can improve nerve growth factors synthesis and contribute to the Schwann cells proliferation and differentiation (29). Isoquercitrin has neuroprotective effect in diseases like Parkinson's and AD by its antioxidant capacity (25). Therefore, isoquercitrin could be a potential therapeutic agent for the treatment of neurodegeneration disease. The antioxidative ability of lycium barbarum polysaccharides could effectively promote nerve regeneration and improve functional recovery following peripheral nerve injury (47). The increased expression of antioxidant enzymes in the stratum pyramidale of the hippocampal CA1 region after subsequent lethal transient forebrain ischemia exhibited neuroprotective effects in the hippocampal CA1 region (46). Navarro-Yepes *et al.* demonstrated that targeted expression of specific antioxidants might be a more promising therapeutic method against oxidative stress, compared with dietary supplements of non-selective antioxidants (44).

Regulation of non-coding RNA in neuronal regeneration

miRNA

miRNAs are a kind of short noncoding RNAs about 22 nt that take part in controlling the balance of gene regulatory networks. Through targeting and controlling gene expression at the post-translational level, miRNAs can control highly complex signal transduction pathways and other biological pathways (48,49). Chip and deep sequencing revealed that miRNA involved in many aspects of rat sciatic nerve regeneration (50-52). The survival of neurons is an essential prerequisite of successful regeneration. The expression profile changes of miRNA in L4-L6 DRG after sciatic nerve injury were systematically analyzed, we found that miR-21 and miR-222 suppressed the apoptosis of DRG neurons by inhibiting TIMP3. In addition, IL-6 could upregulate miR-21 expression by stimulating DRG neurons (53).

At present, many studies have focused on the effect of miRNA on neurite outgrowth, for example, miR-21 is rapidly upregulated in DRG neurons after injury and it promotes the regeneration of DRG neurons by targeting Sprouty2 (54). In addition, miR-431, miR-145, miR-138, miR-214 and miR-132 targeted to Kremen1, Robo2, Sirtuin type 1, Slit-Robo GTPase-activating protein 3 and RASA1 to regulate axonal regeneration, respectively (55-59). We analyzed the expression profile changes of miRNAs of L4-L6 DRG after sciatic nerve injury in rats. It was found that miR-222 promoted the growth of DRG neurons by targeting PTEN. In addition, c-Jun activation can up-regulate miR-222 expression. Regulate CREB phosphorylation by PTEN, and cooperate with cAMP/CREB signaling pathway to promote neuronal regeneration (60). Direct inhibition of let-7 or increased expression level of the intervening gene lin-41 was able to completely restore the regenerative capacity of the larvae neurons (61). After the zebrafish spinal cord injury, the upregulation of miR-133b can target GTPase RhoA to promote axonal regeneration (62).

LncRNA

With the rapid development of functional genomics, lncRNA, an important member of non-coding RNA family, has received increasing attention. It is a kind of gene transcript product that is greater than 200 nt but does not have the function of coding proteins (63). In recent years, it has been found that lncRNA regulates the expression of protein-encoded genes by influencing the transcription, splicing, transport and translation of mRNAs at transcriptional and post-transcriptional levels. LncRNA is highly expressed in the brain, but the vast majority of its effects are unclear (64). We recently studied the expression

profile of lncRNA in DRG after sciatic nerve injury. It was found that 24 lncRNAs were down-regulated by injury, and bioinformatics analysis revealed that the possible target genes were related to MAPK pathway. Further studies have found that interfering with lncRNA BC089918 can promote the growth of DRG neurite processes, which have described the molecular mechanisms of neural regeneration from a new perspective (65). As a conserved lncRNA, Pnky regulates neurogenesis from embryonic and postnatal NSC populations by interacting with a key RNA processing factor (3).

Circular RNAs

CircRNAs as potential regulators of gene expression, have re-emerged as an interesting RNA species, which are highly abundant and evolutionarily conserved non-coding RNAs produced by circularization of specific exons (5,20). Many circRNAs are expressed in a tissue-specific and developmental-stage-specific manner (66), and circRNAs expression are negatively related to the expression of the RNA-editing enzyme ADAR1 (67). CircRNAs can play an essential role in regulating gene expression by the circRNA-miRNA-gene pathway (68). Accumulating data suggest key roles for circRNAs in the CNS. Hanan *et al.* suggested that circRNA levels were dynamically modulated in neurons, and they played important roles in synaptic plasticity and neuronal function (20). CircRNAs displayed an up-regulation during neuronal differentiation, highly concentrated in synapses, and generally displayed differentially expression compared to their mRNAs (67). Circular RNAs not only serve physiological functions in neurons, but have also been linked to brain disease, and therefore have potential as novel biomarkers. The circular RNA CDR1as participates in AD pathogenesis and CDR1as is a negative regulator of miR-7. Investigations have demonstrated that miR-7 could downregulate the expression of α-synuclein, which is the main component of Lewy bodies in the PD brain (69). Further study on circRNA will improve our understanding regarding neurological disease pathogenesis and lead to new diagnostic and treatment methods.

