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RESEARCH ARTICLE

Tau modulates Schwann cell proliferation, migration and differentiation following peripheral nerve injury

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ABSTRACT

Tau protein (encoded by the gene microtubule-associated protein tau, *Mapt*) is essential for the assembly and stability of microtubule and the functional maintenance of the nervous system. Tau is highly abundant in neurons and is detectable in astrocytes and oligodendrocytes. However, whether tau is present in Schwann cells, the unique glial cells in the peripheral nervous system, is unclear. Here, we investigated the presence of tau and its coding mRNA, *Mapt*, in cultured Schwann cells and find that tau is present in these cells. Gene silencing of *Mapt* promoted Schwann cell proliferation and inhibited Schwann cell migration and differentiation. *In vivo* application of *Mapt* siRNA suppressed the migration of Schwann cells after sciatic nerve injury. Consistent with this, *Mapt*-knockout mice showed elevated proliferation and reduced migration of Schwann cells. Rats injected with *Mapt* siRNA and *Mapt*-knockout mice also exhibited impaired myelin and lipid debris clearance. The expression and distribution of the cytoskeleton proteins α-tubulin and F-actin were also disrupted in these animals. These findings demonstrate the existence and biological effects of tau in Schwann cells, and expand our understanding of the function of tau in the nervous system.

KEY WORDS: Tau, Schwann cell, Peripheral nerve injury, Proliferation, Migration, Differentiation

INTRODUCTION

Tau protein is a highly soluble microtubule assembly protein encoded by the gene microtubule-associated protein tau (*Mapt*). Tau is most abundant in neurons of the central nervous system. It performs important functions in modulating the stability of microtubules and promoting tubulin assembly into microtubules (Cleveland et al., 1977; Elie et al., 2015; Janning et al., 2014). Dysfunctional tau is highly associated with many neurodegenerative disorders, such as frontotemporal lobar degeneration, chronic traumatic encephalopathy, Parkinson's disease and Alzheimer's disease (Goedert, 2015; Lei et al., 2010; Li and Götz, 2017). Additionally, tau is present in the astrocytes and oligodendrocytes and glial cells of the central nervous system at very low levels (Lin et al., 2003; Shin et al., 1991). Tau is also present in the peripheral nervous system (Nunez and Fischer, 1997; Zha et al., 2016). However, whether tau is expressed in Schwann cells (the unique glial cells in the peripheral nervous system) and, if so, whether it has any biological function, is largely unknown.

Schwann cells provide necessary physical and trophic support for neurons, and form the myelin sheath that surrounds the axon, and thus are important for maintaining the normal physiological function of peripheral nerves. After damage to the peripheral nervous system, Schwann cells dedifferentiate into an immature cell type, proliferate to generate a larger population, and digest axon and myelin sheath debris to clear a regenerative path for subsequent axon regrowth (Jessen and Mirsky, 2008, 2016; Napoli et al., 2012). Schwann cells then migrate along and wrap around the regrown axons, establish a 1:1 ratio with axons, and undergo redifferentiation and myelination (Ness et al., 2013; Rao and Pearse, 2016). The phenotypes of Schwann cells can be modulated by various cellular and molecular factors, such as the neurotrophic factors nerve growth factor (Li et al., 2015) and brain-derived neurotrophic factor (Yi et al., 2016b). Factors that accelerate Schwann cell proliferation shortly after peripheral nerve injury and/or promote Schwann cell migration and myelination at the later stage after nerve injury will benefit nerve regeneration and functional recovery.

A preliminary study undertaken by our group has shown that tau is present in the rat sciatic nerve stump and that its levels are first downregulated and then upregulated after rat sciatic nerve injury (Zha et al., 2016). Considering that Schwann cells represent the main cell type in sciatic nerve stump, we speculate that tau is present in Schwann cells. Therefore, to explore the presence of tau in Schwann cells, we cultured primary rat Schwann cells and examined the expression of *Mapt* mRNA and presence of tau protein. We then transfected cultured Schwann cells with *Mapt* siRNA to explore the potential roles of tau protein on Schwann cells. Functional studies indicated that *Mapt* siRNA elevated Schwann cell proliferation and suppressed Schwann cell migration and differentiation. *In vivo* study further suggested that *Mapt* knockdown decreased the migration of Schwann cells and impaired the clearance of myelin and lipid debris after rat sciatic nerve injury. Additionally, we used *Mapt*-knockout mice to further investigate the effect of tau protein on Schwann cells, and found that the Schwann cells isolated from *Mapt*-knockout mice had a higher proliferation rate and lower ability migration. *In vivo* experiments also showed that *Mapt*-knockout mice exhibited reduced Schwann cell migration and a suppressed ability to clear debris after sciatic nerve injury.

RESULTS

Tau is present in Schwann cells in peripheral nerves

To investigate the presence of tau in Schwann cells, we cultured primary Schwann cells and performed real-time RT-PCR to examine whether *Mapt*, the mRNA encoding tau, was present in cultured Schwann cells. Representative amplification curves from the real-time RT-PCR showed that, in cultured Schwann cells, the Ct value for *Mapt* was much higher than the Ct value for *Gapdh* (Fig. 1A,B).

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Adult cerebral cortex, a tissue with a high amount of tau, was used as a positive control. The presence of *Mapt* in the cerebral cortex was determined by real-time RT-PCR (Fig. 1A,B) and DNA gel analysis (Fig. 1C). A DNA gel analysis of the PCR amplification products showed that the *Mapt* product in Schwann cells and cerebral cortex had the same size (Fig. 1C). Western blot results also showed a band with the correct the molecular mass for tau protein in both the Schwann cell and the cerebral cortex samples (Fig. 1D). Multiple

