Effects of the Notch Signaling Pathway on Secondary Brain Changes Caused by Spinal Cord Injury in Mice

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Abstract
Spinal cord injury (SCI) can cause secondary brain changes, leading to hypomyelination in the dorsolateral prefrontal cortex (dlPFC). Some studies have shown that notch signaling pathway activation can regulate oligodendrocyte maturation and myelination. The aim of this study was to investigate whether inhibition of the Notch signaling pathway can alleviate hypomyelination in the dlPFC caused by SCI. Moreover, we further investigated whether the changes in myelination in the dlPFC are associated with neuropathic pain following SCI. We established a mouse model of SCI and observed the changes in mechanical and thermal hyperalgesia. Western blotting and immunofluorescence were used to analyze the changes in myelination in the dlPFC. The results indicated the existence of a relationship between activation of the Notch signaling pathway and hypomyelination in the dlPFC and confirmed the existence of a relationship between hypomyelination in the dlPFC and decreases in mechanical and thermal hyperalgesia thresholds. In conclusion, these results suggested that the Notch signaling pathway is activated after SCI, leading to hypomyelination in the dlPFC, and that DAPT can inhibit the Notch signaling pathway and improve mechanical and thermal hyperalgesia thresholds. Our findings provide a new target for the treatment of neuropathic pain caused by SCI.

Keywords Notch signaling pathway · Hypomyelination · NG2 cell · Secondary brain change · Dorsolateral prefrontal cortex

Abbreviations
SCI Spinal cord injury
dlPFC Dorsolateral prefrontal cortex
CNS Central nervous system
NICD Notch intracellular domain
BMS Basso mouse scale
PWT Paw withdrawal threshold
PWL Paw withdrawal latency
BSA Bovine serum albumin
Olig2 Oligodendrocyte transcription factor 2
MBP Myelin basic protein
PS1 Presenilin1
Aβ β-Amyloid peptide protein
APP Amyloid precursor protein

Introduction
Oligodendrocytes are the only myelinated cells in the brain and have the ability to continuously regenerate. The myelin sheath, a membrane that wraps around neuronal axons, is formed by cell protrusions emitted by mature oligodendrocytes. Myelin plays a role in providing nutritional support and protecting axons [1]. A reduction of myelin formation leads to loss of axonal protection, leading to neuronal degeneration, changes in normal neural function, and even abnormal neurobehavior [2]. Myelination in the central nervous system (CNS) is a continuous process. Myelin is formed not only during development but also through middle age. Effective remyelination plays a very important role in maintaining normal nerve function and preventing abnormal neurobehavior. The regenerative ability of mature oligodendrocytes is the key factor affecting myelination in the CNS.
In the CNS, mature oligodendrocytes are mainly differentiated from NG2 cells. Therefore, normal formation of mature oligodendrocytes depends on normal differentiation and maturation of NG2 cells. NG2 cells are one of four glial cell types in the CNS. A large number of studies have found that NG2 cells remain in the CNS in large numbers and retain the ability to divide and proliferate in adulthood [3]. Under the influence of a series of internal and external factors, they can differentiate into mature oligodendrocytes [4, 5]. When the CNS is affected by demyelinating diseases such as ischemia, infection and trauma, NG2 cells, as the precursor cells of mature oligodendrocytes, can proliferate and differentiate in the lesion area to promote myelin repair [6].

However, the differentiation of NG2 cells and the maturation of oligodendrocytes are affected by a series of factors in vivo and in vitro. Previously, a large number of studies on the regulation of oligodendrocyte formation and myelin regeneration were performed, and it was found that the Wnt/β-catenin, Notch and Sonic hedgehog signaling pathways play an important regulatory role in oligodendrocyte formation and myelin regeneration; however, the specific mechanism is still not fully understood [7]. A large number of studies have proven that activation of the Notch signaling pathway can negatively regulate the differentiation and maturation of NG2 cells [8]. NG2 cells express Notch1 receptor. When the ligand Jagged1 binds to the Notch1 receptor, the Notch signaling pathway can be activated, which can inhibit the differentiation and maturation of NG2 cells [9]. Inhibiting of Notch signaling pathway activation can promote the differentiation and maturation of NG2 cells [10]. Deletion of Notch1 can also enhance the differentiation of NG2 cells and myelin regeneration [11]. These results all indicate that the Notch signaling pathway has an inhibitory effect on the formation of oligodendrocytes and remyelination.

Notch signaling pathway is an important pathway to determine cell fate. Once the Notch receptor is activated, the intracellular domain of Notch (NICD) will be released under the mediation of gamma secretase. Therefore, gamma secretase is a key protease that causes NICD release, and the regulation of gamma secretase has become a key point in the regulation of the Notch signaling pathway. Gamma secretase inhibitors can block the release of NICD and inhibit the activation of Notch signaling pathway [12]. A large number of studies have applied gamma secretase inhibitors to the treatment of Alzheimer’s disease and tumors [13]. DAPT is a new type of gamma secretase inhibitor, which has been widely used in the study of Notch signaling pathway and can effectively inhibit the expression of Hes1 gene [14]. Hes1 is an important negative regulator in the Notch signaling pathway [15]. Notch1 protein is an important receptor in Notch signaling pathway and a key regulator of CNS development [16]. The Notch1 receptor and its downstream Hes1 gene play an important role in the regulation of cell proliferation, differentiation and apoptosis [17].

