Discussion on the mechanism of glial scar inhibiting axonal regeneration in CNS and new techniques of anti-inhibition regeneration

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Abstract. Nerve regeneration is a process of self-recovery after nerve injury. Nerve regeneration is more difficult in the central nervous system (CNS) than in the peripheral nervous system (PNS). So far, there are few cases of CNS cure. The difficulty of CNS regeneration brings great loss to patients and society. In recent years, scientific research has found that damage to the CNS is caused by mechanisms that hinder its regeneration. This review has provided a comprehensive summary of recent studies on the inhibitory mechanism of glial scar, which are the main factors affecting axonal regeneration in the CNS, and deeply discussed the mechanism of a series of factors related to glial scar, such as CSPGs, Nogo family proteins, NgR1, LINGO-1 and p75, on the production of glial scar. After this, the idea of their ability to study the regeneration of the CNS after injury was analyzed, and the new technology of anti-inhibition of CNS regeneration therapy was looked forward.

Keywords: CNS Regeneration, Glial Scar, Inhibitory Mechanisms.

1. Introduction

In recent decades, scientists have devoted much effort to studying the mechanisms of nerve regeneration and proposing treatments for nerve regeneration. Although research has shown that the PNS has the ability to repair and regenerate itself, the CNS is largely lacking this ability. There are currently no treatments that can restore neurological function after damage to the CNS. The reason is that it is subject to the inhibitory effect of some factors after CNS damage, mainly the proliferation of glial scar hinders nerve self-repair [1]. Studies on glial scar cells [2-3] and their inhibitory factors. These inhibitory factors, such as Chondroitin Sulfate Proteoglycans (CSPGs), can joint to receptors and exert inhibitory effects. For this reason, scientific agents proposed a solution to destroy themselves [4-5] and destroy receptors [6]. For Nogo family proteins [7], according to its mechanism of action, the way of RNA interference was proposed [8-9]. Composed of Nogo Receptor 1 (NgR1), the NgR1/ LINGO-1 / p75 complex can interact with the Nogo proteins[10], scientists found that disrupting any component of the complex prevented Nogo protein binding [11-14]. In the past two years, RNA-targeted therapy [15], nanotechnology therapy [16-17] and neural stem cells [18] have been developed for the difficulty of central nervous regeneration. This review will introduce different mechanisms to explain the inhibition of axon regeneration by glial scar, and elaborate the possible solutions to relieve the inhibition of central nerve regeneration by combining the inhibition mechanism and recent medical technology.

2. Inhibitory mechanisms in the CNS

Many studies have shown that there are a series of inhibitory mechanisms in the process of axonal regeneration in the CNS. To make the CNS regain the ability of regeneration, the first problem is to overcome or eliminate this inhibition. Studies have found that cicatricial glial scars play an indispensable role in restraining the regeneration of the CNS after injury. The following part will analyze the mechanism of its action in organisms and discuss its related factors.

2.1. Glial scar formation

When the CNS is damaged, a series of damage responses occur, including reactive gliosis, or glial scarring. The consequence of this is that, after the initial stage of neuronal injury, the primary goal of treatment is to repair CNS damage: whether to regenerate axons, induce remyelination, or replace dead neurons, which inevitably must occur in the glial scar environment. [1]

2.1.1. Barrier function

Scientific studies have shown that glial scars prevent further damage through immunity by creating a barrier between damaged and undamaged nerve cells. It has been found that when the CNS is damaged, a number of chemicals and molecules harmful to the organism are released, as well as proinflammatory activated microglia and infiltrating M1 phenotype monocyte derived macrophages (M1 mo-MΦ). If not controlled in time, neurotoxic microenvironment can be further formed, resulting in the appearance of the lesion site. The formation of scar glia plays a role as a barrier at the site of injury, preventing the diffusion of harmful and toxic molecules in the organism.[2]

2.1.2. Immune function

Immune cells may be involved in controlling scar production and degradation, thereby controlling neuronal regeneration. It has been suggested that preventing the formation of chondroitin sulfate proteoglycans (CSPGs) immediately after CNS injury results in the disappearance of well-defined mo-MΦ regionalization. Scarce CSPGs leads to the expression of Insulin Growth Factor 1 (IGF-1),[3] which is an important factor supporting the viability of surviving cells. Therefore, the interaction between CSPGs and anti-inflammatory mo-MΦ is very important after CNS injury. When IGF-1 expression is blocked, microglia production can be reduced, thereby avoiding glial scar formation.

