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Satb1 promotes Schwann cell viability and migration via activation of PI3K/AKT pathway

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Abstract. – OBJECTIVE: Satb1 regulates chromatin structure and gene expression, and is aberrantly expressed in many tumors. However, there is still no report about Satb1 functions in peripheral nerve injury until now. In this study, we explored the regulatory effect of Satb1 on Schwann cells.

MATERIALS AND METHODS: MTT assay, transwell assay, and flow cytometry assay were respectively used to determine Schwann cell viability, migration, and apoptosis. The mRNA and phosphorylation levels of Satb1 and SHIP1 were assessed by RT-PCR and Western blotting analysis, respectively. The correlation between Satb1 and SHIP1 was examined by ChIP assay. The expressions of PI3K/AKT pathway related factors were detected by Western blotting.

RESULTS: In the present study, we found that knock-out of Satb1 significantly inhibited cell viability and migration, and promoted Schwann cells apoptosis. Conversely, over-expression of Satb1 promoted cell viability, migration, and inhibited apoptosis. Satb1 inhibited SHIP1 expression by recruiting HDAC1. Furthermore, results showed that Satb1 activated the PI3K/AKT signaling pathway by inhibiting the expression of SHIP1. SHIP1 showed significant reversal effects on the regulatory roles of Satb1 in Schwann cells. Over-expression of Satb1 and SHIP1 inhibited cell viability, migration, and promoted apoptosis.

CONCLUSIONS: Our study demonstrated that the Satb1 knock-out could inhibit the activation of PI3K/AKT pathway by up-regulating SHIP1, thus inhibiting cell viability and migration, and promoting Schwann cell apoptosis.

Key Words:
Satb1, SHIP1, Peripheral nerve injury, PI3K/AKT signaling pathway, Schwann cell.

Introduction

Peripheral nerve injury (PNI) is a clinical disease characterized by nerve axonal disruption, nerve conduction dysfunction or nerve rupture caused by peripheral injury or ischemia. The incidence of PNI increases annually with the development of modern architecture and transportation industry[1]. The common causes of PNI are mechanical injuries, such as distraction injury, incised injury, and compression trauma. However, the prognosis of PNI is poor because of the slow nerve regeneration and axons reach the target organ around for a long time. After nerve injury, the surrounding muscles become malnutrition and disuse atrophy. It will also aggravate connective tissue hyperplasia and the prolongs axonal conduction, eventually leading to irreversible muscle atrophy[2]. Therefore, basic and clinical researches have focused on how to protect the target organ, slow down muscle atrophy and end-plate degeneration, promote nerve regeneration and reduce the degree of physical dysfunction. Successful regeneration of peripheral nerves involves the protection of neuronal cell bodies, axon regeneration and the formation of functional synapses. At present, the implantation of Schwann cells is one of the effective approaches to improve the regeneration and functional rehabilitation of peripheral nerves. Schwann cells are the myelin glial cells of the peripheral nervous system (PNS), which originate from the neural crest. It participates in various biological functions of PNS, including the conduction of nerve impulses, the involvement of nerve growth and regeneration, the regula-

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tion of motor neuron activity, and mediation of antigen[3]. Special AT-rich sequence-binding protein-1 (Satb1) is a T-cell specific transcription factor, which participates in the chromatin higher structural reorganization and tissue-specific gene expression[4-6]. It is mainly expressed in thymocytes[7] and is essential for thymus development and T-cell maturation[8-10]. Moreover, Satb1 plays a key role in maintaining the balance of chromatin transcription and regulating genes transcription, as well as the location of nuclear proteins. Scholars[11,12] have suggested that Satb1 is involved in general tumors, cell development, and apoptosis. Yuan et al[13] has shown that Satb1 expression is associated with distant metastasis. However, there is still no report on Satb1 functions in PNI until now. Therefore, in this study, we investigated the regulatory effect of Satb1 on Schwann cells. This will provide a new theoretical basis for the clinical therapy of PNI.

Materials and Methods

Cell Culture

Schwann cells were prepared from postnatal day 1 (P1) sciatic nerves of Sprague-Dawley (SD) rats (Beijing Laboratory Animal Research Center, Beijing, China), and dissociated with 0.4% (w/v) collagenase and 0.125% (w/v) trypsin (both from Sigma-Aldrich, St. Louis, MO, USA). The cells (5×10^4 cells/well) were seeded onto 24-well plates and cultured in Dulbecco's Modified Eagle's Medium (DMEM; Sigma-Aldrich, St. Louis, MO, USA) with 10% (v/v) fetal bovine serum (FBS; Thermo Fisher Scientific, Waltham, MA, USA). After one day of culture, Schwann cells were treated with 10 μM cytosine β-D-arabinofuranoside (Ara-C; Sigma-Aldrich, St. Louis, MO, USA) twice for 24 h to reduce the proliferation of fibroblast. Then, Schwann cells were passaged, pooled and continued to culture in DMEM containing 10% (v/v) FBS. Experiments using rats were performed in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals and were approved by the university's Institutional Animal Care and Use Committee of Chinese PLA General Hospital (Beijing, China).