Neuropathic pain, one of the most common complications of spinal cord injury (SCI), has a serious impact on patients’ recovery and life, and its impact on patients sometimes even exceeds that of SCI itself. Although many drugs have been used for the treatment of neuropathic pain, their treatment effects are not satisfactory. The pathogenesis of neuropathic pain after SCI is complex. Therefore, to improve the treatment of neuropathic pain, elucidation of the complex pathophysiological changes that occur in the CNS is urgently needed. Increased sensitivity of peripheral receptors, neuroinflammation, abnormal excitability of spinal dorsal horn neurons, abnormal activation of glial cells and changes in the structure and function of the cerebral cortex may all lead to neuropathic pain after SCI [18, 19]. Some scholars have found a decreased volume of the cerebral cortex, such as the anterior cingulate cortex, anterior insula, dorsolateral prefrontal cortex (dPFC), which are related to pain regulation, and decreased metabolism in these regions in SCI patients with neuropathic pain [20, 21]. The dPFC plays an important role in the regulation of pain, and it participates in the regulating ascending and descending pain transmission pathways. A reduction in the gray matter volume of the dPFC reduces the ability to regulate pain, leading to the occurrence and development of neuropathic pain [22]. Therefore, elucidating the series of pathophysiological changes that occurs in the dPFC after SCI will provide an understanding of the pathogenesis of neuropathic pain and will help formulate new treatment plans for early intervention for neuropathic pain in the future.

Thus far, there have been few studies on the effect of SCI on brain myelination. Therefore, in this study, we established a mouse SCI model and observe changes in thermal and mechanical hyperalgesia thresholds on the day of the operation and after the operation. The relationship between the activation of the Notch signaling pathway and the differentiation and maturation of NG2 cells in the dPFC after SCI was explored, and whether these changes are involved in the occurrence and development of neuropathic pain was assessed. Western blot analysis and immunofluorescence were used to observe the changes in the expression of proteins related to the Notch signaling pathway and oligodendrocyte lineages in the dPFC after SCI. We preliminarily discussed the role of the dPFC in the regulation of neuropathic pain after SCI. The purpose of this study was to find a new target for the treatment of neuropathic pain caused by SCI. The timeline and experimental design of this study are shown in Fig. 1.
Materials and Methods

Animals and Grouping

The experimental animals were adult male C57BL/6 J mice (22–26 g) aged 8 to 10 weeks, which were purchased from Hefei City, Anhui Province. All procedures and experiments were approved by the Ethical Review Committee of the First Affiliated Hospital of Nanchang University. We randomly divided all mice into four groups (six in each group): the sham operation group (sham group), sham operation group treated with DAPT (sham + DAPT group), SCI group and SCI group treated with DAPT (SCI + DAPT group).

SCI and Drug Administration

The operation was performed following the Guide for the Care and Use of Laboratory Animals published by the NIH. An aneurysm clip (FT220T, Braun, Germany, calibrated force of 70 g) was used to clamp the T10 spinal cord of mice in the SCI group. Mice in the sham operation group were subjected to T10 laminectomy but not SCI. DAPT (100 mg/kg) was injected intraperitoneally 3 h before the operation [23]. Manual bladder evacuation was performed for mice in the SCI group at least three times a day until bladder function recovered.

Reagents and Materials

DAPT (ab120633) was purchased from Abcam (England). A rabbit monoclonal antibody against Notch1 (ab52627), a rabbit polyclonal antibody against Notch1 intracellular domain (ab83232), a rabbit monoclonal antibody against Hes1 (ab108937), a rabbit monoclonal antibody against NG2 (ab275024), a rabbit polyclonal antibody against Olig2 (ab254043), a rabbit monoclonal antibody against MBP (ab218011), a mouse monoclonal antibody against CNPase (ab6319), and a rabbit monoclonal antibody against PLP (ab254363), were purchased from Abcam (England). A rabbit polyclonal antibody against PS1 (YT3875) and a rabbit polyclonal antibody against Aβ (YT5754) were purchased from Immunoway (USA). A rabbit polyclonal antibody against APP (25524-1-AP) was purchased from Proteintech (USA). A mouse monoclonal β-actin antibody (TA811000) was purchased from Origene Biological Technology (China). The secondary antibodies used for western blotting and immunofluorescence, i.e., HRP-conjugated goat anti-rabbit IgG (BA1054), and goat anti-rabbit IgG (Gb21303) were purchased from Boster Biological Technology and Service Biological Technology (China), respectively.

Behavioral Analysis

The Basso Mouse Scale (BMS) was used to evaluate hindlimb motor function before and after the operation. The paw withdrawal threshold (PWT) was assessed by the Von Frey filament test (Bioseb, France) (force ranging from 0.04 g to 2.0 g). The paw withdrawal latency (PWL) was assessed according to the response to radiant heat stimulation (Ugo Basile, Italy). All evaluations were conducted by two independent investigators blinded to the experimental design.

Analysis of Histomorphological Changes in the Spinal Cord

Hematoxylin and eosin (HE) staining was used to observe injury to the spinal cord. Spinal cord tissue was taken from the site of laminectomy (T10) for the sham operation group and from the injury site for the SCI group (a 1-cm-long piece of tissue centered around the injury site) and embedded in paraffin after fixation and dehydration. Paraffin-embedded spinal cord tissue was cut into 4 μm sections and stained with HE.

