

Delayed peripheral nerve rehabilitation in aquaporin-3 deficiency in mouse models of sciatic nerve contusion.

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Abstract

BACKGROUND: Aquaporin-3 (AQP3) water channels are belonging to the aquaporin water channel family, permeable not only to water but also to some small solutes such as glycerol and lactate. The purpose of this study is to investigate the possible functions of AQP3 in peripheral nerve rehabilitation based on AQP3-deficient mice.

METHODS: Mature 8-week-old female AQP3-deficient (AQP3^{-/-}) mice and C57BL/6 (WT) mice initially weighing 25~30 g were used in this study. Schwann cells were isolated from sciatic nerves of WT and AQP3^{-/-} mice respectively. AQP3 mRNA and protein expression in sciatic nerve tissues and Schwann cells were detected by RT-PCR, immunoblot analysis, and immunofluorescence staining. Sciatic nerve cross sections from the WT and AQP3^{-/-} mice were stained by toluidine-blue agent to identify the potential influence of AQP3 deficiency to the morphology nerve fibers. The proliferation and migration ability of AQP3^{-/-} and WT Schwann cells were observed in primary cell cultures. To explore the possible role of AQP3 in nerve repair processes, sciatic nerve contusion models were established and walking track analysis was performed on both WT and AQP3^{-/-} mice.

RESULTS: AQP3 was localized in the membrane of Schwann cells. AQP3-deficiency did not alter the morphology of fibers in the sciatic nerve. There was an increase of AQP3 protein expression in the sciatic nerve of wild-type mice after injury. Primary culture of Schwann cells and *in vitro* wound healing model revealed that AQP3-deficient Schwann cells exhibited the same morphology, while showing lower proliferation and migration ability compared with wild-type Schwann cells. There was obvious delay in motor function rehabilitation in AQP3-deficient mice compared with that of wild-type mice.

CONCLUSION: Our study suggested that AQP3 localized in the membrane of Schwann cells and facilitated Schwann cells' proliferation and migration. AQP3 deficiency impaired nerve rehabilitation in wound healing model both *in vitro* and *in vivo*. The study support our hypothesis that AQP3 participates in myelin damage and repair course and the mechanisms underlying the AQP3 in the field of myelin repair and regeneration in peripheral nerves deserves further investigation and exploration in detail.

Abbreviations:

AQPs	- aquaporins
AQP3	- aquaporin-3
AQP3 ^{-/-}	- AQP3-deficient
WT	- wild-type
SFI	- sciatic functional index

INTRODUCTION

Aquaporins (AQPs) water channels are small hydrophobic membrane proteins facilitate bi-directional water transport across the plasma membrane in a number of tissues (Verkman, 2005; Carbrej and Agre, 2009). Thirteen subtypes of AQPs (AQP 0-12) have been identified in mammals so far. Numerous studies reported the localization of six AQPs including AQP1, AQP3, AQP4, AQP5, AQP8, and AQP9 in the central nervous system (Tait *et al.* 2008). Functional studies revealed important roles of AQPs in cerebral spinal fluid secretion (Oshio *et al.* 2005; Filippidis *et al.* 2011), brain edema (Zador *et al.* 2009), neurogenesis (Zheng *et al.* 2010) and glioma (Saadoun *et al.* 2002; Albertini and Bianchi, 2010). The expression and function of AQPs in the peripheral nervous system are less investigated, especially in pathogenic conditions (Ma *et al.* 2011). Matsumoto (Matsumoto *et al.* 2004) and Oshio K (Oshio *et al.* 2006) reported AQP1 expression in the trigeminal ganglion and responsible for pain signal transmission in the peripheral nervous system. Our group reported earlier AQP3 was expressed in Schwann cells, which were glial cells of the peripheral nervous system (Zhang *et al.* 2010).

AQP3 water channels are permeable not only to water but also to some small solutes such as glycerol and lactate, also called 'aquaglyceroporins'. Recent studies provide evidence for AQP3 plays an important role in proliferation, differentiation and migration of tumor cells and epithelial cells (Nong *et al.* 2021; Bollag *et al.* 2020; Arif *et al.* 2018; Marlar *et al.* 2017). Aquaporins participate in wound healing (Hamed *et al.* 2017; Hara-Chikuma and Verkman, 2008). However, the functions of AQP3 in the peripheral nervous system are still largely unknown.

Schwann cells are target cells in myelin demyelination and repair course in most cases. In the present study, we focused on localization and possible functions of AQP3 in the peripheral nervous system. Sciatic nerve contusion models were established and walking track analysis were performed on both wild-type (WT) and AQP3-deficient (AQP3^{-/-}) mice. We investigated the motor function rehabilitation after sciatic nerve contusion and AQP3 protein expression after the nerve injury. Besides the influence of AQP3 on morphology of the peripheral nerve fibers and primary cultured Schwann cells, we also investigated the proliferation and migration ability of AQP3^{-/-} Schwann cells by wound healing model in vitro.