Plasmids Transfection

The Satb1, HDAC1, and SHIP1 expression vectors were constructed by sub-cloning the full-length coding sequences into pcDNA3.1 and confirmed as oeSatb1, oeHDAC1, and oeSHIP1. The empty construct pcDNA3.1 was transfected as oecontrols. The target sequences for small interfering RNA (siRNA) were as follows: Satb1: 5′-GGTGGCAGACATGCTTCAA-3′, HDAC1: 5'-GGAGAGTACTTCCCAGGAA-3', SHIP1: 5'-GGTGGAACCATGGCAACAT-3'. siRNAs were synthesized by GenePharma Co. (Shanghai, China), and named as siSatb1, siHDAC1, and siSHIP1. Cell transfections were conducted using Lipofectamine® RNAiMAX Transfection Reagent (Invitrogen™, Carlsbad, CA, USA) according to the manufacturer's instructions.

Cell Viability Assay

The cells were seeded in 24-well plates at a density of 5×10^4 cells/well. The cell viability was determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) colorimetric assay according to the standard method described previously[14]. Briefly, 20 μl of MTT (Sigma-Aldrich, St. Louis, MO, USA) was added into each well after 48h transfection and incubated for 4 h at 37°C. Then, 150 μl of dimethylsulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA) was added, and the plates were shaken for 10 min. The absorbance was detected at 570 nm (OD_{570}) using a microplate enzyme-linked immunosorbent assay (ELISA) reader (Bio-Rad Laboratories, Hercules, CA, USA). Each experiment was performed three times.

Apoptosis Assay

Cell apoptotic analysis was performed using propidium iodide (PI) and fluorescein isothiocyanate (FITC)-conjugated Annexin V staining. Briefly, Schwann cells were washed in phosphate-buffered saline (PBS) and fixed in 70% ethanol (Sigma-Aldrich, St. Louis, MO, USA). Fixed cells were then washed twice in PBS and stained in PI/FITC-Annexin V with the presence of 50 μg/ml RNase A (Sigma-Aldrich, St. Louis, MO, USA), and incubated for 1 h at room temperature in the dark. Flow cytometry analysis was done using a FACS can (Beckman Coulter, Brea, CA, USA). The data were analyzed by the FlowJo V10.4 software (TreeStar, Ashland, OR, USA).

Migration Assay

The migration of Schwann cells was assessed by transwell migration assay previously described[15]. The 6.5 mm transwell chambers with 8 μm pores (Costar, Cambridge, MA, USA) were used, and the bottom surface of each membrane

was coated with 10 μg/ml fibronectin (Sigma-Aldrich, St. Louis, MO, USA). Then, 100 μl of primary Schwann cells (3×10^5 cells/ml) were seeded onto the upper chamber of each transwell. The cells were incubated at 37°C in 5% CO_2, and 600 μl of complete medium was added to the lower chambers. Non-traversed Schwann cells on the upper surface of the membranes were removed using a cotton swab, and traversed Schwann cells on the lower surface were stained with 0.1% crystal violet (Sigma-Aldrich, St. Louis, MO, USA) for 15 min at room temperature. Stained cells were imaged and randomly counted using a DMR inverted microscope (Leica Microsystems, Wetzlar, Germany). Each experiment was performed three times.

Quantitative Real-time Polymerase Chain Reaction (qRT-PCR)

Total RNA was isolated from transfected cells by using TRIzol reagent (Invitrogen™, Carlsbad, CA, USA) and treated with DNaseI (Promega Corporation, Madison, WI, USA). Reverse transcription was performed using the Multiscribe™ Reverse Transcriptase kit (Applied Biosystems, Foster City, CA, USA) and random hexamers or oligo (dT). The reverse transcription conditions were 5 min at 95°C, followed by 40 cycles of 10 s at 95°C, 30 s at 58°C, 30 s at 72°C, and a final step of 5 min at 72°C. The sequences of the primers were as follows: Satb1-forward, 5'-CGGGGTACCCGCCACCATGGCGATGCTGCTATCCGTGCCG-3'; Satb1-reverse, 5'-CCGGAATTCCAGCTCGTCCTTGGCCTG-3'. GAPDH-forward, 5'-GCACCGTCAAGGCTGAGAAC'-3, GAPDH-reverse, 5'-TGGTGAAGACGCCAGTGGA-3'.

Western Blotting Analysis

The proteins used for Western blotting were extracted using RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China) supplemented with protease inhibitors (Roche, Guangzhou, China). The proteins were quantified using the BCA™ Protein Assay Kit (Pierce Biotechnology, Waltham, MA, USA). The Western blotting system was established using a Bio-Rad Bis-Tris Gel system according to the manufacturer's instructions (Hercules, CA, USA). Rabbit anti-mouse CRT antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). GAPDH antibody was purchased from Sigma-Aldrich (St. Louis, MO, USA). Primary antibodies were prepared in 5% blocking buffer at a dilution of 1:1,000, incubated with the membrane at 4°C overnight. Then, they were washed and incubated with secondary antibody (Sigma-Aldrich, St. Louis, MO, USA), and were marked by horseradish peroxidase for 1 h at room temperature. After rinsing, the polyvinylidene difluoride (PVDF) membrane carried blots, while antibodies were transferred into the Bio-Rad ChemiDoc™ XRS system, and then 200 μl Immobilon Western Chemiluminescent HRP Substrate (Millipore, Billerica, MA, USA) was added to cover the membrane surface. The signals were captured and the intensity of the bands was quantified using the Image Lab™ Software (Bio-Rad Laboratories, Hercules, CA, USA).