Western Blot Analysis

Western blot analysis referred to previous study [24]. Mice dlPFC tissues were weighed, placed in a clean mortar and pestle and rapidly ground. Then RIPA lysis buffer containing PMSF (the volume fraction of PMSF was 1%) was added; the tissues were lysed on ice for 20 min, shaken on a 4 °C shaker, centrifuged at 14,000×g for 15 min at 4 °C; and the supernatant was carefully aspirated and stored in a − 80 °C freezer. The protein content of each sample was determined.
according to the manufacturer’s instructions. The samples to be examined were mixed with 6× buffer loading buffer in a 5:1 ratio, shaken well, and boiled in a water bath for 10 min. A stacking gel and separating gel were made, and equal amounts of protein from each sample were added to each well. After the proteins were successfully electrophoresed and transferred to a membrane, the PVDF membrane was blocked with 5% bovine serum albumin (BSA), incubated in primary antibody overnight on a shaker at 4 °C and rinsed three times with TBST for 15 min each. The membrane was incubated with secondary antibody for 1 h on a shaker at 37 °C and rinsed three times with TBST for 15 min each. Development solution was prepared by mixing liquid A with liquid B in a 1:1 ratio in a darkroom. We used ImageJ software (National Institutes of Health) to quantify the protein bands.

**Immunofluorescence Analysis**

The mice were anesthetized by intraperitoneal injection of sodium pentobarbital and perfused with normal saline and 4% paraformaldehyde. The brains were removed and fixed in paraformaldehyde solution and then embedded in paraffin. dlPFC sections (4 μm thick) were prepared by stereotactic localization using a mouse brain atlas. The tissue sections were dewaxed, incubated in hydrogen peroxide solution and blocked in 5% BSA. The liquid around the tissue was removed, primary antibody was added, and the sections were incubated overnight at room temperature and washed 4 times with PBS for 8 min each. A fluorescent secondary antibody was added, and the sections were incubated for 2 h at room temperature. Then, 50% neutral glycerine to seal the sections, and the sections were observed under a fluorescence microscope (Olympus company, Japan). The experimental process referred to previous study [25].

**Double Immunofluorescence Analysis**

The Notch1 receptor is expressed on the cell membrane, and the NG2 protein is a specific marker of NG2 cells. In this study, activation of the Notch signaling pathway in NG2 cells after SCI was observed by double immunofluorescence for the Notch 1 protein and NG2 protein. The tissue sections were dewaxed in xylene, incubated with citrate buffer (pH 6.0) for antigen repair, and blocked at room temperature for 30 min. After being washed three times, the sections were respectively incubated with a rabbit anti-Notch1 antibody (1:200) mixed with a rabbit anti-NG2 antibody (1:200), a rabbit anti-PS1 antibody (1:200) mixed with a rabbit anti-Aβ antibody (1:200) overnight at 4 °C and then incubated with secondary antibody at room temperature. DAPI was added to the sections and mixed well. The sections were observed under an Olympus fluorescence microscope. The experimental process referred to previous study [26].

**Statistical Analysis**

Quantitative data are presented as the mean ± SD. Statistical analysis was performed using SPSS 18.0. Different statistical methods were used to analyze different types of data. BMS scores, mechanical allodynia and thermal hyperalgesia were compared between different groups by two-way ANOVA followed by the Bonferroni post hoc test. Differences in the data shown in Figs. 4B, 5C 6C, 7B, 8B, 9B 10B, E between each group at each time point were analyzed using one-way ANOVA followed by Tukey’s post hoc test. *p* < 0.05 was considered statistically significant.

**Results**

The regulation of Notch signaling pathway on the differentiation and maturation of NG2 cells has been extensively studied. However, the effect of Notch signaling pathway on the differentiation and maturation of NG2 cells in the brain after SCI is still less discussed. The present work investigated the effect of the activation of Notch signaling pathway in the dlPFC on the differentiation and maturation of NG2 cells after SCI, and further discussed the correlation between these changes in the dlPFC and the occurrence and development of neuropathic pain. For this purpose, SCI was successfully induced in mice with an aneurysm clip. The activation of the Notch signaling pathway in the dlPFC and its effect on the differentiation and maturation of NG2 cells was analyzed by western blotting and immunofluorescence, and changes in the PWT and PWL of mice in each group were assessed before and after the operation.

**Locomotion Analysis**

BMS is a scoring method based on the characteristics of motor function of SCI mice, which is a sensitive and reliable scoring method for mice [27]. The BMS score of the sham operation group at each time point was 9, and there were no obvious abnormalities in hindlimb motor function. The BMS score of the SCI group was 0 on the first day after the operation, at which point the hindlimbs were completely paralyzed. After 24 h, the BMS score gradually recovered. The average BMS score of the SCI group was significantly lower than that of the sham operation group. From the seventh day after operation, the average BMS score in the SCI + DAPT group was significantly higher than that in the SCI group (**p < 0.001) (Fig. 2).
HE Staining of Spinal Cord Tissue

HE staining was used to observe the morphological changes of spinal cord tissue. 14 days after the operation, HE staining of spinal cord tissues from the sham group and sham + DAPT group showed that the gray and white matter of the spinal cord were structurally intact, with uniformly distributed cells; normal morphology; and clear Nissl bodies; and no hemorrhagic necrosis, edema, or degeneration. HE staining of spinal cord tissues from the SCI group and SCI + DAPT group indicated that the structure of the spinal cord in the injured area was destroyed, the gray matter and white matter were edematous, the boundaries were unclear, Nissl bodies were absent, the neuronal structure was destroyed, some of the nuclei were pyknotic, and vacuoles were present in the gray and white matter (Fig. 3).