The above studies indicate that the glial scar formation is a vital factor hindering axon regeneration in the CNS, and its immune effect can become the focus of treatment of CNS injury. CSPGs play a significant role in the formation of glial scar. By studying the properties of CSPGs, researchers can further understand the effect of removing or reducing CSPGs, so as to achieve the purpose of relieving the CNS inhibition.

2.2. Inhibition of CSPGs

Chondroitin sulfate proteoglycans (CSPGs) play an indispensable role in the formation of extracellular matrix in the CNS. As an important part of glial scar, it can inhibit the regeneration of damaged nerves after CNS injury. Scientific studies have suggested that reducing the content of CSPGs in the injured nerve can relieve its inhibitory effect on the regeneration of the injured nerve. The mechanism of operation can be divided into two parts, one is to reduce the CSPGs, the other is to reduce the activity of CSPGs receptor.

2.2.1. Degradation of CSPGs

CSPGs are composed of core proteins and glycosaminoglycans (GAGs) containing chondroitin sulfate (CS) disaccharide repeat units. Some scientists have proposed and proved through in vivo studies that enzymatic hydrolysis (cartilage sulfase) to remove GAG from the core protein of CSPGs, thereby degrading CSPGs, is a successful treatment strategy to overcome CSPGs-mediated axonal growth inhibition.[4]

Chondroitinase ABC (ChABC) can digest GAG chains on CSPGs proteoglycans. Studies have shown that ChABC only degrades GAG side chains that play an inhibitory role in CSPGs, thus achieving the inhibitory effect of eliminating CSPGs on nerve regeneration. In addition, some carbohydrate residues from CSPGs remained attached to core proteins after ChABC treatment. The degree of damage to CSPGs by ChABC can be determined by detecting the specific residual content of ChABC. CSPGs play a vital role in restraining axonal regeneration in the CNS, and experimental data show that most of the inhibition of CSPGs functions on GAG side chains. Thus, blocking the GAG chain effectively increases the chance of nerve regeneration.[5]

2.2.2. Destroy CSPGs receptors

The majority of CSPGs receptors in the nervous system include PTP, LAR, and Nogo receptor 1/3 (NgR1/3). A series of scientific studies have shown that CSPGs can activate RhoA-Rho kinase after contact with its receptor, thereby inhibiting AkT-GSK3β and Ras-Raf-MEK-ERK channels, thus inhibiting axonal cell regeneration. Therefore, they can treat CSPGs receptors to make CSPGs unable to bind, so as to achieve the purpose of axon regeneration.[6]

The above studies indicate that CSPGs, as an inhibitory factor of axon regeneration in the CNS, are proposed to solve the problem of inhibited nerve regeneration from two basic directions: degradation of CSPGs and blocking of CSPGs receptors according to their mechanism of action in nerve injury. The method of degrading CSPGs has been successfully implemented in some laboratories and has obvious effect on nerve regeneration. There have been few experiments on strategies to block the CSPGs receptor because of the limitations of the scientific community's understanding of the receptors.

2.3. The protein family Nogo

Axonal regeneration in the CNS is inhibited after injury. Scientists have proposed a range of factors that inhibit nerve regeneration, such as Myelin-Associated Glycoproteins (MAG), suppressive Myelin-Associated Inhibitors (MAIs) and Oligodendrocyte Myelin Glycoproteins (OMGP). One of the most studied inhibitors is the Nogo protein of the reticulin family in MAIs, which has A variety of isoforms, and usually NoGO-A. So far, many studies on Nogo family proteins have been aimed at exploring pathways to inhibit the expression of Nogo family proteins, thereby alleviating the inhibitory effect of Nogo family proteins on nerve regeneration. [7]

2.4. Inhibition of Nogo protein by RNA interference[8]:

Scientific studies have found that RNA interference can inhibit the expression of Nogo protein, which can be used as a new treatment for the regeneration of the CNS after arrest. Recombinant adenovirus short hairpin RNA was used to transfect spinal cord tissues *in vivo* and adenovirus transfected oligodendrocytes *in vitro*. The expression of Nogo protein was detected by reverse transcription PCR at mRNA level, and the protein expression was detected by Western Blot.