MATERIALS AND METHODS*Animals*

Mature 8-week-old female AQP3-deficient (AQP3^{-/-}) mice (C57BL/6 genetic background), which were generated by targeted gene disruption (Ma *et al.* 2002), and

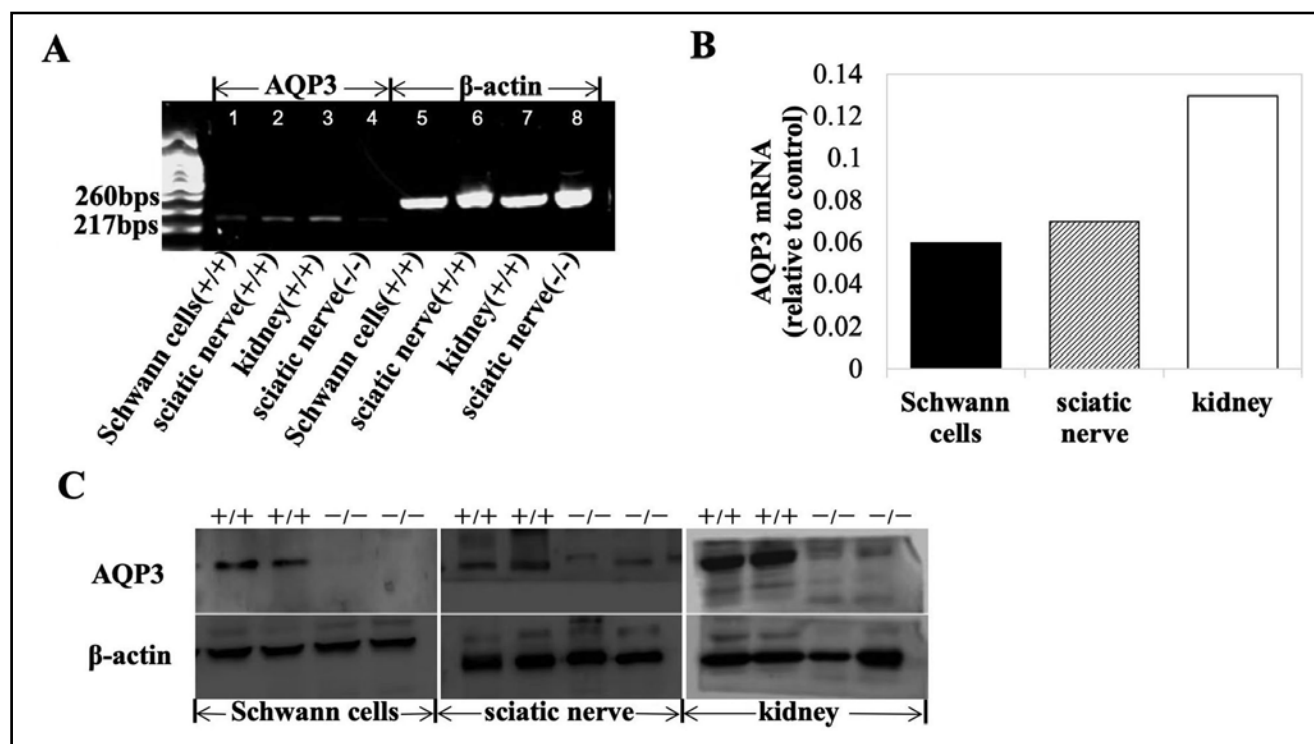


Fig. 1. AQP3 mRNA and protein expression in Schwann cells, sciatic nerve and kidney tissues. A: AQP3 mRNA (217 bps) was detected in primary cultured Schwann cells, sciatic nerve tissues and kidney tissues from WT mice. B: Relative quantification of AQP3 mRNA in sciatic nerve and kidney tissues (n = 4). C: AQP3 protein, approximately 31 kDa, was determined by immunoblot analysis in primary cultured Schwann cells, sciatic nerve tissues and kidney tissues from WT mice, while null in samples from AQP3^{-/-} mice.

C57BL/6 (WT) mice initially weighing 25~30 g were used in this study. All mice were maintained in a specific pathogen-free grade animal facility of the Animal Service Center of Jilin University, with temperature/humidity-controlled conditions and a 12-hour light/dark cycle. All animal experimental procedures were approved by the Committee on Animal Research of Jilin University (Permit No: SCXK 2016-048).

Isolation and Primary cell culture of Schwann cells

Schwann cells were isolated from sciatic nerves of WT and AQP3^{-/-} mice respectively according to previously published protocols (Schmid *et al.* 2014). Sciatic nerve samples were washed with sterile DMEM thrice to remove accompanying blood or scar tissue, and transferred to supplement DMEM medium, consisting of 10% Fetal Bovine Serum (FBS) and 1% Penicillin/Streptomycin mix (Thermo Scientific, MA). Under a dissecting microscope, sciatic nerve samples were cut into 1-2 mm segments, then incubated in an enzymatic mixture containing 1.25U dispase (Sigma) and 0.125% Collagenase Type IV (Sigma) in DMEM medium. Nerve pieces were incubated for 3.5 h at 37°C with 5% CO₂ levels. The cells were recovered by centrifugation at 600 g for 5 min at 37°C. The pellet was resuspended in supplemented DMEM and FBS medium and plated on poly-L-Lysine pre-coated 12-well dishes and glass coverslips. Culture medium (95%DMEM + 5%FBS) was replaced with fresh medium after 36 h, then every 2 days. SCS were observed and taken photos under microscopy.