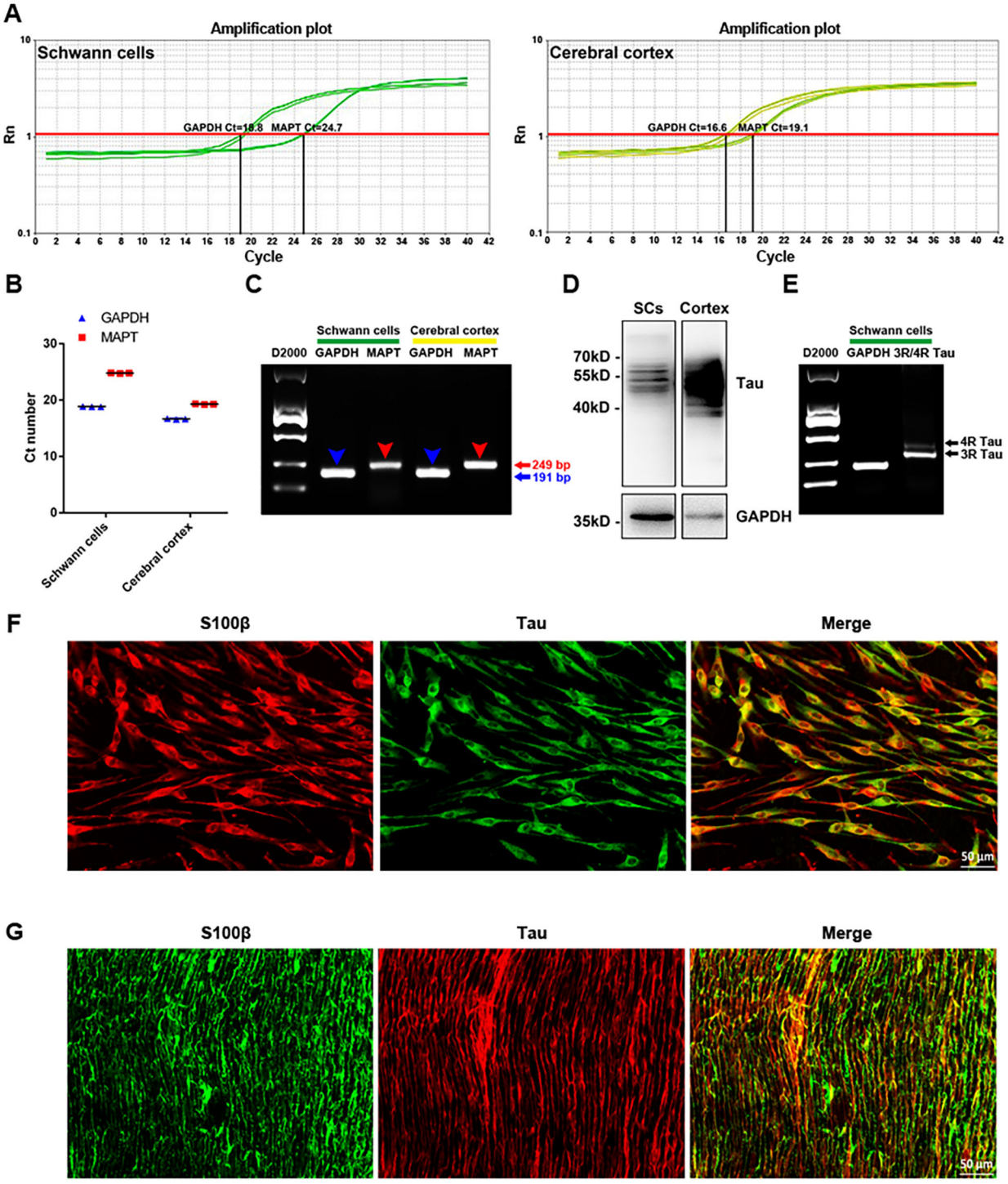

Fig. 1. The presence and localization of tau in Schwann cells. (A) Typical amplification curves of real-time RT-PCR examining the expression of mRNAs coding for tau protein (*Mapt*) in Schwann cells and cerebral cortex. Although the expression of mRNAs coding for tau protein in the cerebral cortex is much higher than that of Schwann cells, there is indeed tau expression in Schwann cells. (B) Summarized Ct values for *Gapdh* and *Mapt* in Schwann cells and cerebral cortex. (C) DNA gel image of *Mapt* and *Gapdh* in Schwann cells and cerebral cortex. (D) Tau protein expression in Schwann cells (SCs) and cerebral cortex. This indicates that there is indeed tau protein expression in Schwann cells. (E) DNA gel image of genes coding for 3R and 4R tau isoforms in Schwann cells. (F) Localization of tau protein in cultured primary Schwann cells. Red indicates S100β and green indicates tau protein. (G) Localization of tau protein in rat sciatic nerve stumps. Green indicates S100β and red indicates tau protein. Scale bars: 50 µm.

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bands were observed when using a tau antibody that recognized total tau, suggesting that different isoforms of tau might exist in Schwann cells. But the number of bands in Schwann cells seemed smaller when compared with that of the cerebral cortex (Fig. 1D). Moreover, primers that could recognize mRNAs encoding three-repeat (3R) tau and four-repeat (4R) tau, two different Tau isoforms, were used to examine the different tau isoforms in Schwann cells. DNA gel images demonstrated that both 3R and 4R tau isoforms were present in Schwann cells (Fig. 1E). Immunohistochemical labeling was then applied to analyze the protein expression of tau. In cultured Schwann cells, immunohistochemistry with anti-tau and the Schwann cell marker anti-S100β antibodies showed that most of the tau signals intermingled with S100β signals, indicating that tau protein was present in Schwann cells (Fig. 1F). In the rat sciatic nerve stump, immunohistochemistry with anti-tau and anti-S100 antibodies showed that tau protein was colocalized with S100-positive Schwann cells (Fig. 1G).

Tau modulates Schwann cell proliferation

Primary Schwann cells were transfected with *Mapt* siRNA to examine whether reduced expression of *Mapt* might affect the phenotype of Schwann cells. Results from real-time RT-PCR and western blotting showed that *Mapt* siRNA could robustly downregulate *Mapt* expression (Fig. 2A,B).

Schwann cells transfected with *Mapt* siRNA or with non-targeting negative control (NC siRNA) were first subjected to a cell proliferation assay and EdU-positive cell counting, and, from these data, the proliferation rate was calculated. There was a much larger