Chromatin Immunoprecipitation (ChIP) Assay

ChIP assays were performed by ChIP Assay Kit (Millipore, Billerica, MA, USA) following standard protocol. Schwann cells were treated with 1% formaldehyde (Sigma-Aldrich, St. Louis, MO, USA) for 10 min, cracked with sodium dodecyl sulfate (SDS) lysis buffer followed by ultrasonication, and then were incubated with proper antibodies. After washing with high salt, low salt, and LiCl buffer, the elution buffer was used to harvest the chromatin fragments. Finally, the enrichments were examined using RT-PCR.

Statistical Analysis

All experiments were repeated three times. The results of multiple experiments are presented as the mean ± standard deviation (SD). Statistical analyses were performed using SPSS 19.0 statistical software (SPSS Inc., Chicago, IL, USA). *p*-values were calculated using a one-way analysis of variance (ANOVA) with Dunnett's *t*-test. *p*-value < 0.05 was considered to indicate a statistically significant result.

Results

Knock-out of Satb1 Inhibited the Ciability and Migration, and promoted Apoptosis in Schwann Cells

The expression of Satb1 was detected at mRNA and phosphorylation level by RT-PCR and Western blotting, respectively. The results (Figure 1A and 1B) showed that Satb1 expression level was significantly decreased in the siSatb1 group ($p <$

0.05). As shown in Figure 1C and 1D, knockout Satb1 significantly inhibited Schwann cells viability and migration ($p < 0.05$). By contrast, flow cytometry analysis showed cell apoptosis was evidently increased when Satb1 knockout ($p < 0.05$, Figure 1E).

Over-expression of Satb1 Promoted the Viability and Migration, and Inhibited Apoptosis in Schwann Cells

The transfection efficiency of oeSatb1 was detected by RT-PCR and Western blotting, respectively. In Figure 2A, we found that oeSatb1 was efficiently transfected into Schwann cells, that the mRNA and phosphorylation levels of Satb1 were both remarkably increased ($p < 0.05$). As shown in Figure 2B and 2C, over-expression (OE) of Satb1 significantly promoted the viability and migration of Schwann cells ($p < 0.05$). Moreover, the Western blotting analysis showed that the expression of Bax and Bid were decreased, while the expression of Bcl-2 was clearly increased when Satb1 over-expression (Figure 2D), thus illustrating that Satb1 over-expression could promote apoptosis in Schwann cells.