Administration of DAPT Inhibited Activation of Notch Signaling, Promoted NG2 Cell Differentiation in the dIPFC, and Decreased the PWT and PWL

Gamma secretase inhibitors are specific blockers of the Notch signaling pathway. Presenilin1 (PS1) is the active center of gamma secretase [28]. And gamma secretase is also an important factor in promoting the production of β-amyloid peptide protein (Aβ), which is a normal metabolite of amyloid precursor protein (APP). Thus, gamma secretase inhibitors can not only inhibit the activity of the Notch signaling pathway, but also inhibit the production of Aβ. Studies have confirmed that changes in APP and Aβ expression will occur after CNS injury [29].

DAPT is a new type of gamma secretase inhibitor. One study found that the application of DAPT could inhibit the expression of APP and Aβ, and the decrease of Aβ could affect the functional recovery of SCI animals [30]. In our study, DAPT pretreatment inhibited Notch signaling pathway activity. The western blot results showed that there was a significant reduction in Notch1 levels in the dIPFC in the SCI + DAPT group (**p < 0.001). The expression of NG2 was also downregulated in the SCI + DAPT group, while the expression of oligodendrocyte transcription factor 2 (Olig2) was upregulated in the SCI + DAPT group, indicating that DAPT suppressed Notch signaling pathway activation and promoted NG2 cell differentiation in the dIPFC (**p < 0.001). Mechanical and thermal hyperalgesia thresholds, as indicated by the PWT and PWL, gradually decreased in the SCI group but showed no obvious changes in the sham operation group. Administration of the Notch inhibitor DAPT markedly prevented the development of thermal and mechanical hyperalgesia, as the PWT and PWL were significantly attenuated in the SCI + DAPT group (**p < 0.001). Our studies showed that downregulation of Notch1 expression in the dIPFC promoted NG2 cell differentiation and attenuated the development of neuropathic pain (Fig. 4).

In our study, we also found that DAPT not only inhibited the activation of Notch signaling pathway, but also inhibited the expression of PS1, APP and Aβ. The western blot results showed that PS1, APP and Aβ in the dIPFC increased significantly after SCI; while PS1, APP and Aβ in the SCI + DAPT group were significantly reduced compared with the SCI group (**p < 0.001). The immunofluorescence results showed that PS1 and APP increased significantly after SCI, but decreased significantly in the SCI + DAPT group (Fig. 5). However, there is still some controversy as to whether the reduction of Aβ protein will have a negative effect on the functional recovery of CNS injury, and further research is still needed [31].

DAPT Inhibited the Expression of Downstream Molecules of Notch Signaling Pathway After SCI

DAPT can inhibit the formation of NICD by preventing gamma secretase from cleaving the S3 site of the intracellular segment of the Notch receptor, preventing the translocation of NICD to the nucleus and thus inhibiting the activation of the Notch
signaling pathway [12]. In the SCI group, the immunofluorescence results showed that the expression of NICD increased significantly. DAPT treatment could significantly reduce the expression of NICD (Fig. 6A). The western blot results showed that the protein level of NICD in the SCI + DAPT group were significantly decreased compared with the SCI group (**p < 0.001).

Fig. 4  A Immunoreactive bands for Notch1, NG2, Olig2 and β-actin. B Changes in the optical densities of Notch1, NG2, and Olig2 in each group. After DAPT treatment, the activity of the Notch signaling pathway was inhibited, the protein levels of NG2 in the dlPFC were significantly decreased, and the protein levels of Olig2 were significantly increased (**p < 0.001). 
C The PWL of the SCI group was significantly lower than that of the SCI + DAPT group (*p < 0.05, **p < 0.001). 
D The PWL of the SCI group was significantly lower than that of the SCI + DAPT group (*p < 0.05, **p < 0.001).

Fig. 5  DAPT treatment inhibited the expression of PS1, APP and Aβ in the dlPFC after SCI.  A Immunofluorescence analysis of PS1 and APP expression in the dlPFC in each group. B Immunoreactive bands for PS1, APP, Aβ and β-actin. C Changes in the optical densities of PS1, APP and Aβ in each group. The protein levels of PS1, APP and Aβ in the SCI + DAPT group were significantly decreased compared with the SCI group (**p < 0.001). Scale bar: 20 μm.
Hes1 is an important effector molecule in the Notch signaling pathway and a negative transcriptional regulator that is mainly expressed in the nucleus. Continuous high expression of Hes1 leads to inhibition of cell differentiation. In our study, immunofluorescence showed that the expression of Hes1 in the nucleus was significantly higher in the SCI group than in the sham operation group, indicating that the Notch-Hes1 signaling pathway was activated in the dlPFC after SCI and that the expression of Hes1 in the SCI + DAPT group was significantly decreased. These results were consistent with the western blotting results mentioned above, further indicating that DAPT inhibited the activation of the Notch signaling pathway in the dlPFC after SCI (Fig. 7).

The Inhibitory Effects of SCI on NG2 Cell Differentiation and Maturation via the Notch Signaling Pathway

It was shown previously that NG2 cell differentiation and maturation are regulated by the Notch signaling pathway [10]. Notch receptors are expressed on the surface of NG2 cells, and the activation of Notch signaling pathway can inhibit the differentiation and maturation of NG2 cells [32]. To investigate the effect of the Notch signaling pathway on NG2 cells, we performed coimmunostaining using anti-NG2 and anti-Notch1 antibodies. The data showed that the number of NG2 cells increased and that Notch1 was expressed at high levels in the dlPFC after SCI, whereas the number of NG2 cells decreased and Notch1 was expressed at low levels in the SCI + DAPT group. These results further confirmed that the Notch signaling pathway has a negative regulatory effect on the differentiation and maturation of NG2 cells (Fig. 8).