RNA extraction and RT-PCR

Primary cultured Schwann cells, sciatic nerve tissues and kidney tissues from WT and AQP3^{-/-} mice were isolated and RT-PCR was performed to evaluate the expression of AQP3 mRNA. SCS were harvested on the 10th day of culture. Total RNA was isolated using TRIzol reagent (Invitrogen, CA, USA) according to the manufacturer's instructions. Two micrograms of total RNA were reversely transcribed with random priming. The cDNA was then amplified by PCR with primers specific against AQP3 (forward primer, 5'-GGCTAAAAACGCTCCCTGTATCCA-3'; re-verse primer, 5'-GGAGTTTCCCACCCC-TATTCCTAAA-3'; product length 217 bp). The PCR products were electrophoresed in 1.5 % agarose gels, and the bands were visualized by ethidium bromide staining. Densitometric analysis was performed and corrected for loading using glyceraldehyde 3-phosphatedehydrogenase. β -actin, forward primer, 5'-ACATGCCGCTGGAGAAACC -T-3'; reverse primer, 5'-TCCACCACCCTGTTGCTGTAG-3'; product length 260 bp, a housekeeper gene.

Immunofluorescence staining

Schwann cells were cultured on glass coverslips for 7-10 days then harvested. Sciatic nerves were harvested from WT and AQP3^{-/-} mice (n = 3, each group) and

immediately fixed in liquid nitrogen. Serial 10 μ m frozen acetone-fixed sciatic nerve cross sections were prepared. Indirect fluorescence immunohistochemistry was performed on both cultured Schwann cells and frozen sections of sciatic nerve according to established protocols. After washing 3 times with PBS, the cells or frozen sections were blocked with 3 % bovine serum albumin (BSA) at room temperature for 1 h, then incubated with primary antibody (goat anti-AQP3 polyclonal antibody 1:100, Abcam/ab153694) or rabbit anti-S100 antibody 1:200, Sigma) overnight at 4°C. After washing in PBS, FITC-conjugated rabbit anti-goat or sheep anti-rabbit IgG secondary antibody (1:1000, Sigma) was applied for 30 minutes at room temperature. Samples were washed in PBS and cell nuclei were stained with DAPI or PI. The samples were mounted on slides with antifade solution (Vector Laboratories, Inc) and examined/photographed under fluorescence microscopy or confocal scanning laser microscopy (CSLM).

Western blot

Schwann cells, sciatic nerve tissues and kidney tissues were lysed on ice with RIPA buffer, supplemented with halt protease and phosphatase inhibitor cocktail (Thermo Scientific). Total cytoplasmic and membrane proteins were extracted according to established protocols. Equal amounts of protein were denatured, reduced and separated by sodium dodecyl sulfate (SDS) gel electrophoresis using the Bio-Rad MiniProtein III Apparatus, then electrotransferred to polyvinylidene fluoride membranes for Western blot. After 3 h of blocking with Tris-buffered saline containing 5 % nonfat dry milk and 0.1 % Tween 20, the membranes were incubated overnight with anti-AQP3 polyclonal antibody (1:100) or β -actin (1:500, Sigma) diluted in the blocking solution. The membranes were washed and incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h (1:10000). Finally, the signals were detected by enhanced chemiluminescence detection kit (Amersham, NJ, USA). The chemiluminescent signal was captured by a UVP BioSpectrum500 imaging system (UVP, CA, USA).

Cell proliferation detection

Freshly isolated Schwann cells were seeded in 48-well microplates at a density of 1.0×10^5 cells /well and cultured for 10 days to monitor cell proliferation. The Schwann cells were cultured and measured for proliferation at time points of 7 d and 10 d. The luminescence signals were measured immediately using PerkinElmer VICTORTM X2 (Waltham, MA, USA) to determine the cell viability. Six wells were measured for each group, and the assay was repeated for three times.

In vitro wound healing model

Schwann cells were cultured on poly-L-Lysine pre-coated 12-well dishes for 7-10 days. Culture medium