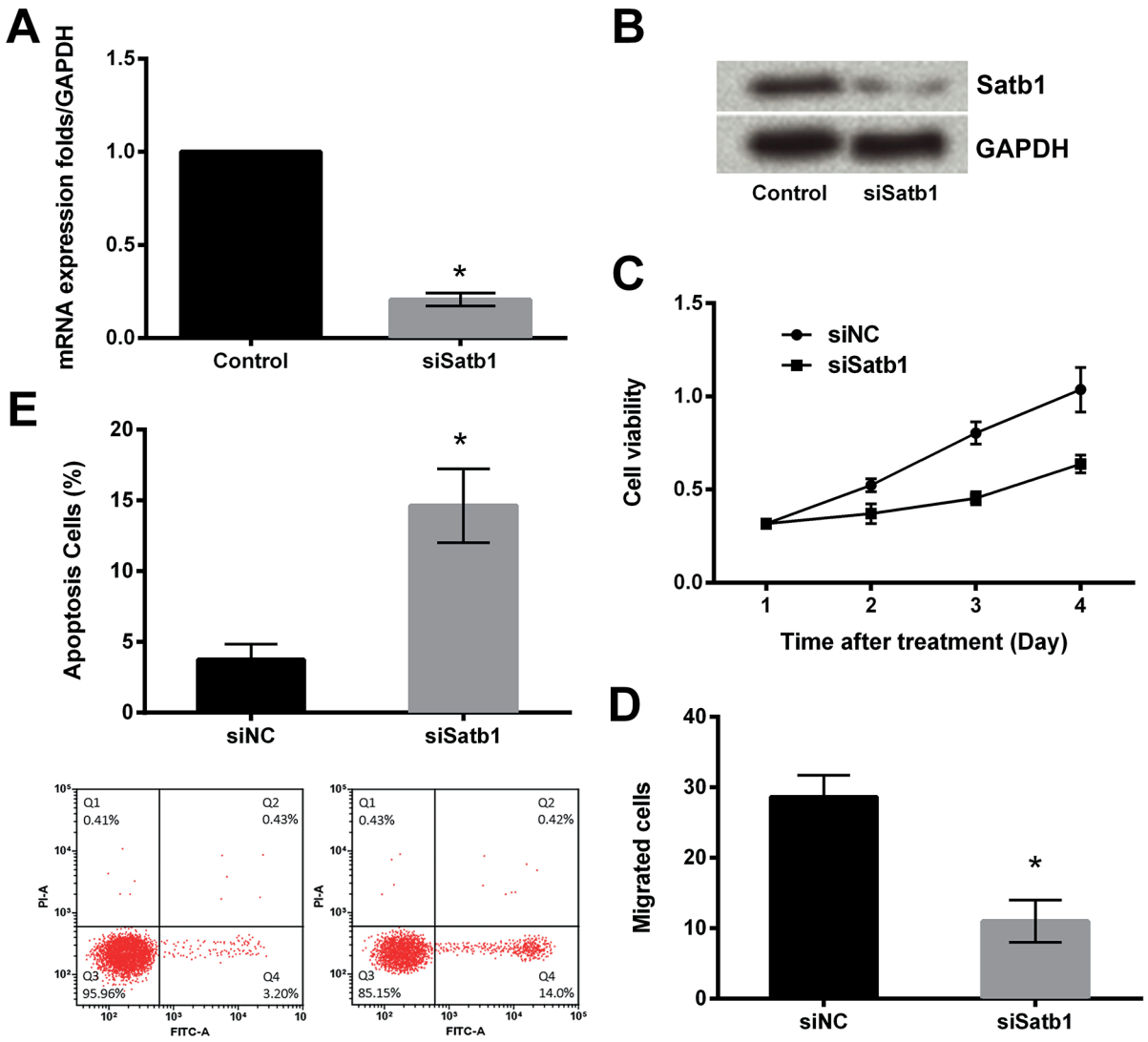

Figure 1. Knock-out of Satb1 inhibited the viability and migration, and promoted apoptosis in Schwann cells. ***(A*** and ***B)*** The expression of Satb1 was detected after transfection of siSatb1 at mRNA and phosphorylation level by RT-PCR and Western blotting, respectively. ***(C)*** The cell viability was detected by MTT assay. ***(D)*** The migration of Schwann cells was detected by transwell assay. ***(E)*** The apoptosis was detected by flow cytometry assay. Error bars represent mean ± SD, *$p < 0.05$.

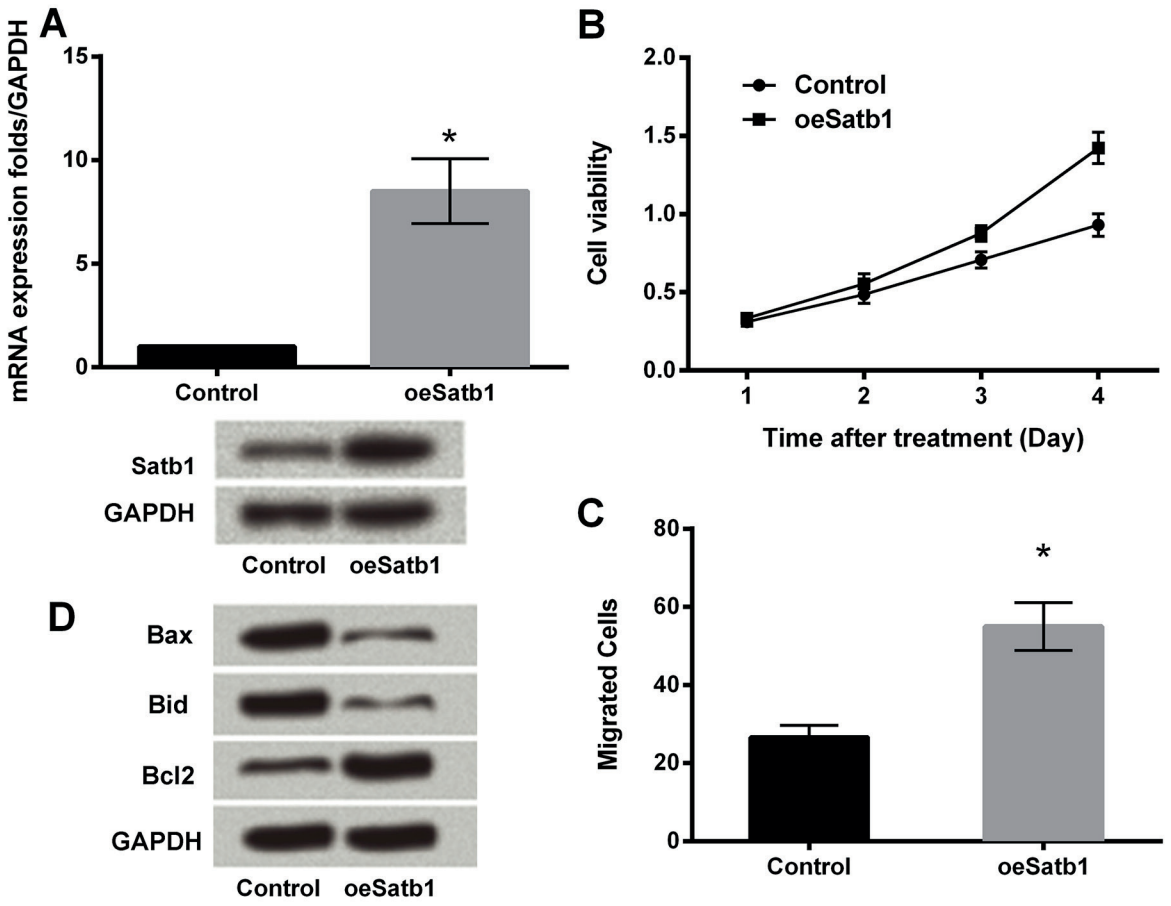

Figure 2. Over-expression of Satb1 promoted the viability and migration, and inhibited apoptosis in Schwann cells. ***(A)*** The expression of Satb1 was detected after transfection of oeSatb1at mRNA and phosphorylation level by RT-PCR and Western blotting, respectively. ***(B)*** The cell viability was detected by MTT assay. ***(C)*** The migration of Schwann cells was detected by transwell assay. ***(D)*** The apoptosis-related proteins were detected by Western blotting. Error bars represent mean ± SD, *$p < 0.05$.