SCI Induced Notch Signaling Pathway Activation Restrained the Maturation of Oligodendrocytes

NG2 cells are the main source of mature oligodendrocytes. Inhibiting the activation of Notch signaling pathway can promote the formation of oligodendrocytes [10]. Olig2 is a specific marker of mature oligodendrocytes. We observed the changes in the number of Olig2 + oligodendrocytes in the dlPFC after SCI by immunofluorescence. The results showed that the number of Olig2 + oligodendrocytes in the dlPFC was significantly decreased in the SCI group compared with the sham operation group (**p < 0.001). The number of oligodendrocytes in the SCI + DAPT group was significantly increased compared with the SCI group (**p < 0.001). However, the number of oligodendrocytes in the SCI + DAPT group was still significantly lower than that in the sham + DAPT group (**p < 0.001). The above results indicated that SCI inhibited the differentiation of NG2 cells into mature oligodendrocytes and that DAPT pretreatment reversed the reduction in the number of oligodendrocytes in the dlPFC after SCI (Fig. 9A, B). Based on these results, we further observed the changes in the number of CNPase + oligodendrocytes. 2',3'-cyclic nucleotide phosphohydrolase (CNPase) is also a marker of mature oligodendrocytes. The same results were obtained as above, CNPase + oligodendrocytes in the SCI + DAPT group increased significantly compared with the SCI group.
Fig. 8 Immunofluorescence staining showing DAPI-labeled nuclei (blue), Notch1 (red) and NG2 cells (green) in the dlPFC in each group on the 14th day after the operation. B The bar graph showed that the number of Notch1 + and NG2 + cells in the dlPFC was significantly increased in the SCI group compared with the SCI + DAPT group (**p < 0.001). Scale bar: 20 μm

Fig. 9 Immunofluorescence staining of Olig2 + oligodendrocytes in the dlPFC. A Immunofluorescence staining showing DAPI-labeled nuclei (blue) and Olig2 + oligodendrocytes (red) in the dlPFC in each group on the 14th day after the operation. B The bar graph showed that compared with that in the SCI group, the number of Olig2 + oligodendrocytes/mm³ in the SCI + DAPT group was significantly increased (*p < 0.05). The number of Olig2 + oligodendrocytes/mm³ in the SCI + DAPT group was still lower than that in the sham + DAPT group (**p < 0.001). C Immunofluorescence staining showing CNPase + oligodendrocytes in the SCI + DAPT group were significantly increased compared with the SCI group. Scale bar: 20 μm
Moreover, our results were consistent with previous studies [32].

**SCI Induced Hypomyelination in the dlPFC**

Myelin basic protein (MBP) and proteolipid protein (PLP) are the component of myelin sheath, which play an important role in maintaining the function of the CNS. They are secreted by mature oligodendrocytes in the CNS. When the maturation of oligodendrocyte was inhibited, the expression of MBP and PLP also decreased. To confirm whether the reduction in the number of Olig2+ oligodendrocytes resulted in reduced myelin production, MBP expression in the dlPFC was assessed by immunofluorescence. Immunofluorescence analysis showed that MBP protein expression was reduced after SCI, but this decrease was reversed by pretreatment with DAPT. The protein expression of MBP was significantly lower in the SCI group than the SCI + DAPT group on the 14th day after operation (*p < 0.05). And the expression of MBP in the SCI + DAPT group was also significantly lower than that in the sham + DAPT group (**p < 0.001). These data indicated that the decrease in the number of oligodendrocytes after SCI resulted in reduced myelin protein production and hypomyelination in the dlPFC and that myelin regeneration was restored by inhibition of the Notch signaling pathway (Fig. 10A, B). Based on these results, we further studied the expression of PLP. The same results were obtained as above, the expression of PLP increased significantly in the SCI + DAPT group compared with the SCI group (Fig. 10C). The western blot results showed that the protein level of PLP in the SCI + DAPT group was significantly increased compared with the SCI group (**p < 0.05) (Fig. 10D, E).

**Discussion**

The most common complications of SCI are sensory, motor, and sphincter dysfunction. Neuropathic pain is also a persistent complication of SCI. The incidence of neuropathic

**Fig. 10** Expression of MBP in each group. A Immunofluorescence analysis of MBP expression in the dlPFC in each group on the 14th day after operation. B The bar graph showed the immunofluorescence density of MBP. The immunofluorescence intensity in the SCI + DAPT group was significantly higher than that in the SCI group (*p < 0.05), and was still lower than that in the sham + DAPT group (**p < 0.001). C Immunofluorescence analysis of PLP expression in the dlPFC in each group. D Immunoreactive bands for PLP and β-actin. E Changes in the optical densities of PLP in each group, the protein level of PLP in the SCI + DAPT group were significantly increased compared with the SCI group (*p < 0.05). Scale bars: 20 μm
pain in patients with SCI is approximately 65%-85%, and approximately 1/3 of patients have severe pain, which causes great pain to the patient and seriously affects the quality of life of the patient [33]. However, the specific pathogenesis of neuropathic pain after SCI is still unclear, thus effective treatment measures are lacking. Therefore, neuropathic pain seriously affects human health, and treatment strategies for this condition urgently need to be identified. Although scholars at home and abroad have explored the pathogenesis of neuropathic pain from different aspects in recent years and proposed different hypotheses, the pathogenesis of neuropathic pain after SCI is complex, and there is no single mechanism underlying this condition. Current research only addresses neuropathic pain from a single perspective. This study aims to preliminarily explore the pathogenesis of neuropathic pain after SCI. As a result, our study found that: (1) Activation of the Notch signaling pathway in the dIPFC after SCI is involved in the differentiation and maturation of NG2 cells; and (2) Deficits in the generation of oligodendrocytes and remyelination in the dIPFC may be related to the occurrence and development of neuropathic pain after SCI.