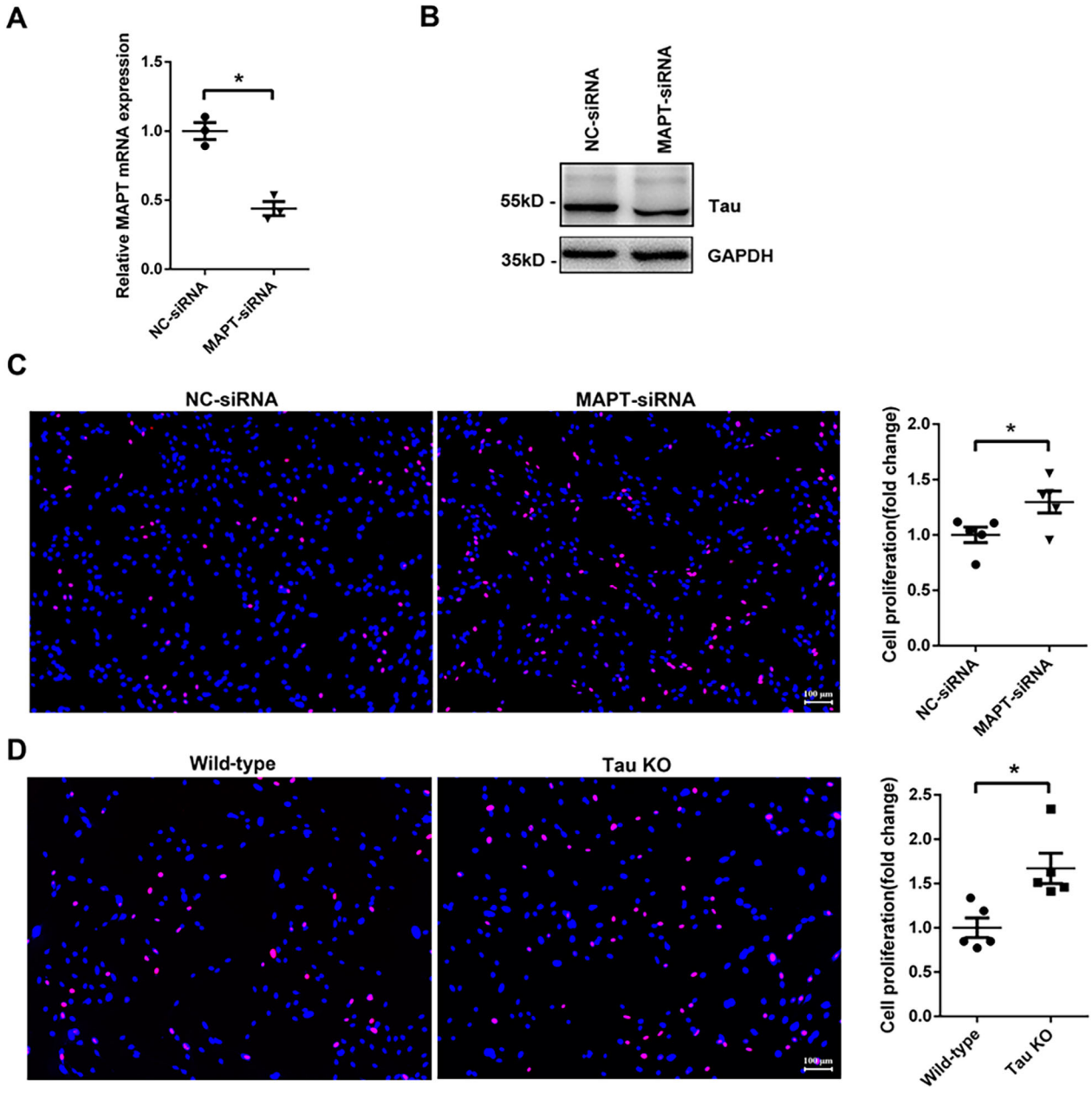

Fig. 2. Tau affects Schwann cell proliferation. (A) The mRNA and (B) protein expression levels of tau in Schwann cells were decreased by *Mapt* siRNA transfection (MAPT-siRNA) as compared to siRNA control (NC-siRNA). (C) Transfection of Schwann cells with *Mapt* siRNA, compared with siRNA control, increased Schwann cell proliferation. Scale bars: 100 µm. (D) Schwann cells isolated from *Mapt*-knockout mice (Tau KO), compared with wild-type mice, showed elevated cell proliferation. In C and D, the magenta color indicates EdU staining and blue indicates Hoechst 33342 staining.

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proportion of EdU-positive cells in Schwann cells transfected with *Mapt* siRNA than for Schwann cells transfected with NC siRNA. Summarized data showed that the proliferation rate of Schwann cells transfected with *Mapt* siRNA was significantly increased to ∼1.3 fold of the control value (Fig. 2C).

The biological function of tau on Schwann cells was further determined by analyzing *Mapt*-knockout mice. Primary Schwann cells isolated from *Mapt*-knockout mice or wild-type c57bl/6 mice were subjected to a cell proliferation assay. Results from the EdU proliferation assay showed that the proliferation rate of Schwann cells isolated from *Mapt*-knockout mice was obviously higher than cells isolated from wild-type mice (Fig. 2D).

Tau modulates Schwann cell migration

Transfected Schwann cells were also subjected to a Transwell-based migration assay and Crystal Violet staining to examine the effect of *Mapt* on Schwann cell migration. The number of cells that had migrated through the Transwell chamber, as assessed by Crystal Violet staining, was substantially fewer for the Schwann cells transfected with *Mapt* siRNA than the number for Schwann cells transfected with negative control, indicating that *Mapt* siRNA transfection suppresses Schwann cell migration (Fig. 3A). This assay was also performed on Schwann cells from *Mapt*-knockout mice, and this also revealed that the migration ability of Schwann cells isolated from *Mapt*-knockout mice was noticeably lower than that for cells isolated from wild-type mice (Fig. 3B).

Tau modulates Schwann cell differentiation

The effect of tau on Schwann cell differentiation was also determined. Schwann cells were cultured in control medium [Dulbecco's modified Eagle's medium nutrient mixture F12 (DMEM/F12) and 0.5% fetal bovine serum (FBS)] or differentiation medium [DMEM/F12, 0.5% FBS, 20 ng/ml heregulin (HRG) and 1 mM db-cAMP] prior to subsequent real-time RT-PCR experiments. Compared with cells cultured in the control medium, Schwann cells cultured in the differentiation medium expressed higher levels of the myelin-related genes *Mbp* and P0 (also known as *Mpz*) (Fig. 4A). Notably, the amount of *Mapt* mRNA in Schwann cells cultured in the differentiation medium was also increased, indicating that Schwann cell differentiation might upregulate *Mapt* expression (Fig. 4A). Subsequently, Schwann cells transfected with *Mapt* siRNA or negative control were cultured in the differentiation medium to examine the effect of *Mapt* on Schwann cell differentiation. Compared with cells transfected with negative control, Schwann cells transfected with *Mapt* siRNA had a relatively lower mRNA amount of *Mbp* and P0, suggesting that *Mapt* affects the differentiation of Schwann cells (Fig. 4B).