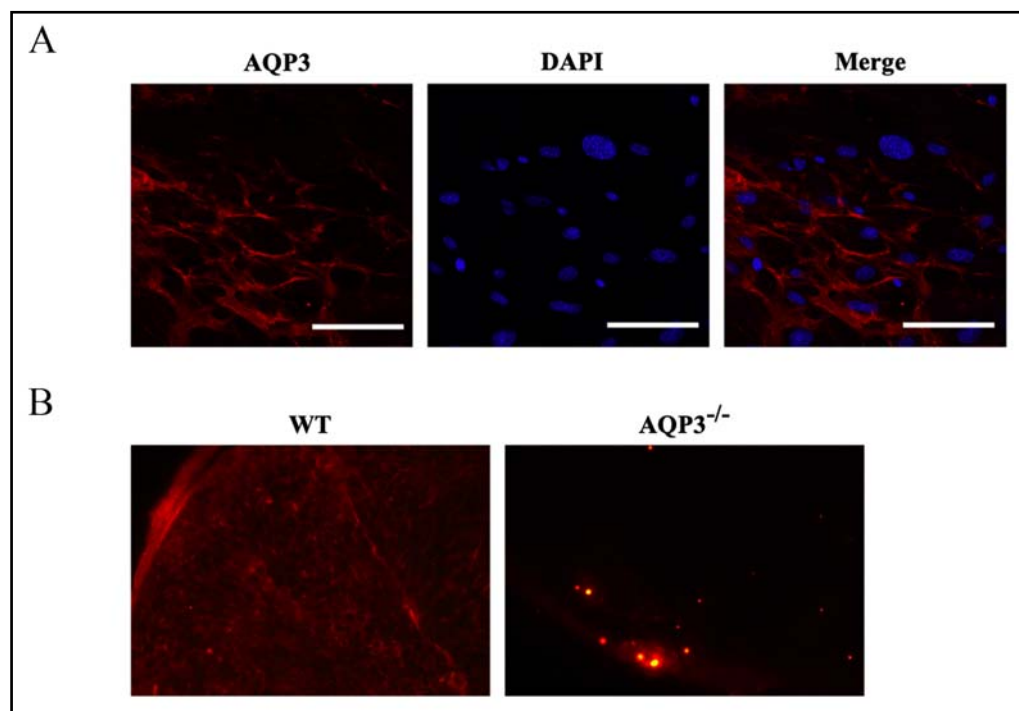


Fig. 2. Immunofluorescence staining of AQP3 in primary cultured Schwann cells and frozen cross sections of sciatic nerve. A: AQP3 (red) was observed in the membrane of primary cultured WT Schwann cells under confocal scanning laser microscopy (scale bars 25 μ m). B: Left: AQP3 (red) staining in frozen cross section of WT sciatic nerve (scale bar 50 μ m). Right: No immunofluorescence staining in frozen cross section of AQP3^{-/-} sciatic nerve (scale bar 50 μ m).

(95%DMEM + 5%FBS) was replaced with fresh medium every 2 days and the cells were observed every 2 days under microscopy. Cells were scraped in an approximately 300 μ m wide strip using a standard 50- μ l pipette tip. Cells were washed twice to remove non-adherent cells. Schwann cells were observed 24 h after scraping to detect Schwann cells migration, as described (Saadoun *et al.* 2005). Phase contrast micrographs at 4 $\times$ were taken just after cells were scraped and after 24 h. Wound healing was quantified as the area covered by the wound edges over 24 h.

Morphology analysis of sciatic nerve

Sciatic nerves were harvested from at least 4 mice per experimental group and immediately fixed in 3% buffered glutaraldehyde in 0.1 M phosphate buffer overnight and stored in 0.1 M phosphate buffer at room temperature until further processed. The samples were dehydrated in graded acetone, which was then replaced with acetone, and embedded in epoxy resin for sectioning. The transverse semi-thin sections were stained with a 1% toluidine blue/borax solution for light microscope. The parameters measured included the

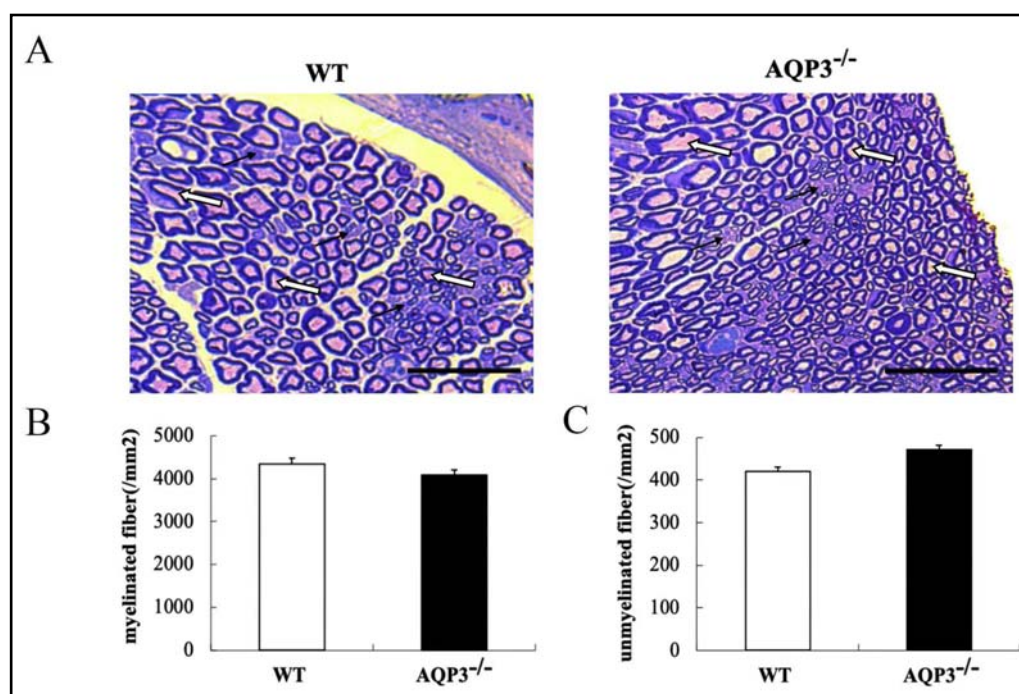


Fig. 3. Morphology properties of sciatic nerve cross sections from WT and AQP3^{-/-}. A: Cross sections of sciatic nerve from WT and AQP3^{-/-} mice (toluidine-blue stained, Dark arrows, myelinated fibers; White arrows, unmyelinated fibers, scale bars 50 μ m). B: Quantifications of myelinated fiber (n = 4). C: Quantifications of unmyelinated fiber (n = 4).