Satb1 Inhibited the Expression of SHIP1 by Recruiting HDAC1

The influence of Satb1 on SHIP1 expression was analyzed by Western blotting. As shown in Figure 3A, knockout of Satb1 increased the expression of SHIP1. Conversely, over-expression of Satb1 inhibited the expression of SHIP1. ChIP assay showed that a specific region of the SHIP1 promoter was enriched after Satb1 precipitation ($p < 0.05$, Figure 3B). Then, from Figure 3C, we found that Satb1 knock-out significantly promoted the enrichment of H3K4me3, while over-expression of Satb1 inhibited the gathering of H3K4me3 in the SHIP1 promoter region ($p < 0.05$). But the effect of Satb1 on H3K27me3 showed the reverse trend that Satb1 over-expression clearly promoted H3K-27me3 enrichment ($p < 0.05$, Figure 3D). Subsequently, we also performed ChIP assay to assess the relationship between Satb1 and HDAC1. The result showed that HDAC1 was enriched in the Satb1 deposition, which proved that HDAC1 interacted with Satb1 (Figure 3E). Furthermore, we found that over-expression of Satb1 significantly promoted the enrichment of HDAC1 in the SHIP1 promoter ($p < 0.05$, Figure 3F). Western blotting results suggested that over-expression of HDAC1 decreased the expression of SHIP1, while knockout of HDAC1 increased SHIP1 expression (Figure 3G).

Satb1 Activated the PI3K/AKT Signaling Pathway by Inhibiting SHIP1 Expression

Further, Schwann cells were transfected with siSHIP1 and oeSHIP1, respectively. As shown in Figure 4A, we found that siSHIP1 and oeSHIP1 were successfully transfected into cells. Then, we focused on PI3K/AKT signaling pathway and examined the expression of the pathway-related