Tau modulates Schwann cell migration *in vivo*

Considering the significant *in vitro* effect of *Mapt* siRNA on Schwann cell migration, *Mapt* siRNA was applied to rats to determine the *in vivo* effect of *Mapt*. *Mapt* siRNA was directly injected at the injury site immediately after rat sciatic nerve crush, and sciatic nerve stumps were collected for immunofluorescence labeling at 4 days post surgery. We divided the crushed site into three equal parts, from the proximal end to the distal end of the sciatic nerve, called the proximal, middle and distal sites. Relatively less tau was detected in the sciatic nerve stumps after *Mapt* siRNA injection, suggesting that *Mapt* siRNA caused a high level of knockdown (Fig. 5A). Immunostaining with anti-S100β antibody showed that the relative fluorescence intensity of S100β at the proximal, middle and distal injury sites in rats injected with *Mapt* siRNA was significantly less than in rats injected with negative control, indicating that *Mapt* siRNA inhibits Schwann cell migration *in vivo* (Fig. 5B).

The effect of tau on the *in vivo* migration of Schwann cells after nerve injury was also determined in *Mapt*-knockout mice, which

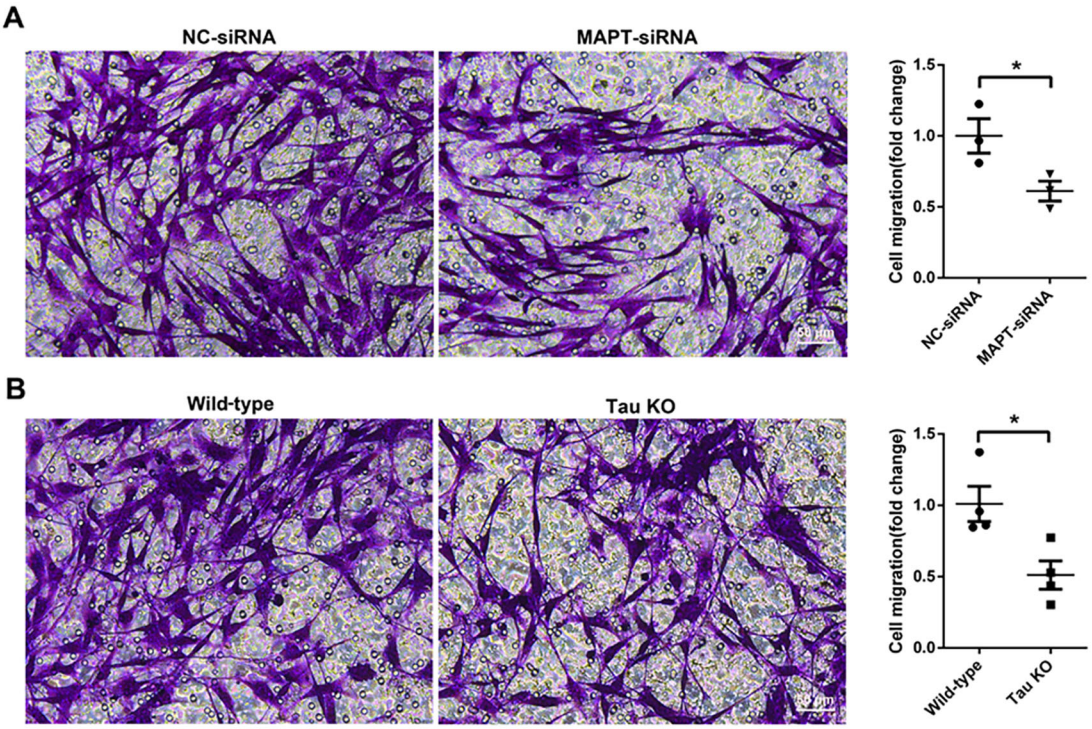

Fig. 3. Tau affects Schwann cell migration. (A) Transfection of Schwann cells with *Mapt* siRNA (MAPT-siRNA) decreased Schwann cell migration compared with transfection of siRNA control (NC-siRNA). (B) Schwann cells isolated from *Mapt*-knockout mice (Tau KO), compared with wild-type mice, showed reduced cell migration. See Materials and Methods for a detailed description of the assay. **P*<0.05. Scale bars: 50 µm.

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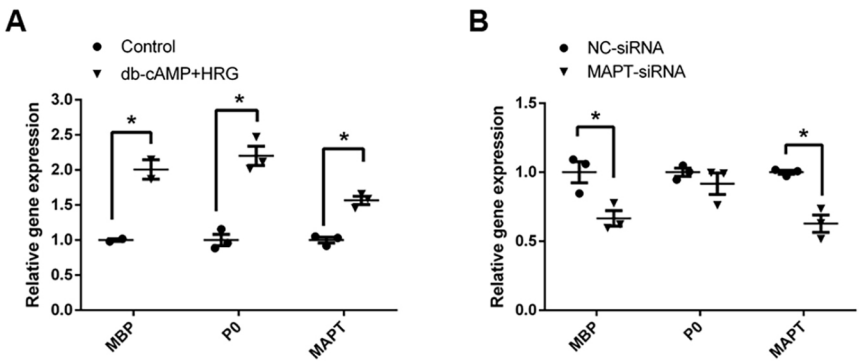

Fig. 4. Tau affects Schwann cell differentiation. (A) The mRNA levels of *MBP*, P0 and *Mapt* were higher in Schwann cells cultured in differentiation culture medium (db-cAMP+HRG) than those cultured in control medium. (B) The mRNA levels of *Mbp*, P0 and *Mapt* were higher in siRNA control (NC-siRNA)-transfected Schwann cells cultured in differentiation culture medium than *Mapt* siRNA-transfected Schwann cells (MAPT-siRNA) cultured in differentiation culture medium. **P*<0.05.

have no detectable tau signal in sciatic nerve stumps (Fig. 5C). Results from anti-S100β antibody immunostaining showed that, after sciatic nerve injury, the relative fluorescence intensity of S100β at the injury site, especially the proximal and middle sites, was significantly less in *Mapt*-knockout mice as compared with in wild-type mice (Fig. 5D).

Tau modulates debris clearance after nerve injury

A precondition of peripheral nerve regeneration is that axon and myelin debris need to have been engulfed in order to build a regenerative path. An Oil Red O staining experiment was conducted to determine the effect of tau on myelin and lipid debris clearance. Oil Red O staining images showed that more myelin and lipid debris was accumulated in distal nerve stumps in rats injected with *Mapt* siRNA (Fig. 6A) or in *Mapt*-knockout mice (Fig. 6B). These results suggest that tau affects myelin and lipid debris removal after peripheral nerve injury.