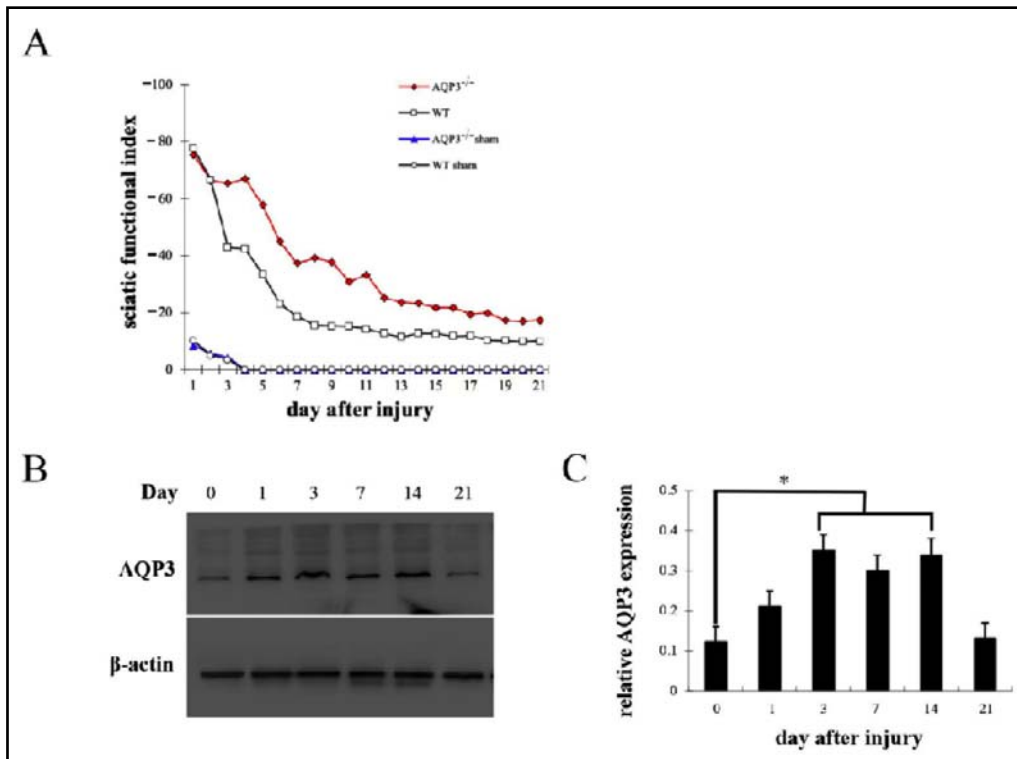


Fig. 4. Motor function analysis and AQP3 protein expression after sciatic nerve injury.

A: SFI after sciatic nerve injury in AQP3^{-/-} and WT mice (n = 24 for each operative group, n = 6 for each sham operative group). B-C: AQP3 protein levels in the sciatic nerve at determined time (n = 4, *p < 0.05).

total number of myelinated and unmyelinated fibers of the cross sections of the sciatic nerve from WT and AQP3^{-/-} mice.

Sciatic nerve contusion model

Mature 8-week-old female WT and AQP3^{-/-} mice were used in this study (25–30 g, n = 24 for each operative group, n = 6 for each sham operative group). Unilateral crush of sciatic nerves were accomplished following previously described methods (Bauder and Ferguson, 2012; Gougoulas *et al.* 2004; Wansink *et al.* 2004). Once anesthetized, mice were placed in prone position. Superficial sterilization was achieved using polyvinylpyrrolidone iodine solution to cover the sacral area and both lower extremities. After making a skin incision at the sciatic notch level in the back, the right sciatic nerve was exposed using the gluteal muscle splitting incision under a surgical microscope. Using a pair of microforceps with interlocking teeth, the sciatic nerve was crushed at the proximal segment 5 mm before the bifurcation by the application of a defined pressure for 3 times (20 s/each time, 10 s interval). The locking mechanism of the hemostatic forceps with a series of interlocking teeth ensured reproducibility and standardization of injury. A sham operation was performed on the right sciatic nerve of other animals, and the sciatic nerve was exposed in the same way, but not crushed. Subsequently, the skin was closed with 6-0 stitches and all mice were kept in animal rooms under standard housing conditions. Animals were submitted to a functional gait analysis everyday after surgery for motor function evaluation at determined

time points. Mice were deeply anesthetized (ketamine 200 mg/kg and xilasin 40 mg/kg) and euthanized and crushed nerves were dissected out for further procedures.