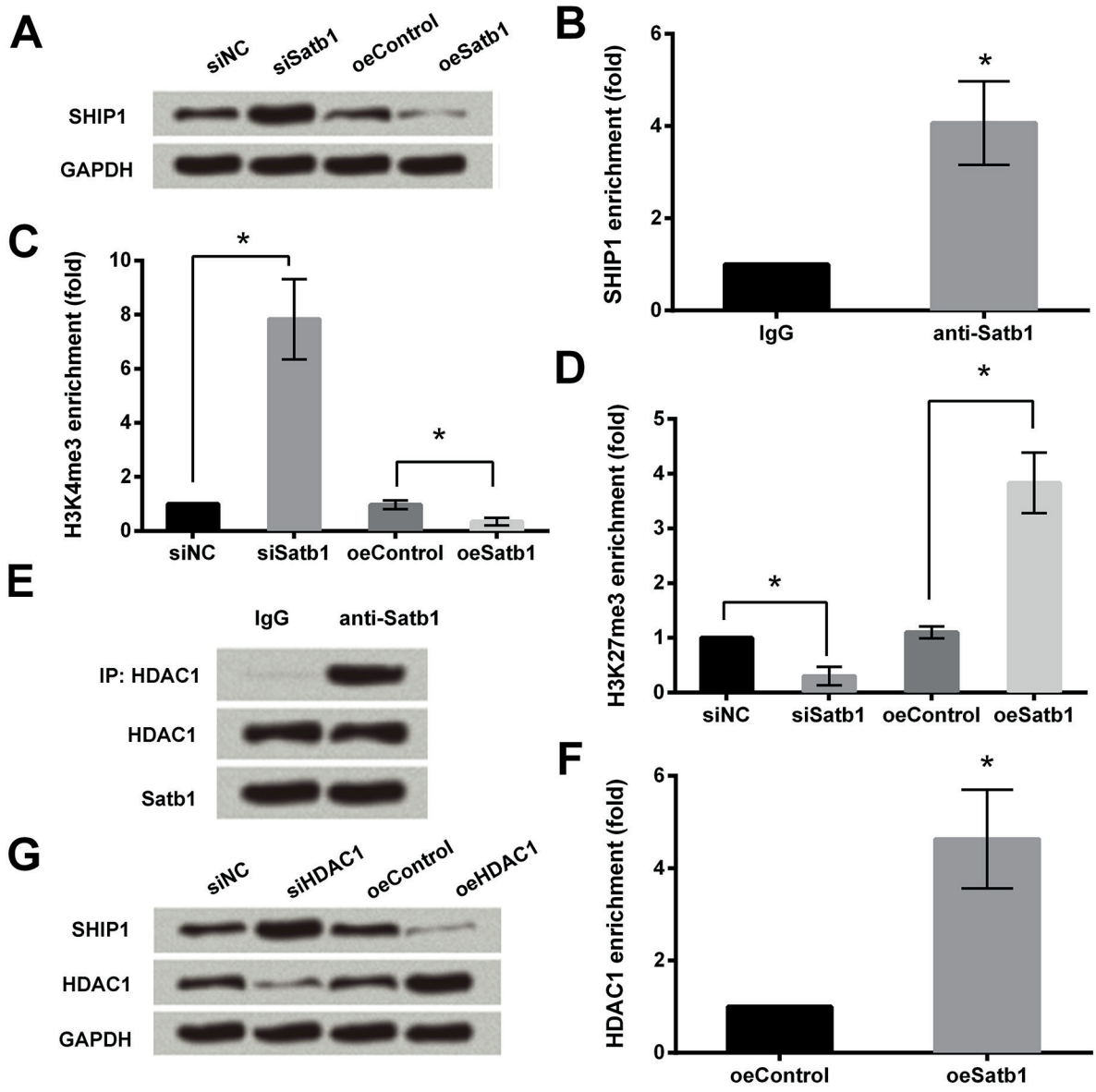

Figure 3. Satb1 inhibited the expression of SHIP1 by recruiting HDAC1. ***(A)*** The expression of SHIP1 was detected after cells were transfected with siSatb1 and oeSatb1 by Western blotting. ***(B)*** Satb1 binds to SHIP1 promoter. ChIP assays were performed using control lgG antibody and anti-Satb1, and enrichment region of SHIP1 promoter was examined using RT-PCR. ***(C*** and ***D)*** The enrichment of H3K4me3 and H3K27Me3 were examined using RT-PCR. ***(E)*** The correlation of Satb1 with HDAC1 was detected by ChIP assay. The fold enrichment of HDAC1 was examined using Western blotting. ***(F)*** The fold enrichment of HDAC1 was examined using RT-PCR. ***(G)*** The expression of SHIP1 was detected after transfection of siHDAC1 and oeHDAC1 by Western blotting. Error bars represent mean ± SD, *$p < 0.05$.

factors by Western blotting. The expressions of p-AKT and p-PI3K were decreased in the siSatb1 group, while they were significantly increased in the siSatb1+siSHIP1 group (Figure 4B). However, over-expression of Satb1 and SHIP1 showed the reverse trend that Satb1 and SHIP1 over-expression alleviated the increase of p-AKT and p-PI3K expressions (Figure 4C). These results indicated that Satb1 could activate the PI3K/AKT signaling pathway by inhibiting SHIP1 expression.

Reverse effect of SHIP1 on Satb1-Regulated Schwann Cells

As shown in Figure 5A and 5B, over-expression of Satb1 increased cell viability and migration. Meanwhile, SHIP1 over-expression inhibits

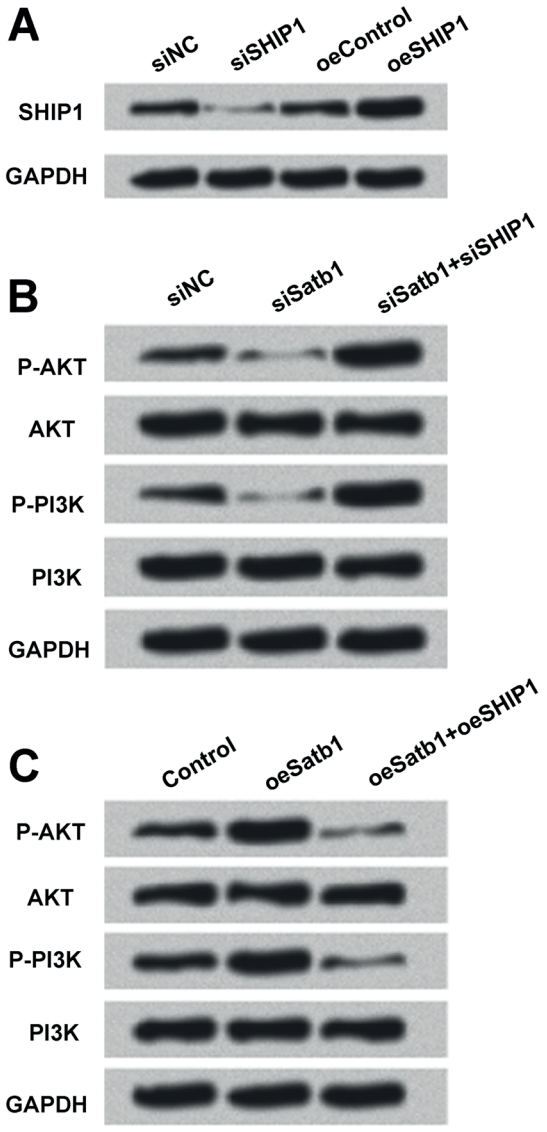

Figure 4. Satb1 activated the PI3K/AKT signaling pathway by inhibiting SHIP1 expression. ***(A)*** The expression of SHIP1 was detected after cells were transfected with si-SHIP1 and oeSHIP1 by Western blotting at phosphorylation level. ***(B*** and ***C)*** The expressions of PI3K/AKT pathway related proteins were detected by Western blotting. Error bars represent mean ± SD.

these additions ($p < 0.05$). Similarly, apoptosis was increased in the siSatb1 group, and that was decreased in the siSatb1+siSHIP1 group ($p < 0.05$, Figure 5C).