NG2 is a glycoprotein expressed on the membrane of oligodendrocyte precursor cells. It is a specific marker of oligodendrocyte precursor cells. Therefore, oligodendrocyte precursor cells are also called NG2 cells. NG2 cells can respond to CNS damage, differentiate to replace damaged oligodendrocytes, and aid remyelination and are considered stem cells that are conducive to repair after CNS injury [34]. Therefore, whether NG2 cells can differentiate into oligodendrocytes during CNS injury is of great significance for myelin regeneration and repair after CNS injury. However, NG2 cells not only differentiate into oligodendrocytes as precursor cells but also act as precursors of neurons and other glial cells. NG2 cells have the potential to differentiate into a variety of cells, such as astrocytes and neurons [35]. Therefore, the complex role and regulatory mechanism of NG2 cells in the repair after CNS injury still need to be further explored and studied.

The Notch signaling pathway is an important signaling pathway that is involved in CNS development and damage repair [36]. Activation of the Notch signaling pathway can inhibit the differentiation and maturation of NG2 cells and reduce the generation of oligodendrocytes and myelin regeneration, and the application of Notch signaling pathway inhibitors can reverse the above effects [9, 37]. Notch1 expression was found to be increased in NG2 cells in multiple sclerosis, leading to decreased myelin regeneration [38]. This study revealed that the expression of Notch1 was significantly increased in the dIPFC in the SCI group. Double immunofluorescence showed that Notch1 was localized in NG2 cells, indicating that the Notch signaling pathway may be involved in the differentiation and maturation of NG2 cells in the dIPFC after SCI. However, different findings related to the role of the Notch signaling pathway in regulating oligodendrocyte maturation and myelination have been reported [39]. First, a large amount of evidence has shown that, in addition to the inhibitory Jagged1/Notch1 signaling pathway, other pathways act via the Notch signaling pathway to mediate oligodendrocyte differentiation [40]. The glycosyl phosphatidylinositol-anchored neural cell adhesion molecule F3/contactin is clustered at the paranodal region, a vital site for axoglial interaction, during development; acts as a functional ligand of Notch; can trigger gamma-secretase-dependent nuclear translocation of the NICD; and promotes oligodendrocyte maturation and myelination [41]. Second, in the CNS, NG2 cell-derived glial cells, oligodendrocytes, astrocytes, microglia and neurons can express receptors or ligands of the Notch signaling pathway and participate in the activation of the Notch signaling pathway, which may play a role in the pathogenesis of CNS diseases. Moreover, various cytokines or signaling pathways may interact with the Notch signaling pathway. Therefore, the role of the Notch signaling pathway in regulating the differentiation and maturation of NG2 cells still needs to be further clarified.

The activation of Notch signaling pathway is closely related to neuropathic pain. The application of DAPT can alleviate pain sensitivity, but the exact mechanism remains unclear [42]. DAPT can not only inhibit the activation of Notch signaling pathway, but also inhibit the production of Aβ. Some studies have found that the increase of APP and Aβ is closely related to the severity of traumatic brain injury [43]. However, another study found that although the application of DAPT could inhibit the expression of APP and Aβ, but the functional recovery of SCI animals was affected [30]. In our study, the application of DAPT significantly inhibited the activation of the Notch signaling pathway, significantly reduced the effect of the Notch signaling pathway on the differentiation and maturation of NG2 cells, and at the same time inhibited the production of Aβ, but the motor function of the injured mice did not fully recover, and the pain sensitivity was not completely alleviated. In addition to the influence of complex pathophysiological changes in the CNS after SCI on the recovery of neural function, it may be related to the complex regulatory mechanism of neuropathic pain, and may also be related to the complex regulatory mechanism of the Notch signaling pathway. Therefore, in view of the important relationship of Notch signaling pathway with gamma secretase, PS1 and APP, when DAPT is applied to experimental research, the interrelationship between Notch signaling pathway and other factors should be fully considered. Even though DAPT is a specific inhibitor of the Notch signaling pathway, it remains to be further investigated whether it can become an effective drug for the treatment of SCI and neuropathic pain in the future.

The CNS is a unified whole, and our data revealed that the impact of SCI on the CNS is widespread. Local changes

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in the spinal cord occur at the injured site, and complex pathophysiological changes also take place throughout the CNS; these complex secondary changes may be the main factors affecting the recovery of patients and leading to complications [44, 45]. The Notch signaling pathway may play an important role in these complex secondary pathophysiological changes. In this study, the administration of a Notch signaling pathway inhibitor significantly ameliorated the effect of SCI on the differentiation and maturation of NG2 cells in the dIPFC, and the preliminary results also suggested that these changes may be related to the occurrence and development of neuropathic pain. These findings will deepen our understanding of secondary brain changes caused by SCI and are of great significance, as they provide new insight into the pathogenesis of neuropathic pain. Remyelination is very important for functional repair after CNS injury. This process involves the proliferation, differentiation and maturation of NG2 cells, and each of these processes is affected by many factors, including many signaling pathways, a large number of cytokines and other glial cells. With the deepening of research, a detailed understanding of every aspect of remyelination regulation and identification of strategies to prevent the vicious cycle of demyelination and neurodegeneration after SCI may aid the treatment of new treatment methods for SCI, which is conducive to recovery after SCI.