Tau modulates cytoskeleton proteins

The effect of tau on the expression and localization of cytoskeleton proteins in Schwann cells was further investigated to decipher whether tau regulated Schwann cell movement by modulating cytoskeleton proteins. Immunostaining for α-tubulin showed that, compared with Schwann cells isolated from wild-type mice, the expression and localization of α-tubulin was disrupted in Schwann cells isolated from *Mapt*-knockout mice (Fig. 7A). α-Tubulin was evenly distributed in Schwann cells isolated from wild-type mice; however, in Schwann cells isolated from *Mapt*-knockout mice, α-tubulin was less abundant and aggregated (Fig. 7A). Similarly, immunostaining for F-actin also showed that the expression and localization of F-actin was affected by tau (Fig. 7B). Changes of these cytoskeleton proteins may disrupt the formation of lamellipodia and filopodia, impair cellular movement and suppress Schwann cell migration.

DISCUSSION

In the current study, we examined whether tau was present in primary Schwann cells by using real-time RT-PCR and immunofluorescence labeling, and determined the biological effects of tau on Schwann cells by performing cell proliferation, cell migration and cell differentiation assays, and examining sciatic nerve crush models. Our results show, for the first time, that tau is expressed in Schwann cells in rat sciatic nerves. Functional results showed that silencing of *Mapt*, the gene encoding tau, would increase the proliferation of Schwann cells and decrease the migration and differentiation of Schwann cells. Experiments from *Mapt*-knockout mice further identified that tau protein modulates Schwann cell proliferation and migration. Moreover, a previous study has suggested that there are reduced MBP protein levels in the sciatic nerves of *Mapt*-knockout mice (Lopes et al., 2016). This is consistent with our observation from the aspect of *Mbp* gene expression, and further demonstrates that tau affects the abundance of myelin-related genes and the process of Schwann cell differentiation.

Phenotype modulation of Schwann cells, especially strictly controlled dedifferentiation, proliferation, migration and redifferentiation of Schwann cells, is essential for peripheral nerve repair and regeneration (Chen et al., 2007). Our previous studies have identified numerous factors that could affect Schwann cell behaviors. For instance, sciatic nerve injury-induced upregulates the microRNA miR-sc8 and downregulates epidermal growth factor, which could inhibit Schwann cell proliferation and migration (Yi et al., 2016a). Injury-induced downregulated miR-1 and upregulated brain-derived neurotrophic factor can also promote Schwann cell proliferation and migration (Yi et al., 2016b).

Notably, the regulating factors discussed above are mainly consistently up- or down-regulated following sciatic nerve injury, and their effects on Schwann cell proliferation and migration are also consistent. Schwann cells, however, have two distinct different phenotypes during peripheral nerve regeneration. At an early stage following peripheral nerve injury, Schwann cells go through dedifferentiation and proliferation while, at relatively later time points, Schwann cells go through migration and redifferentiation (Glenn and Talbot, 2013; Ness et al., 2013; Zhou and Notterpek, 2016). Therefore, molecules that exhibit an expression trend whereby their levels change over time might affect both the dedifferentiation and the redifferentiation processes in Schwann cells. Our previous data showed that *Mapt* is first downregulated and then upregulated after peripheral nerve injury (Zha et al., 2016). Here, the biological functions of tau in different aspects of Schwann cell phenotype were examined. Functional studies showed that tau inhibited Schwann cell proliferation, but promoted Schwann cell migration and differentiation. Therefore, the downregulation of tau at the early stage after peripheral nerve injury might benefit Schwann cell proliferation while the upregulation of tau at later stages might benefit Schwann cell migration and differentiation. Moreover, upregulation of tau might help with the removal of myelin and lipid debris and the construction of a regenerative path. By having different roles in different aspects of the Schwann cell phenotype, dysregulated tau might collectively contribute to a disruption in peripheral nerve repair and regeneration.

Tau is highly associated with the physiological roles of central nervous system as well as its pathological states. Beside its widely accepted role in neurodegenerative diseases, in has been shown that tau is upregulated and hyperphosphorylated following injury to the central nervous system (Caprelli et al., 2019; Liao et al., 2008; Qi et al., 2016; Zhu et al., 2013). It has been reported that the neuroprotective effect of rosiglitazone against diffuse axonal injury is mediated by it acting to decrease the amount of hyperphosphorylated tau and increasing the amount of total tau,

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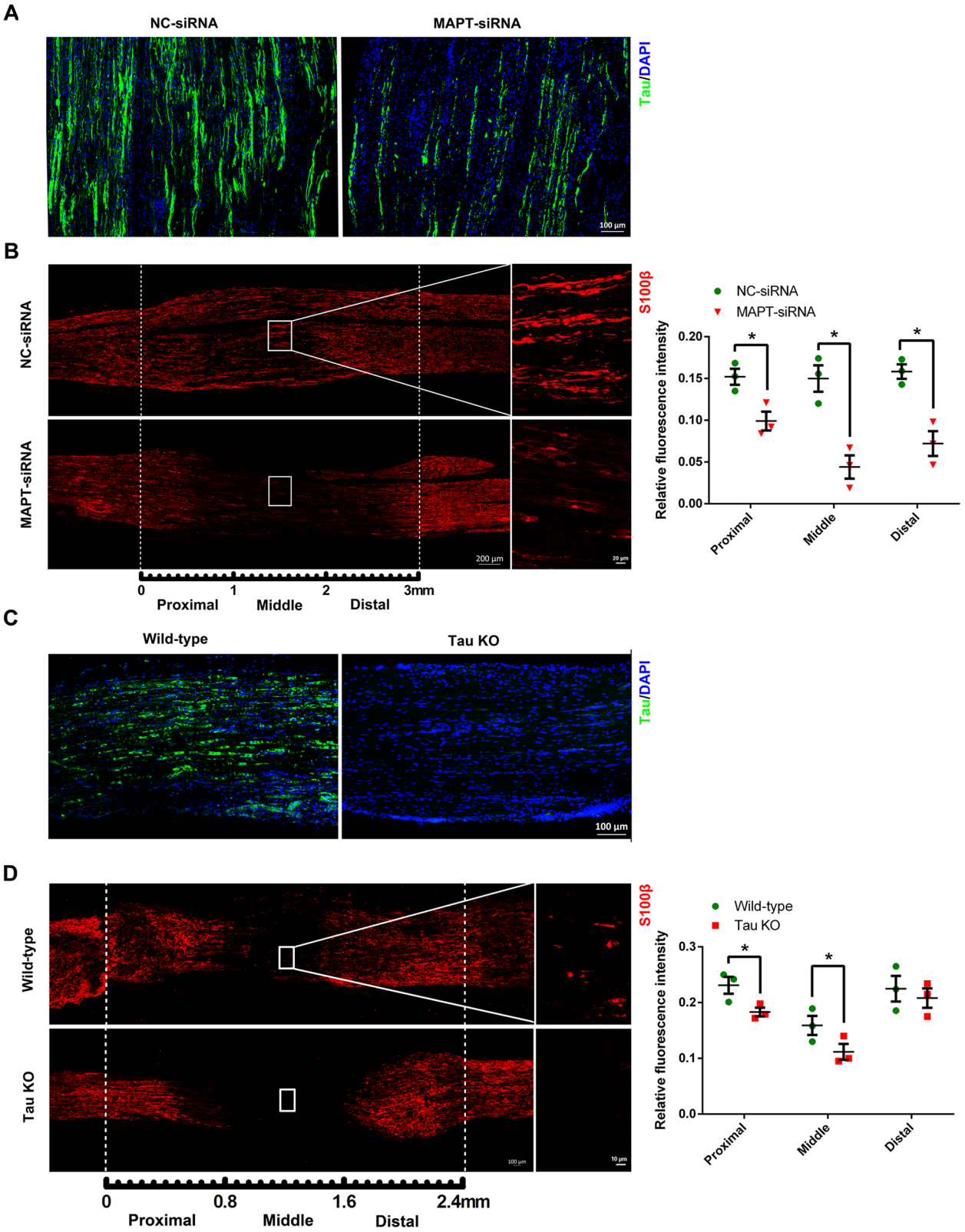