Discussion

PNI can result in partial or complete loss of motor, sensory, and autonomic functions, thus leading to severe disability[16]. Schwann cells are the myelin-forming cells of PNS. After the peripheral nerves were injured, the transition of Schwann cells from axon myelination to demyelinated state, and ultimately re-myelination of axons[17]. Notably, Schwann cells play an important role in the regeneration of injured peripheral nerves. Therefore, in-depth study of PNI cellular and molecular mechanisms will be conducive to the development of peripheral nerve repair strategies. Previous studies[18,19] have

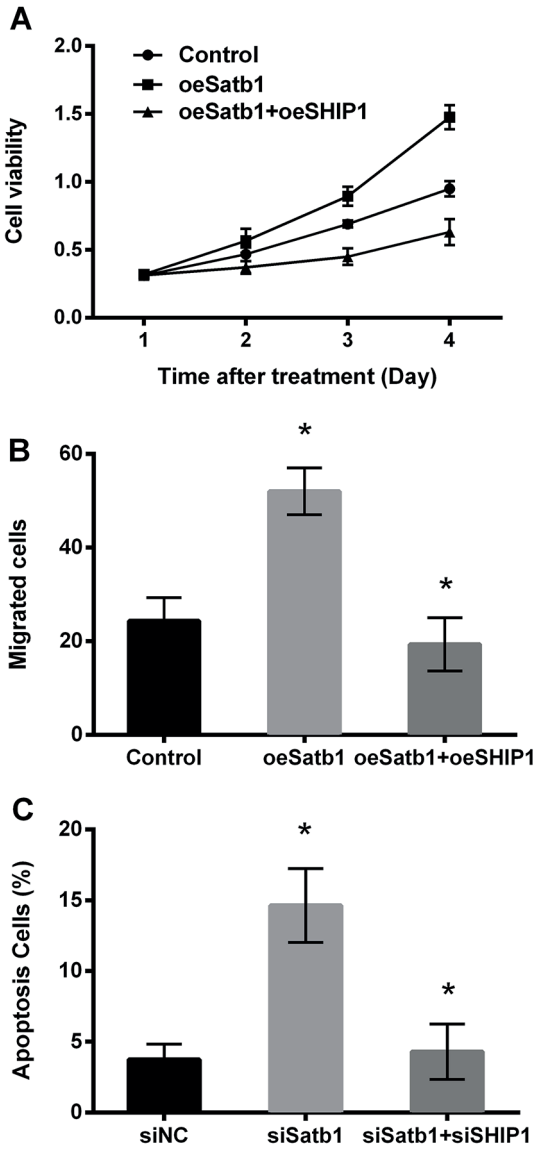

Figure 5. Reverse effect of SHIP1 on Satb1-regulated Schwann cells. ***(A)*** The cell viability was detected by MTT assay. ***(B)*** The migration of Schwann cells was detected by transwell assay. ***(C)*** The apoptosis-related proteins were detected by flow cytometry assay. Error bars represent mean ± SD, * $p < 0.05$.

proved that Satb1 could regulate the self-renewal of hematopoietic stem cells and the differentiation of embryonic stem cell. Also, over-expression of Satb1 is correlated with the progression of various human cancers[20-23]. Thus, we speculated that Satb1 might play a key role in regulating the phenotype of Schwann cells. Our study confirmed that knockout of Satb1 inhibited the viability and migration of Schwann cells, on the contrary, over-expression of Satb1 promoted them. These results suggested that Satb1 was positively correlated with Schwann cells. To further study the mechanism of Satb1 in regulating Schwann cells, we focused on the relationship between Satb1 and Src homology 2 (SH2)-containing inositol phosphatase-1 (SHIP1). Then, we found that Satb1 could interact with histone deacetylase 1 (HDAC1) and inhibit the expression of SHIP1 by regulating HDAC1 binding to the promoter region of SHIP1. However, the protein encoded by HDAC1 gene belongs to the histone deacetylase/acuC/AphA family and is a component of the histone deacetylase complex. A report said that HDAC1 interacts with retinoblastoma tumor-suppressor protein, and is a key factor in the control of cell proliferation and differentiation[24,25].

SHIP1 is the inositol 5'-phosphatase contained SH2 structure, which could inhibit the activation of phosphatidylinositol 3-kinase (PI3K) signaling pathway. Some evidences[26,27] showed that SHIP1 could induce cell apoptosis by activating caspase-3 and caspase-9, up-regulation of Bad and p27, and down-regulation of Bcl-xL. Liu et al[27] reported that over-expression of SHIP1 decreases cell viability and induces cell apoptosis in myeloid cell lines. Moreover, over-expression of SHIP1 could promote cell apoptosis by inducing specific caspase cascades, which are independently regulated by the 5-phosphatase activity of SHIP1 in the erythrocytes[26]. However, previous researches mainly concentrated on the effect of SHIP1 on the hematopoietic system. Our study found that over-expression of SHIP1 could inhibit cell viability and migration of Schwann cells, and knock-out of SHIP1 inhibited apoptosis, indicating that SHIP1 had a negative regulatory effect on Schwann cells.