Two groups of Nogo-specific and an unrelated group of short hairpin RNAs (controls) were cloned into intracellular adenoviruses and then tested for Nogo expression in adenovirus-transfected nerve cells. Results *in vitro,* the expression of Nogo mRNA was reduced by nearly half (51% and 49%) in two groups of Nogo-specific short hairpin RNA, and the expression of Nogo protein was also reduced by 50% and 48%. The expression of Nogo mRNA *in vivo* was also decreased compared with the control group.

For the study of Nogo-A protein in the Nogo protein family, Guzik-Kornacka A.M. [9] In their study, they summarized the results of current clinical trials on the use of No-A or Nogo-A signaling inhibition, and proposed the use of glia-derived synaptic growth inhibitors in the treatment of CNS regeneration.

2.5. MAG binds receptors NgR1, GT1b, NgR1, p75 and LINGO-1

Further studies of Nogo family proteins in the scientific community have shown that myelin inhibitors, including Nogo proteins, MAG and OMGP, regulate the regeneration of damaged nerves through postganglionic signaling in individual neurons. The receptor for myelin inhibitors is a receptor complex consisting of NgR1、LINGO-1 and p75. [10] Multiple research teams have found that inhibition of any element of the complex can undo the inhibitory effect on nerve regeneration after CNS injury

2.5.1. Inhibition of p75

NgR1 is a receptor that can be anchored to the cell surface by glycophosphatidyl (GPI). In earlier studies, two gangliosides in the nervous system, such as Trisialoganganglioside (GT1b) and

Disialoganganglioside (GD1b), have been shown to play a role in linking MAG partners and signaling to p75 [11].

Nayanendu's team found that Ganglioside mediates NgR1 and LINGO-1 in experiments with Ganglioside, NgR1, LINGO-1and p75 and confirmed that NgR1 and LINGO-1 bind more strongly to Nogo in the presence of p75. The p75 acts as a signal sensor to perform intramemmal proteolysis through the combined action of secretase activity, which induces Rho GTPase activity and thereby inhibits axonal growth.

To demonstrate the mediating effect of ganglioside on NgR1 and LINGO-1, they set up two groups with and without ganglioside respectively, and studied the binding of NgR1 with LINGO-1 and p75 in these two conditions by ELISA. The experimental results showed that the binding between NgR1 and LINGO-1 was much stronger in the presence of ganglioside than in the absence of ganglioside, but the presence or absence of ganglioside did not affect NgR1 and p75, nor did LINGO-1 and p75. Therefore, GT1b factor from ganglioside can directly affect NgR1 and LINGO-1, indicating that GT1b is a mediator of NgR1 and LINGO-1.

Their study showed that Ganglioside is involved in regulating myelin inhibitory receptor signaling. When ganglioside is reduced, the interaction between NgR1 and LINGO-1 is weakened, and the inhibitory effect of Nogo protein on neural regeneration in the CNS is weakened. Reducing ganglioside can help promote central nerve regeneration, and these studies provide ideas for the treatment of nerve regeneration.

2.5.2. LINGO1 antagonist

As a special protein in the CNS, LINGO-1 is a vital component of NgR1 / LINGO-1 / p75 that inhibits nerve regeneration in the CNS and is also an vital factor in inhibiting axonal regeneration in the CNS.

Ji's team tested whether treatment with the antagonist LINGO-1-Fc could improve spinal cord injury by promoting axonal bud and reducing RhoA activation to investigate the effects of Lingo1 *in vivo* after CNS damage. [12]

To confirm whether LINGO-1-Fc could act as an inhibitor to inhibit the action of Lingo-1, they designed an ELISA assay to test the inhibition of AP-LINGO-1 binding to NgR1 by LINGO-1-Fc. The experimental results show that LINGO-1-Fc can reduce the connection between AP-LINGO -1 and NgR1. Meanwhile, in an i*n vivo* study of RhoA inhibition by LINGO-1-Fc, they used a mouse model of SCI to measure Rho-GTP inhibitor levels and observed that the LINGO-1-Fc treatment group had significantly lower levels than the control group without antagonist treatment.