Fig. 5. See next page for legend.

suggesting that tau is involved in traumatic brain injury (Zhao et al., 2016). Tau was even considered as a diagnostic biomarker for spinal cord injury (Caprelli et al., 2019; Yokobori et al., 2015). In addition, it has been found that, in the spinal cord, tau influences the expression of MBP and the differentiation of oligodendrocyte progenitor cells (Ossola et al., 2016). This indicates that tau affects both neurons and glial cells in the nervous system. Our previous work (Zha et al., 2016) and the present study has shown that tau is differentially expressed in the peripheral nervous system after peripheral nerve injury and that altered expression of tau modulates

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Fig. 5. Tau affects Schwann cell migration *in vivo*. (A) Tau immunoblotting in the sciatic nerve stumps of rats injected with siRNA control (NC-siRNA) and rats injected with *Mapt* siRNA (MAPT-siRNA). Scale bar: 100 µm. (B) The *in vivo* migration of Schwann cells was impaired in rats injected with *Mapt* siRNA as compared with rats injected with siRNA control. Immunostaining with anti-S100β was performed at 4 days post rat sciatic nerve crush and was used to determine Schwann cell migration. The area with the dashed line indicates the crush area, and the areas set as the proximal, distal and middle site are indicated. Boxed areas are shown at a higher magnification on the right. Scale bars: 200 µm (main image), 20 µm (magnification). The relative fluorescence intensity (calcaulated with Image Analysis System Software, Leica) of S100β at the proximal, middle and distal sites were summarized and shown as a histogram. (C) Tau immunostaining (green) in the sciatic nerve stumps of wild-type mice and *Mapt*-knockout mice (Tau KO). DAPI staining is shown in blue. Scale bar: 100 µm. (D) The *in vivo* Schwann cell migration in *Mapt*-knockout mice was slower than in wild-type mice. Immunostaining with anti-S100β (red) was performed at 3 days post mouse sciatic nerve crush and was used to determine Schwann cell migration as in B. The area with the dashed line indicates the crush area, and the areas set as the proximal, distal and middle site are indicated. Boxed areas are shown at a higher magnification on the right. Scale bars: 100 µm (main image), 10 µm (magnification). **P*<0.05.

the Schwann cell phenotype. Immunostaining studies further demonstrated that tau regulated cytoskeleton proteins. Disruption of cytoskeleton proteins would delay Schwann cell migration and impair peripheral nerve regeneration (Wang et al., 2018). Therefore, tau might also play significant roles in the peripheral nervous system and strongly affect the peripheral nerve regeneration process. Another recent study has also shown that the beneficial effect of polyaniline on peripheral nerve regeneration might be related to an increased amount of tau (Fan et al., 2017).

Taken together, this study, to our knowledge, has shown for the first time that tau is expressed in Schwann cells and contributes to the modulation of the Schwann cell phenotype. These outcomes provide new insight into the biological role of tau and imply that interventions that affect tau could have a role in the treatment of peripheral nerve injury.

MATERIALS AND METHODS

Animals

Male adult Sprague-Dawley (SD) rats weighing 180–220 g were used to perform sciatic nerve crush. Neonatal 1-day-old SD rats were used to isolate Schwann cells. Rats were purchased from the Experimental Animal Center of Nantong University of China. Male adult *Mapt*-knockout mice [tau KO; B6.Cg-Mapttm1(EGFP)Klt] were homozygous for the tau-null mutation and were littermates derived from JAX stock #005491 [B6.Cg-Mapttm1(EGFP)Klt Tg(MAPT)8cPdav/J]. Wild-type c57bl/6 mice were used as controls. All procedures were ethically approved by the Administration Committee of Experimental Animals and were performed in accordance with the Institutional Animal Care Guidelines of Nantong University.

Cell culture and transfection

Schwann cells were isolated from the sciatic nerve stumps of neonatal SD rats and purified to remove fibroblasts as previously described (Li et al., 2015). Harvested Schwann cells were cultured in DMEM (Invitrogen, Carlsbad, CA) containing 10% FBS (Invitrogen), 1% penicillin and streptomycin (Invitrogen), 2 µM forskolin (Sigma, St Louis, MO) and 10 ng/ml HRG (Sigma) in a humidified 5% CO_2 incubator at 37°C. For cell transfection, primary cultured Schwann cells were transfected with *Mapt* siRNA (MAPT-siRNA-1, MAPT-siRNA-2 or MAPT-siRNA-3; in the experiments shown here, we used MAPT-siRNA-3 as this gave the best knockdown; data not shown) or non-targeting negative control (random sequence, RiboBio, Guangzhou, China) by using Lipofectamine RNAiMAX transfection reagent (Invitrogen). The cells were used 48 hours after transfection.