Regulation of regeneration pathway

Currently, seven classical regulatory pathways are known, and they are cAMP/PKA/CREB, JNK/c-JUN, ATF3, JAK/STAT3, CBP/p300/PCAF-p53, KLF4 and BMP4/Smad1 (70). These pathways are interrelated, such as c-Jun and ATF3 and STAT3 and other transcription factors may work together to start the peripheral nerve regeneration (18). KLF4 can collaborate with p53 to transactivate the p21Cip1/Waf1 enhancer. KLF4 can also bind to the STAT3 705 tyrosine phosphorylation site, thereby inhibiting the regeneration of the optic ganglion axons (71).

Previous studies have shown that Bcl-2 regulates the concentration of Ca^{2+} on the endoplasmic reticulum by reducing the intake of Ca^{2+} on the endoplasmic reticulum and increasing the outflow of Ca^{2+}. In neurons, Bcl-2 enhances intracellular Ca^{2+} concentration after nerve damage, activating mitogen-activated protein kinases and CREB to promote axonal regeneration (72).

On the other hand, knockout of SOCS3 in RGC can upregulate mTOR levels at 3rd and 7th day after nerve injury, while it can promote RGCs response to injury-inducing factors (73). It was found that co-deletion of SOCS3 and PTEN could greatly promote the strength and persistence of axon regeneration, indicating that PTEN and SOCS3 promote the regeneration of axons through two independent pathways (74).

A recent study showed that cAMP was able to prolong the axonal growth of RGC after optic nerve injury in PTEN knockout mice. At 10 weeks after injury, most of the regenerated axons crossed the middle of the optic chiasma. Mice with impaired optic nerve begin to respond to similar optokinetic response and diel movement (75). This suggests that the regenerated RGC axons are reestablished with the target neurons. PI3K/Akt signal pathway and its downstream targets (GSK3 and mTOR) have indicated to play an essential role in regulating the intrinsic axonal regeneration (76).

Exosomes

Exosomes are small membranous vesicles that are released by almost all cell type. Exosomes generally comprise mRNAs and miRNAs as well as proteins, and play a variety of important functions in intercellular communication for many physiological and pathological processes (77). The increasing evidence supports that exosomes play a potentially essential role in the physiology and pathology of adult neurogenesis (78). Oligodendrocytes could release exosomes. Neurons internalize the released exosomes by endocytosis. These data demonstrated that the exosomes derived from oligodendrocytes could participate in a

novel mode of bidirectional neuron-glia communication contributing to neuronal integrity (79). The exosomes derived from Schwann cell could mediate neuron-glia communication contributing to axonal regeneration, and neuronal survival (80). Jarmalavičiūtė *et al.* demonstrated that exosomes derived from SHEDs (Stem cells derived from the dental pulp of human exfoliated deciduous teeth) are considered as new potential therapeutic tool for Parkinson's disease (81). Guitart *et al.* suggested that astrocytes could mediate the release of exosomes carrying prion protein and other molecules, resulting in improved survival of neurons under hypoxic and ischemic conditions (82). Exosomes might also participate in the disseminating of pathological proteins such as phosphorylated tau, APP fragments, prion protein or α-synuclein across the nervous system (83). Exosomes derived from neurons were found to transfer α-synuclein toxic forms between neuronal and non-neuronal cells, which contributed to PD spreading. Exosomes have been redesigned for targeted therapy. Exosomes are an ideal vector for gene therapy because they are comprised of non-synthetic and non-viral components (44). I believe that exosome will have significant prospects in the field of neural regeneration.

Prospect

In summary, on the one hand the activation of regeneration capacity is controlled by a series of complex extracellular signals; on the other hand it is related to the intrinsic regeneration capacity of neurons. The activation of intrinsic regeneration capacity of neurons mainly related to the regulation of the transcription factors, the regulating factors, the regulation of non-coding RNA after transcription, the regulation model of regenerative pathway, and the regulation of exosomes in neural regeneration. The difficulty of the nerve intrinsic regeneration ability study is the comparison of the length of neuron regeneration axon in vitro. In recent years, the establishment and improvement of micro fluid culture (84) and "spot" culture (85) have solved these problems. In addition, due to the presence of the blood-brain barrier, the drug is difficult to effect on the neurons of the CNS. In recent years, intrathecal injection of siRNA and the injection of siRNA (86) and miRNA (87,88) in peripheral nerve regeneration chamber have achieved good results. The establishment of these methods greatly broadens the way to study the ability of nerve regeneration, which helps to deepen the understanding of the neural regeneration mechanism. In-depth clarification of the mechanism of neural regeneration will provide new ideas and strategies for neurological diseases, including neurodegenerative lesions, central and peripheral nerve injury, etc., and provide useful scientific guidance for clinical application.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/biotarget.2017.05.01). HS serves as an unpaid Executive Editor-in-Chief of *Biotarget*. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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