Ji's team experimentally reported that LINGO-1-Fc effectively restrains the junction of NgR1 and LINGO-1, reduces RhoA, a repressor that inhibits the recovery of damaged nerves, and demonstrated that it contributes to central nerve regeneration in a mouse model of SCI *in vivo.*

2.5.3. Targeted blocking of LINGO1

Leucine-rich repeats (LRR) on LINGO-1 protein were found to interact with the Ig domain of Nogo receptor. This interaction is specifically expressed in oligodendrocytes in the CNS and can be an important direction for the research of therapeutic regeneration in the CNS.

In order to further study the mechanism of LINGO-1 inhibition and promote oligodendrocyte survival and axonal regeneration in different animal models by immunological methods, S. Mi's team proposed that some proteins rich in leucine repeats (LRR) play an crucial role in the CNS. [13] Therefore, it can be used as a therapeutic target for CNS regeneration.

LINGO-1 binds to neuronal or axonal NgR1 complexes and inhibits axonal regeneration by activating Rho-A channels. There is another mechanism: in animal experiments, antibodies are used to block LINGO-1 function *in vitro* to accelerate myelin regeneration, axonal regeneration, and functional recovery. There are scientific studies showing that in Northern Blot analysis, scientists found LINGO-1 in the CNS of mice, but not in the non-CNS.[14] This condition raises the possibility of targeted therapy.

The above scientific studies indicate that the inhibitory effect of NgR1/ LINGO-1 / p75 complex on axon regeneration in CNS can be relieved by blocking one of the three functions. Nayanendu's Team experiment proved that the reduced content of Gangliosides could reduce the content of p75, thereby reducing the role of NgR1 and LINGO-1. Ji's team demonstrated the existence of a LINGO-1 antagonist, which inhibited the concatenate of NgR1 and LINGO-1 and reduced Rho-A. S. Mi's Team demonstrated that targeting blocking LINGO-1 through immune principles makes this new therapeutic approach clinically possible Drugs targeting genes/protein. These studies on the mechanism of NgR1/ LINGO-1 / p75 complex inhibition and release provide ideas for the treatment of central nervous regeneration.

3. New technology of anti-inhibition regeneration

3.1. Target remyelinating inhibitor proteins or other inhibitors

In the study of CNS regeneration, scientists have found that the inactivation of endogenous regeneration pathway is the main cause of axonal failure in the CNS, and the Mechanistic Target of Rapamycin (mTOR) signaling pathway is one of the important endogenous regeneration pathways driving axonal regeneration in various CNS injuries. Therefore, blocking the mTOR signal can achieve the CNS regeneration, and usually by using phosphatase and tensin homologue (PTEN) to prevent the signal from being received by the receptor. The scientists found that RNA may block mTOR signaling binding through targeted drug delivery to stimulate axonal regeneration in the CNS.[15]

Studies have found that there are two kinds of mTOR complexes. One is mTOR complex 1 (mTORC1), which is highly sensitive and can be inhibited by rapamycin. Another is that the sensitivity of mTOR complex 2 (mTORC2) is not high. Therefore, most of the regulation of mTOR is caused by mTORC1. MTORC1 can control cell growth and proliferation by regulating cellular genes, and its regulatory sites are upstream of cell growth and proliferation. Scientific studies have found that mTOR can act as a regulator and participate in the regulation of MTORC1 in the ribosome, while Sod1 is an effector downstream of MTORC1, which can catalyze the conversion of superoxide to hydrogen peroxide and plays an important role in neural repair in mouse models. MAF1 is also a downstream factor regulated by mTOR and can control RNA polymerase III in ribosomes to regulate mRNA synthesis and tRNA recruitment. The expression of PTEN can enhance the inhibitory signaling pathway after interaction with MAF1, so promoting PTEN expression can block the signaling pathway and lead to neural regeneration in the CNS.