Real-time RT-PCR

Real-time RT-PCR was performed as previously described (Li et al., 2015). Briefly, RNA was isolated from cultured Schwann cells and reverse transcribed to cDNA by using the Omniscript Reverse Transcription Kit (Qiagen, Valencia, CA). RT-PCR was performed by using the QuantiNova SYBR Green PCR Kit (Qiagen) on an Applied Biosystems StepOne real-time PCR system. The sequences of primer pairs were follows: *Mapt* (total tau) (forward) 5′-CAAGTGTGGCTCAAAGGACAATATC-3′ and (reverse) 5′-TCAATCTTCTTATTCCCTCCTCCAG-3′; *Mapt* (3R/4R tau) (forward) 5′-GGTGAACCACCAAAATCCGGAGAACG-3′ and (reverse)

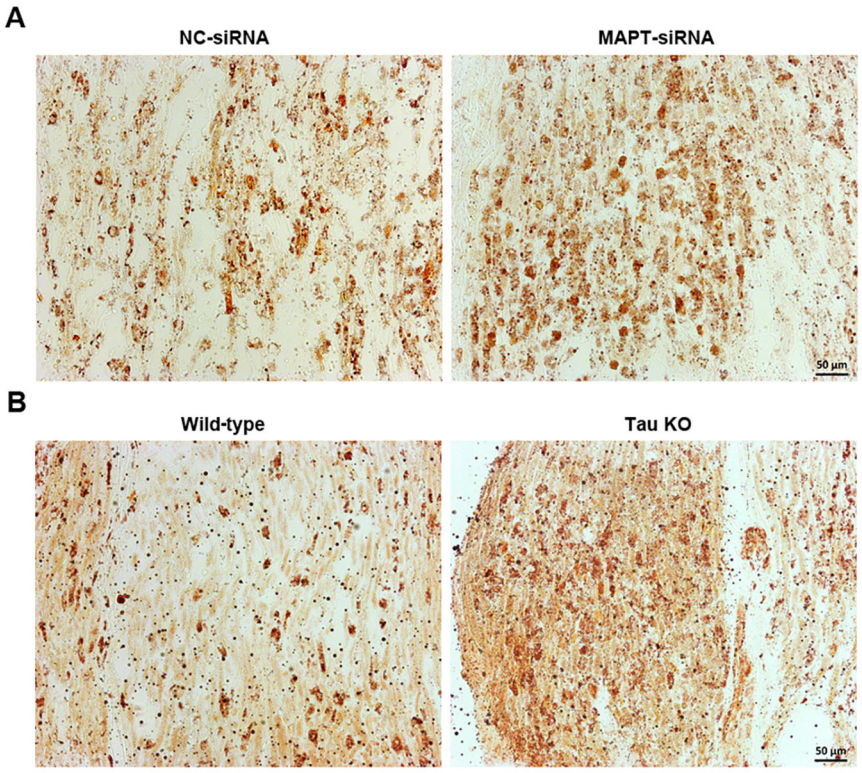

Fig. 6. Tau affects debris clearance. (A) The amount of remaining myelin and lipid debris was higher in rats injected with *Mapt* siRNA (MAPT-siRNA) as compared with rats injected with siRNA control (NC-siRNA). (B) The amount of remaining myelin and lipid debris was higher in *Mapt*-knockout mice (Tau KO) as compared with wild-type mice. Scale bars: 50 µm. Images show Oil Red O staining.

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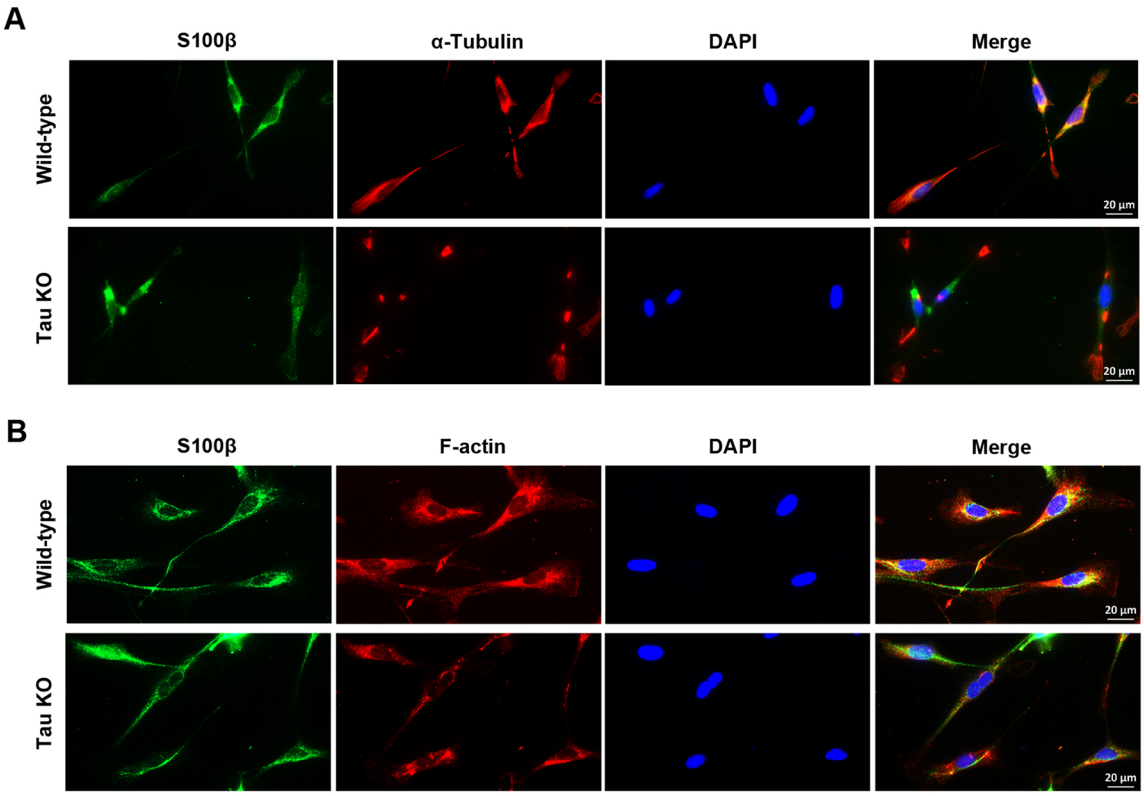

Fig. 7. Tau affects cytoskeleton proteins. (A) Immunostaining of S100β, α-tubulin and DAPI in Schwann cells isolated from wild-type mice and *Mapt*-knockout mice (Tau KO). (B) Immunostaining of S100β, F-actin and DAPI in Schwann cells isolated from wild-type mice and *Mapt*-knockout mice. Scale bars: 20 µm.

5′-CCACACTTGGAGGTCACCTTGC-3′; *Mbp* (forward) 5′-CCGACGAGCTTCAGACCATC-3′ and (reverse) 5′-AGTACTTGGATCCGTGTCGC-3′; P0 (forward) 5′-CGTGATCGGTGGCATCCTC-3′ and (reverse) 5′-GGCATACAGCACTGGCGTCT-3′; and GAPDH (forward) 5′-AACGACCCCTTCATTGAC-3′ and (reverse) 5′-TCCACGACATACTCAGCAC-3′. Relative quantification of *Mapt*, *Mbp* or P0 was performed by using the ΔΔCt method with GAPDH as the reference gene.