**Limitations of this Study**

First, our experimental results showed that the Notch signaling pathway is involved in the regulation of NG2 cell differentiation, maturation and myelination. However, regulation of the CNS involves many factors. Therefore, the existence of other regulatory mechanisms that play an even more important role cannot be ruled out. Second, because pain regulation in the CNS involves a complex neural network in which the dIPFC constitutes only one node, the specific role of the dIPFC in the regulation of neuropathic pain needs to be further studied. Third, our results preliminarily suggest that Notch signaling pathway inhibitors can alleviate neuropathic pain and may affect secondary changes in the dIPFC. However, whether Notch signaling pathway inhibitors act on the dIPFC only or on other brain regions as well remains to be further studied. Due to limitations of time and manpower, we did not systematically describe the secondary changes at the injury site of the spinal cord and in other brain regions in this study. Functional magnetic resonance technology can be used in the future to observe the changes in the structure, function and metabolism of the whole brain and spinal cord after SCI to reveal the regulatory network of neuropathic pain and the interaction and relationship between the regulatory networks and thus elucidate the mechanism of neuropathic pain caused by SCI.

**Conclusions**

There are no satisfactory methods for the treatment of neuropathic pain caused by SCI in the clinic. Although a large number of studies on the pathogenesis of neuropathic pain at the cellular and molecular levels have been carried out, the specific pathophysiological mechanism is still unclear. However, in recent years, secondary brain changes after SCI have attracted increasing attention from researchers. Both clinical studies and animal studies have shown that external factors can cause myelin abnormalities, which have a profound impact on nerve function. Therefore, considering the current status of treatment approaches for neuropathic pain after SCI, we focused on the influence of SCI on myelination in the dIPFC to explore the pathogenesis of neuropathic pain. In conclusion, our results provide new data for the study of neuropathic pain pathogenesis and a novel target for the treatment of neuropathic pain after SCI.

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**Data Availability** Data and materials are available from the corresponding author on reasonable request.