RNA targeting mTOR channel blockade to promote CNS regeneration is a gene-level treatment method for CNS regeneration. Currently, it has been tested on mouse CNS. However, for human treatment, the development of other corresponding technologies is needed, such as nanoparticles that can become RNA carriers.

3.2. Nanotechnology

With the development of nanotechnology, it can be used in medicine and biology for allowing cells and tissues with nanoengineered substrates to have extraordinory functional specificity and control at the molecular, that is, subcellular, level. In the treatment of central nerve regeneration, nanotechnology can be used for drug delivery, cell development and so on. In the study of Dr. Gabriel A. et al. [16], amphipathic peptides can be designed to self-assemble into nanofiber scaffold networks based on their hydrophilicity. This structure can hold the water molecules in place, and if the cell culture medium is mixed with it, the nerve cells are encapsulated in the gel, limiting the proliferation of reactive glia and thereby relieving the inhibition of nerve regeneration caused by it.

The treatment of CNS by nanotechnology can be combined with a variety of different targeted drugs, which has become a new way of modern drug delivery. From the cellular level, you can control the formation of scar tissue, from the genetic side you can deliver RNA, from the molecular side you can deliver influence factors or some proteins. But now that nanotechnology is in its infancy, the risks

are unclear. The remained question is if inhaled nanoparticles, or if they spread throughout the body, can have a fatal effect, causing a cascade of inflammatory reactions.[17]

3.3. Stem cell

After injury to the CNS, there is an inflammatory response in which reactive glia, astrocytes and bone marrow cells, along with various other fine cells, form complexes around the wound--scar cells that block endogenous regeneration. For this reason, scientists have spent the past few years investigating ways to eliminate or inhibit scar cell production so that the CNS can regenerate. Neural stem cells give scientists an idea of how to regenerate the CNS.

Neural stem cells (NSCs) have anti-inflammatory properties and regenerative function, which may provide a new idea for replacing scar cells in CNS regeneration. Therefore, in the treatment of nerve injury, where exogenous NSCs have been found to heal by reducing inflammation and providing cells that replace scar cells, endogenous NSCs establish response conditions in a different way. In 2021, a research team proposed to use artificial 3D model system to study scar cells and combine exogenous or endogenous NSCs to treat the problem of scar cells in central nervous regeneration. [18]

In rodent experiments, the scientists found that endogenous NSCs promote the reactivity of astrokeratinocytes in scars by differentiating, and also that their differentiation restores demyelinating areas. At the same time, exogenous NSCs treatment has obvious effect, has the effect of overcoming inflammation, is conducive to nerve transplantation. The development of a 3D stem cell model system means that a large number of the cells found in scars can be differentiated, enabling endogenous NSCs to function in the area of nerve damage.

4. Conclusion

This review mainly discussed how to relieve the inhibitory effect of scar cells, which is the main factor inhibiting CNS regeneration, so as to achieve the purpose of CNS regeneration. According to CSPG, one of the factors of scar cell formation, the methods of CSPG degradation and CSPG receptor destruction by ChABC are discussed in this chapter. After that, according to the Nogo family proteins of cicatricial cells, the inhibition mechanism of Nogo family proteins was analyzed in depth, and the inhibition of Nogo proteins by RNA interference and the degradation of elements in the complex composed of NgR1, LINGO-1 and p75 were comprehensively discussed. In order to achieve the release of CNS inhibition method. There are some merging medical technologies related to CNS regeneration, the way of RNA targeted blocking to inhibit regeneration, nano-drug delivery technology, and the new treatment method of stem cells replacing the position of glial scar to achieve endogenous regeneration.

In recent years, scientists have had a deeper understanding of central nerve regeneration. A series of studies on the inhibition of CNS regeneration have been carried out around scar cells, from the study on the composition of scar to the discovery of factors related to scar. Although many factors inhibiting CNS regeneration have been found so far, among which the mechanism of action itself or between them has also been discussed, and some researchers have proposed theoretical solutions, but there are few cases that can be used in clinical treatment. Although scientists have studied CNS regeneration in mice and found it to be effective, treatments for humans have yet to be developed. The advent of nanotechnology makes targeted therapy possible, and the use of neural stem cells makes it a completely new treatment idea. However, the side effects of these new technologies are still unclear, which need to be further studied.

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