Western blotting

Proteins were extracted from cell cultures and lysed with Laemmli sample buffer containing 2% SDS, 52.5 mM Tris-HCl and protease inhibitors (Roche Diagnostics, Indianapolis, IL). Protein lysate mixed with SDS-PAGE loading buffer (Beyotime, Shanghai, China) was incubated at 100°C for 5 min. Equal amounts of protein from each treatment were loaded onto 10% SDS-polyacrylamide gels and transferred onto PVDF membranes (Millipore, Bedford, MA). Membranes were blocked with 5% non-fat dried milk in PBS, incubated with primary anti-tau antibody (1:100, Abcam, ab64193, Cambridge, MA) or anti-GAPDH antibody (1:1000, Proteintech Group, Rosemont, IL), probed with horseradish peroxidase (HRP)-conjugated secondary antibody (Pierce, Rockford, IL), and developed with Pierce ECL Western Blotting Substrate (Thermo Scientific, Rockford, IL). Quantification of western blot bands was performed with Quality One software (Bio-Red, Hercules, CA).

Immunofluorescence labeling

Sciatic nerve tissues or cell cultures were fixed with 4% paraformaldehyde, washed with PBS, blocked with Immunol Staining Blocking Buffer (Beyotime), incubated with primary anti-S100β antibody (1:100, Abcam, ab52642 or 1:400, Sigma S2532), anti-tau antibody (1:100, Abcam, ab64193), anti-α-tubulin antibody (1:500, Abcam, ab52866) or anti-F-actin antibody (1:100, Abcam, ab205), and then incubated with Cy3-conjugated secondary antibody (Proteintech) prior to mounting on slides. Finally, nuclear staining was performed using DAPI (Thermo Scientific, Rockford, IL) staining. Tissue slides were visualized by using fluorescent signals from different lasers. Images were captured by using an optical and epifluorescence microscope (Axio Imager M2, Carl Zeiss Microscopy, Jena, Germany).

Cell proliferation assay

Schwann cells were seeded at a density of 2×10^5 cells/ml onto 96-wells plates pre-coated with 0.01% poly-L-lysine. After cellular adherence, 50 µM EdU was added to the cell culture medium and cells were cultured for an additional 24 h. Cell cultures were fixed with 4% paraformaldehyde and cell proliferation was measured by using a Cell-Light EdU DNA Cell Proliferation Kit (RibiBio, C10310-1), via EdU and Hoechst 33342 staining. Images were captured with a DMR fluorescence microscope (Leica Microsystems, Bensheim, Germany). The proliferation rates of Schwann cells were determined from randomly selected images and calculated by dividing the number of EdU-positive cells by the number of total cells.

Cell migration assay

100 µl DMEM containing of 3×10^5 cells/ml Schwann cells was added to the upper chamber of a 6.5 mm Transwell chamber with 8 µm pores (Costar, Cambridge, MA) and 600 µl cell culture medium was added to the bottom chamber. After 24 h of incubation, the upper surface of the insert membrane was cleaned with a cotton swab and Schwann cells attached to the bottom surface of the insert membrane were stained with 0.1% Crystal Violet. The migration abilities of Schwann cells were detected in two ways. The randomly selected images captured by using a DMR inverted microscope (Leica) reflect the migration ability of the photomicrographed cells in the lower chamber of Transwell migration chamber after cells were allowed to migrate for 24h. The dot-plot reflects the intensity of the absorbance of Crystal Violet staining from the cells in the lower chamber of Transwell migration chamber corresponding to the images (dissolved in 33% acetic acid solution to provide a statistical standard).

Cell differentiation

The differentiation of Schwann cells was induced by adding HRG and cAMP as previously described (Arthur-Farraj et al., 2011) with modifications. Cells in the control group were cultured in DMEM/F12

containing 0.5% FBS and 1% penicillin and streptomycin. Cells in the differentiation group were cultured in DMEM/F12 containing 0.5% FBS, 1% penicillin and streptomycin, 20 ng/ml HRG and 1 mM db-cAMP (Sigma). Media were refreshed every day, and cells were cultured for 3 days prior to subsequent real-time RT-PCR experiments.

In vivo experiments

Animals were anaesthetized and underwent sciatic nerve crush surgery as previously described (Yi et al., 2015). For rat *in vivo* studies, rats were randomly divided into two groups to receive the injection of *Mapt* siRNA or corresponding control (RibiBio). *Mapt* siRNA or negative control was dissolved in saline and injected perineurally at the injury site directly after nerve crush. At 4 days after surgery, rats were euthanized and rat sciatic nerve stumps were harvested for immunostaining with anti-S100β antibody to evaluate Schwann cell migration. For mice *in vivo* studies, both wild-type mice and *Mapt*-knockout mice received surgery and mouse sciatic nerve stumps were collected at 3 days after surgery for subsequent immunofluorescence labeling. We used the Image Analysis System Software (Leica Microsystems GmbH) to calculate the relative fluorescence intensity of the images. The value of the fluorescence intensity in the dot-plot is the value automatically generated after the background is removed.

Oil Red O staining

For Oil Red O staining, sciatic nerve stumps were fixed in 10% formalin, washed in 0.01 M PBS and 60% isopropanol sequentially, and incubated for 15 min at 37°C in 0.6% Oil Red O (Sigma) pre-filtered with a 0.2 µm filter. The stained sections were rinsed in 60% isopropanol and 0.01 M PBS to diminish the background interference. Randomly selected images were captured by using a DMR inverted microscope (Leica).

Data analysis

Results are presented as means±s.e.m. from 3–5 experiments. *P*-values were determined with a Student's paired two-tailed *t*-test by using GraphPad Prism 6.0 software (GraphPad Software, La Jolla, CA). $P<0.05$ was considered as statistically significant.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: S.Y., X.G., S.L.; Methodology: S.Y., Q.L., X.W., T.Q., H.W., P.W., D.C., S.L.; Software: H.W.; Validation: H.W.; Formal analysis: S.Y., G.Z., J.Y., X.G., D.C.; Investigation: S.Y., Q.L., X.W., T.Q., H.W., G.Z., J.Y., P.W., S.L.; Resources: H.W., D.C.; Data curation: Q.L., X.W., T.Q.; Writing - original draft: S.Y., S.L.; Supervision: X.G., S.L.; Project administration: X.G.; Funding acquisition: S.Y., X.G., S.L.

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