**Declarations**

**Conflict of interest** The authors declare that they have no conflict of interest.

**Consent for Publication** All authors gave consent for publication.

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**References**

1. Nave KA, Werner HB (2014) Myelination of the nervous system: mechanisms and functions. Annu Rev Cell Dev Biol 30:503–533
2. Griffiths I, Klugmann M, Anderson T et al (1998) Axonal swellings and degeneration in mice lacking the major proteolipid of myelin. Science 280(5369):1610–1613
3. Dang TC, Ishii Y, Nguyen V et al (2019) Powerful homeostatic control of oligodendrogial lineage by PDGF-Ralpha in adult brain. Cell Rep 27(4):1073–1089
4. Young KM, Psachoulia K, Tripathi RB et al (2013) Oligodendrocyte dynamics in the healthy adult CNS: evidence for myelin remodeling. Neuron 77(5):873–885
5. Kang SH, Fukaya M, Yang JK et al (2010) NG2+ CNS glial progenitors remain committed to the oligodendrocyte lineage in postnatal life and following neurodegeneration. Neuron 68(4):668–681
6. Valny M, Honza P, Kiska J et al (2017) Multipotency and therapeutic potential of NG2 cells. Biochem Pharmacol 141:42–55
7. Ravanelli AM, Kearns CA, Powers RK et al (2018) Sequential specification of oligodendrocyte lineage cells by distinct levels of Hedgehog and Notch signaling. Dev Biol 444(2):93–106
8. Li C, Xie Z, Xing Z et al (2021) The Notch signaling pathway regulates differentiation of NG2 cells into oligodendrocytes in demyelinating diseases. Cell Mol Neurobiol. https://doi.org/10.1007/s10571-021-01089-0
9. Zang Y, Argaw AT, Gurfein BT et al (2009) Notch1 signaling plays a role in regulating precursor differentiation during CNS remyelination. Proc Natl Acad Sci USA 106(45):19162–19167
10. Wang S, Sdrulla AD, Disibio G et al (1998) Notch receptor activation inhibits oligodendrocyte differentiation. Neuron 21(1):63–75
11. Genoud S, Lappe-Stiefke C, Goebels S et al (2002) Notch1 control of oligodendrocyte differentiation in the spinal cord. J Cell Biol 158(4):709–718
12. Doye HF, John V, Anderson JP et al (2001) Functional gamma-secretase inhibitors reduce beta-amyloid peptide levels in brain. J Neurochem 76(1):173–181
13. Evin G, Sernee MF, Masters CL (2006) Inhibition of gamma-secretase as a therapeutic intervention for Alzheimer’s disease: prospects, limitations and strategies. CNS Drugs 20(5):351–372
14. Geling A, Steiner H, Willem M et al (2002) A gamma-secretase inhibitor blocks Notch signaling in vivo and causes a severe neurogenic phenotype in zebrafish. EMBO Rep 3(7):688–694
15. Sueda R, Imai Y, Harima Y et al (2019) High Hes1 expression and resultant Ascl1 suppression regulate quiescent vs. active neural stem cells in the adult mouse brain. Genes Dev 33(9–10):511–523
16. Shi M, Liu Z, Lv Y et al (2011) Forced notch signaling inhibits commissural axon outgrowth in the developing chick central nerve system. PLoS ONE 6(1):e14570
17. Artavanis-Tsakonas S, Rand MD, Lake RJ (1999) Notch signaling: cell fate control and signal integration in development. Science 284(5415):770–776
18. Hains BC, Waxman SG (2006) Activated microglia contribute to the maintenance of chronic pain after spinal cord injury. J Neurosci 26(16):4308–4317
19. Li Y, Cao T, Ritzel RM et al (2020) Dementia, depression, and associated brain inflammatory mechanisms after spinal cord injury. Cells 9(6):1420
20. Yoon EJ, Kim YK, Shin HJ et al (2013) Cortical and white matter alterations in patients with neuropathic pain after spinal cord injury. Brain Res 1540:64–73
21. Gustin SM, Wrigley PJ, Siddall PJ et al (2010) Brain anatomy changes associated with persistent neuropathic pain following spinal cord injury. Cereb Cortex 20(6):1409–1419
22. Apkarian VA, Hashmi JA, Baliki MN (2011) Pain and the brain: specificity and plasticity of the brain in chronic clinical pain. Pain 152(3 Suppl):S49–S64
23. Yang Q, Yan W, Li X et al (2012) Activation of canonical notch signaling pathway is involved in the ischemic tolerance induced by sevoflurane preconditioning in mice. Anesthesiology 117(5):996–1005
24. Xie Z, Huang S, Xie S et al (2021) Potential correlation between depression-like behavior and the mitogen-activated protein kinase pathway in the rat hippocampus following spinal cord injury. World Neurosurg 154:e29–e38
25. Huang P, Chen X, Hu X et al (2020) Experimentally induced sepsis causes extensive hypomyelination in the prefrontal cortex and hippocampus in neonatal rats. Neuromolecular Med 22(3):420–436
26. Aparicio E, Mathieu P, Pereira LM et al (2013) The Notch signaling pathway: its role in focal CNS demyelination and apoptosis-related remyelination. J Neurochem 127(6):819–836
27. Basso DM, Fisher LC, Anderson AJ et al (2006) Basso mouse scale for locomotion detects differences in recovery after spinal cord injury in five common mouse strains. J Neurotrauma 23(5):635–659
28. Cai T, Morishima K, Takagi-Niidome S et al (2019) Conformational dynamics of transmembrane domain 3 of presenilin 1 is associated with the trimming activity of gamma-secretase. J Neurosci 39(43):8600–8610
29. Tian L, Guo R, Yue X et al (2012) Intranasal administration of nerve growth factor ameliorates beta-amyloid deposition after traumatic brain injury in rats. Brain Res 1440:47–55
30. Pajoohesh-Ganjii A, Burns MP, Pal-Ghosh S et al (2014) Inhibition of amyloid precursor protein secretases reduces recovery after spinal cord injury. Brain Res 1560:73–82
31. Kobayashi S, Sasaki T, Katayama T et al (2010) Temporal-spatial expression of presenilin 1 and the production of amyloid-beta after acute spinal cord injury in adult rat. Neurochem Int 56(3):387–393
32. Du M, Tan Y, Liu G et al (2017) Effects of the Notch signalling pathway on hyperoxia-induced immature brain damage in newborn mice. Neurosci Lett 653:220–227
33. Siddall PJ, Mcclelland JM, Rutkowski SB et al (2003) A longitudinal study of the prevalence and characteristics of pain in the first 5 years following spinal cord injury. Pain 103(3):249–257
34. Dimou L, Gallo V (2015) NG2-glia and their functions in the central nervous system. Glia 63(8):1429–1451
35. Belachew S, Chittajallu R, Aguirre AA et al (2003) Postnatal NG2 proteoglycan-expressing progenitor cells are intrinsically multipotent and generate functional neurons. J Cell Biol 161(1):169–186
36. Ebehaber M, Hayward P, Arias AM (2006) Notch, a universal arbiter of cell fate decisions. Science 314(5804):1414–1415
37. Hammond TR, Gadea A, Dupree J et al (2014) Astrocye-derived endothelin-1 inhibits remyelination through notch activation. Neuron 81(3):588–602
38. Jurynczyk M, Selmaj K (2010) Notch: a new player in MS mechanisms. J Neuroimmunol 225(1–2):9–17
39. Cui XY, Hu QD, Tekaya M et al (2004) NB-3/Notch1 pathway via Deltex1 promotes neural progenitor cell differentiation into oligodendrocytes. J Biol Chem 279(24):25858–25865
40. Huang P, Zhou Q, Lin Q et al (2020) Complement C3a induces axonal hypomyelination in the periventricular white matter through activation of WNT/beta-catenin signaling pathway in septic neonatal rats experimentally induced by lipopolysaccharide. Brain Pathol 30(3):495–514
41. Hu QD, Ang BT, Karsak M et al (2003) F3/contactin acts as a functional ligand for Notch during oligodendrocyte maturation. J Neurosci 23(5):635–659
42. Chen XF, Johnson VE, Uryu K et al (2009) A lack of amyloid beta plaques despite persistent accumulation of amyloid beta in axons of long-term survivors of traumatic brain injury. Brain Pathol 19(2):214–223

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44. Hilton BJ, Moulson AJ, Tetzlaff W (2017) Neuroprotection and secondary damage following spinal cord injury: concepts and methods. Neurosci Lett 652:3–10
45. Wu J, Zhao Z, Sabirzhanov B et al (2014) Spinal cord injury causes brain inflammation associated with cognitive and affective changes: role of cell cycle pathways. J Neurosci 34(33):10989–11006

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