Motor function evaluation

Walking track analysis was performed everyday day following surgery. All mice were trained to walk along a standard track (100 cm long and 7 cm wide). The hind paws were painted with black ink. Thus, changes of paw prints were recorded. The following measurements were taken from the footprints. The sciatic functional index (SFI) was calculated according to the following equation (Bain *et al.* 1989) for quantification of the motor function and neurological recovery of the sciatic nerve. All evaluations were completed by an investigator blinded to the experimental design.

$$SFI = -38.3 \times (EPL-NPL)/NPL + 109.5 \times (ETS-NTS)/NTS + 13.3 \times (EITS-NITS)/NITS - 8.8$$

PL (print length): distance from the heel to the top of the third toe. ITS (intermediary toe spread): distance from the second to the fourth toe. TS (toe spread): distance between the first and the fifth toe. N (normal): for the non-operated foot. E (experimental): for the experimental foot. The SFI value varies from -100 to 0. The value near 0 reflects normal function, while a value of approximately -100 reflects complete dysfunction.

Statistical analysis

Data are presented as mean standard error, and significance was assessed by two-sided Student's test, unpaired comparison of means (GraphPad Prism). Experimental

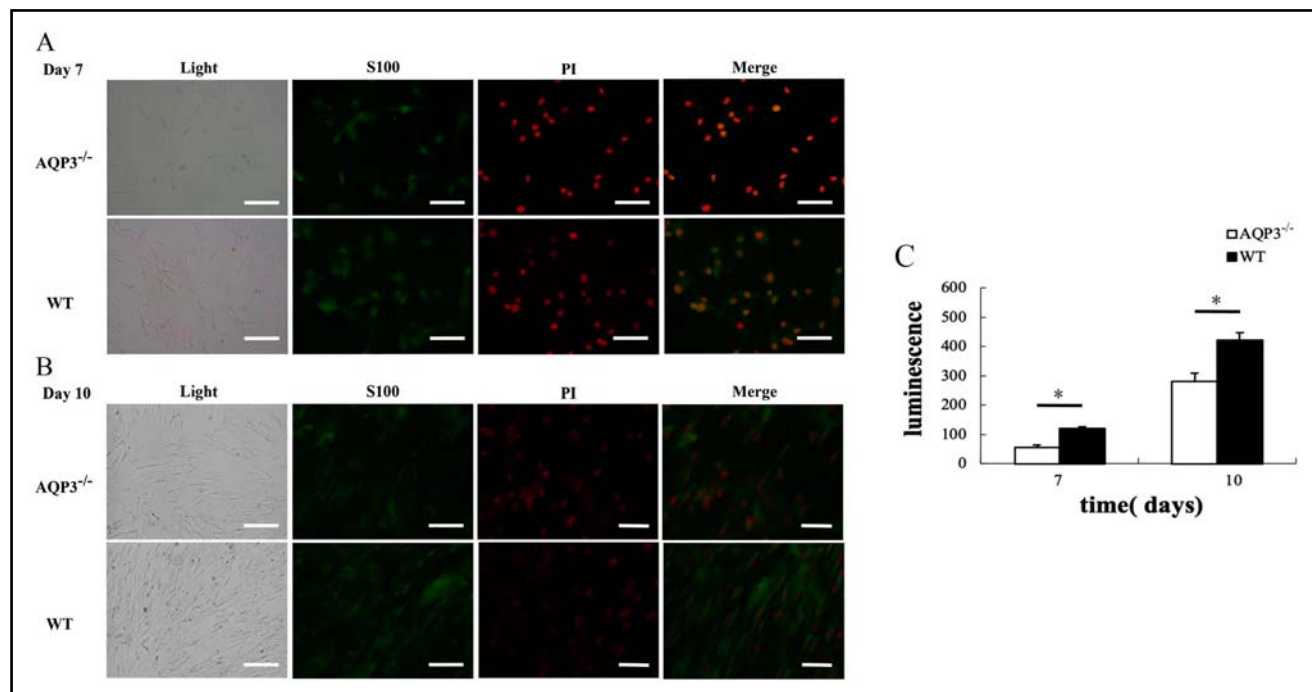


Fig. 5. Proliferation of AQP3^{-/-} and WT Schwann cells and immunofluorescence staining of S100β. A-B: Primary cultured Schwann cells under microscopy and S100 (green) verification (A, 7 d, scale bar: 100 μm; B, 10 d; scale bar: 50 μm; nuclei were stained with PI). C: Schwann cells were measured for proliferation at time points of 7 d and 10 d (n = 6 wells for each group, *p < 0.05).

groups were considered significantly different at *p < 0.05.

RESULTS

AQP3 mRNA and protein were expressed in sciatic nerve tissues and Schwann cells

In order to detect the expression of AQP3 in mouse peripheral nerve, sciatic nerve tissues and Schwann cells of WT and AQP3^{-/-} mice were isolated and conducted to RT-PCR and immunoblot analysis. Meanwhile, mouse kidney tissues were used as positive controls of AQP3 expression. AQP3 mRNA (217 bps) was detected in Schwann cells, sciatic nerve and kidney from the WT mice (Fig. 1A). Expression of AQP3 was normalized against β-actin (260 bps). Relatively gray quantification of AQP3 mRNA in sciatic nerve was about 50% that of the kidney (Fig. 1B). AQP3 protein bands (31 kDa) were observed in the Schwann cells, sciatic nerve tissues, and kidney tissues from WT mice in immunoblot analysis (Fig. 1C). Quantification of AQP3 protein was normalized against β-actin (42 kDa) in each sample. Schwann cell, sciatic nerve and kidney tissues from AQP3^{-/-} mice do not express AQP3 protein, as expected.

Immunofluorescence staining of AQP3 in Schwann cells and sciatic nerve

Immunofluorescence staining was performed on primary cultured Schwann cells and frozen cross sections of the sciatic nerve. AQP3 (red) was observed

in the membrane of primary cultured WT Schwann cells under confocal scanning laser microscopy (Fig. 2A). Immunofluorescence staining in frozen cross sections of WT sciatic nerve showed AQP3 (red) was localized in the membrane of Schwann cells, which presented mesh like appearance around the nuclei of Schwann cells (Fig. 2B left). Immunofluorescence staining was absent in frozen cross section of AQP3^{-/-} sciatic nerve samples (Fig. 2B right).

Morphology property of fibers in sciatic nerve cross sections

In order to identify the potential influence of AQP3 deficiency on the morphology nerve fibers, sciatic nerve cross sections from the WT and AQP3^{-/-} mice were stained by toluidine-blue agent. The morphology and diameters of myelinated fibers (dark arrows) and unmyelinated fibers (white arrows) had no significance difference between the AQP3^{-/-} and WT groups (Fig. 3A, n = 4). Quantification of myelinated fibers and unmyelinated fibers of AQP3^{-/-} and WT samples were shown in the histogram (Fig. 3B, 3C; n = 4). There was no significant difference in the number of myelinated fibers and unmyelinated fibers between the two groups.

Impaired motor function rehabilitation in AQP3^{-/-} mice

SFI was calculated for quantification of the motor function and neurological recovery after contusion. There was an obvious delay in motor function recovery in AQP3^{-/-} group compared with that of the WT group (Fig. 4A, n = 24 for each operative group). In the

operative groups, SFI was less than -60 in the early days after contusion, reflecting severe damnification to the nerve motor function. The motor function evaluation demonstrated that AQP3^{-/-} and WT mice reestablished most operated side hind limb movements (SFI nearly -20) after 7 and 13 days post-injury respectively. In the two sham operative groups, SFI was near to 0 after the third day of contusion. There was an increase of AQP3 protein expression after injury, which reached maximum at about the 3rd day after injury then sustained high level within 2 weeks (Fig. 4B, 4C).

Defective proliferation of AQP3^{-/-} Schwann cells

Schwann cells from AQP3^{-/-} and WT were isolated and cultured (Fig. 5A, 5B). S100 β was used as a labeling protein of Schwann cells (Rezaei et al. 2017). S100 β immunofluorescence staining (green) was performed to verify the purity of Schwann cells, which was above 95%. The Schwann cells were cultured for 10 days and measured at 7 d and 10 d. AQP3^{-/-} Schwann cells demonstrated the same morphology as WT cells. However, AQP3^{-/-} Schwann cells proliferation was significantly slower than WT Schwann cells (Fig. 5 C).

Defective migration of AQP3^{-/-} Schwann cells

Wound healing assay was used to measure migration of Schwann cells. Wound healing was quantified as speed of wound edge covered the scraped edges after 24 h. AQP3^{-/-} Schwann cells showed lower migration ability in contrast with the WT cells (Fig. 6).

DISCUSSION

Despite the wide tissue distribution of AQP3 and its ability to transport water as well as some small solutes, deletion of AQP3 in mice was not associated either with impaired development, perinatal mortality, or growth retardation. The only overt phenotype of the AQP3^{-/-} mice was marked polyuria with urine osmolalities substantially lower than those WT mice (Ma et al. 2002). AQP3^{-/-} mice were precious gift provided by Pr.

Tonghui MA. AQP3 was located in kidney, skin, cornea, digestive tract, genital system, and glial cells of nervous system (Zhang et al. 2010; Hara-Chikuma et al. 2008; Ma et al. 2002, 2000; Levin et al. 2006; Hara et al. 2002; Thiagarajah et al. 2007). In the present study, we focused on localization and possible functions of AQP3 in the peripheral nervous system

AQP3 deficiency does not alter the morphology and the number of myelinated fibers and unmyelinated fibers in cross sections of sciatic nerves. There was no significant difference in the number of myelinated fibers and unmyelinated fibers between the AQP3^{-/-} and WT samples. Primary culture of Schwann cells and in vitro wound healing model revealed that AQP3^{-/-} Schwann cells exhibited the same morphology, while showing lower proliferation and migration ability in contrast with that of the WT cells. AQP3-dependent Schwann cells migration adds to an expanding list of cell types whose migration is increased by AQP-facilitated water permeability. Existing studies provide evidence that AQP3 facilitated proliferation, differentiation and migration of tumor cells and epithelial cells (Nong et al. 2021; Bollag et al. 2020; Arif et al. 2018; Marlar et al. 2017). Mice lacking AQP3 were found to have significantly impaired epidermal proliferation in a wound healing mode (Hara-Chikuma et al. 2008). In the eye, AQP3 facilitated migration of corneal epithelial cells. In the germ cells, AQP3 facilitated sperm osmoadaptation and migration (Chen et al. 2011). Hara M reported selectively reduced glycerol in skin of AQP3-deficient mice may account for impaired skin function (Hara et al. 2002).

This study suggests a certain role for AQP3 in the peripheral nerve myelin sheath. To explore potential AQP3 function in motor functional rehabilitation, sciatic nerve contusion models were established and walking track analyses were performed on WT and AQP3^{-/-} mice. There was an obvious delay in motor functional rehabilitation in AQP3^{-/-} mice compared with that of WT mice. We also observed there was an increase of AQP3 protein expression after injury

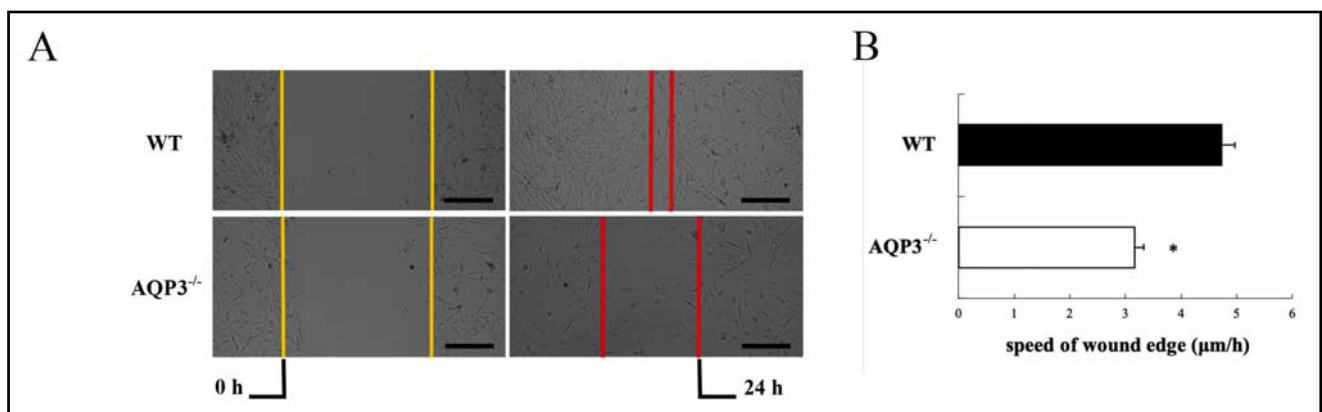


Fig. 6. In vitro wound healing assay. A: Micrographs showing in vitro wound healing after scrape (scale bar: 100 μm). B: Data summary (n = 6, *p < 0.05).

of the sciatic nerve, which was maximum at about the 3rd day after injury then sustained high level within 2 weeks.

AQP3 deficiency impaired nerve rehabilitation in wound healing model both *in vitro* and *in vivo*. These data support our hypothesis that AQP3 participates in myelin demyelination and repair course. The slowed motor functional recovery in AQP3^{-/-} mice may be a consequence of impaired function of Schwann cells in proliferation and migration.

Peripheral nerve rehabilitation following injury involved several processes such as cellular mechanisms and energy supply. Schwann cells are glial cells of the peripheral nervous system, which are target cells in myelin demyelination and repair course. Schwann cells provide myelination and trophic support for axons (Jessen and Mirsky, 2005; Campana, 2007). In response to peripheral nervous injury, Schwann cells were activated and acquired the ability to migrate and proliferate (Lehmann and Höke, 2010; Arthur-Farraj *et al.* 2012), which were essential for nerve remyelination. The sciatic nerve injury model partially simulated myelin demyelination and repair course *in vivo*, while the axons were mostly integrated. AQP3 protein level increased in the sciatic nerve after injury, which probably was related to its water or glycerol transporting function, as a possible mechanism of Schwann cells activation and compensatory increase to solutes transportation during the nerve rehabilitation course. It was reported that AQP3 was involved in energy metabolism and inflammatory processes. AQP3 increased glucose transporter1 expression, glucose uptake, ATP concentration, and lactate production in kidney, suggesting a mechanistic link between AQP3 expression and glycolysis. AQP3 might thus increase glycolysis by increasing cellular H₂O₂ and glycerol permeability (Wang W *et al.* 2019). NLRP3-inflammasome activation induced by reswelling, nigericin, and ATP was blocked when AQP3 was inhibited or silenced in macrophage, suggesting AQP3 as potential players in the setting of the inflammatory response (da Silva IV *et al.* 2021). However, the role of AQP3 in peripheral nerves has not been reported so far. Therefore, AQP3 may be a new determinant of myelin function and may play roles in both water transfer and maintenance of integrity of myelin of the peripheral nervous system. Further investigations should be made to discuss whether AQP3 is the regnant aquaporin responsible for water and energy supply in the peripheral nervous system.

CONCLUSIONS

Our study suggested that AQP3 localized in the membrane of Schwann cells and facilitated Schwann cells' proliferation and migration. AQP3 deficiency impaired nerve rehabilitation in wound healing model both *in vitro* and *in vivo*. AQP3 participates in myelin demyelination and repair course and exhibit increment

after peripheral nerve injury. The study support our hypothesis that AQP3 participates in myelin demyelination and repair course and the mechanisms underlying the AQP3 in the field of myelin repair and regeneration in peripheral nerves deserves further investigation and exploration.

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