PI3K/AKT signaling pathway plays an important role in maintaining the normal function of cells. It is also involved in cell growth, differentiation, and metabolism process. PI3K initiates signaling pathways that regulate cell proliferation, differentiation, inhibition of apoptosis, and actin cytoskeletal rearrangements[28]. Furthermore, aberrant oncogenesis is mostly mediated through PI3K/AKT signaling pathway. This regulatory role involves many human tumors, such as epithelial ovarian cancer[29], prostate cancer[30], breast cancer[31], and gastric cancer[32]. SHIP1, as a key negative regulator in the PI3K/AKT signaling pathway, degrades phosphatidylinositol (3,4,5)-triphosphate (PIP3) into phosphatidylinositol (3,4)-bisphosphate (PIP2) by catalyzing phosphatidylinositol D-5 phosphate dephosphorylation[33,34], thus inhibiting signal transduction in downstream of PI3K[35]. In this study, we found a consistent result with previous studies, which Satb1 could activate PI3K/AKT signaling pathway by inhibiting SHIP1 expression.

Conclusions

We showed that knockout of Satb1 could deactivate the PI3K/AKT signaling pathway by up-regulating the expression of SHIP1, thus inhibiting cell viability, migration and promoting apoptosis of Schwann cells. Our study revealed the mechanism of Satb1 in regulating Schwann cells, which might contribute to the clinical treatment of PNI.

Acknowledgements

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Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Osbourne A, Medicine Y. Peripheral nerve injury and repair. Plast Reconstr Surg 2007; 81: 29-33.

2) Dupont-Versteegden EE, Murphy RJ, Houlé JD, Gurley CM, Peterson CA. Mechanisms leading to restoration of muscle size with exercise and transplantation after spinal cord injury. Am J Physiol Cell Physiol 2000; 279: C1677-1684.

3) Freed CR, Greene PE, Breeze RE, Tsai WY, Dumouchel W, Kao R, Dillon S, Winfield H, Culver S, Troja-

nowski JQ. Transplantation of embryonic dopamine neurons for severe Parkinson's disease. N Engl J Med 2001; 12: 710-719.

4) Cai S, Han HJ, Kohwi-Shigematsu T. Tissue-specific nuclear architecture and gene expression regulated by SATB1. Nat Genet 2003; 34: 42-51.

5) Yasui D, Miyano M, Cai S, Varga-Weisz P, Kohwi-Shigematsu T. SATB1 targets chromatin remodeling to regulate genes over long distances. Nature 2002; 419: 641-645.

6) Purbey PK, Singh S, Notani D, Kumar PP, Limaye AS, Galande S. Acetylation-dependent interaction of SATB1 and CtBP1 mediates transcriptional repression by SATB1. Mol Cell Biol 2009; 29: 1321-1337.

7) Dickinson LA, Joh T, Kohwi Y, Kohwishigematsu T. A tissue-specific MAR SAR DNA-binding protein with unusual binding site recognition. Cell 1992; 70: 631-645.

8) Alvarez JD, Yasui DH, Niida H, Joh T, Loh DY, Kohwi-shigematsu T. The MAR-binding protein SATB1 orchestrates temporal and spatial expression of multiple genes during T-cell development. Genes Dev 2000; 14: 521-535.

9) Ahlfors H, Limaye A, Elo LL, Tuomela S, Burute M, Gottimukkala KV, Notani D, Rasool O, Galande S, Lahesmaa R. SATB1 dictates expression of multiple genes including IL-5 involved in human T helper cell differentiation. Blood 2010; 116: 1443-1453.

10) Notani D, Gottimukkala KP, Jayani RS, Limaye AS, Damle MV, Mehta S, Purbey PK, Joseph J, Galande S. Global regulator SATB1 recruits β-catenin and regulates TH2 differentiation in Wnt-dependent manner. PLoS Biol 2012; 8: e1000296.

11) Tu W, Luo M, Wang Z, Yan W, Xia Y, Deng H, He J, Han P, Tian D. Upregulation of SATB1 promotes tumor growth and metastasis in liver cancer. Liver Int 2012; 32: 1064-1078.

12) Han B, Luan L, Xu Z, Wu B. Expression and biological roles of SATB1 in human bladder cancer. Tumor Biol 2013; 34: 2943-2949.

13) Yuan CL, Li L, Zhou X, Liz H, Han L. Expression of SATB1 and HER2 in gastric cancer and its clinical significance. Eur Rev Med Pharmacol Sci 2016; 20: 2256-2264.

14) Wang R, Wang ZX, Yang JS, Pan X, De W, Chen LB. MicroRNA-451 functions as a tumor suppressor in human non-small cell lung cancer by targeting ras-related protein 14 (RAB14). Oncogene 2011; 30: 2644-2658.

15) Li S, Wang X, Gu Y, Chen C, Wang Y, Liu J, Hu W, Yu B, Wang Y, Ding F. Let-7 microRNAs regenerate peripheral nerve regeneration by targeting nerve growth factor. Mol Ther 2015; 23: 423-433.

16) Navarro X, Vivó M, Valero-Cabré A. Neural plasticity after peripheral nerve injury and regeneration. Progr Neurobiol 2007; 82: 163-201.

17) Hung HA, Sun G, Keles S, Svaren J. Dynamic regulation of schwann cell enhancers after peripheral nerve injury. J Biol Chem 2015; 290: 6937.

18) Will B, Vogler TO, Bartholdy B, Garrett-Bakelman F, Mayer J, Barreyro L, Pandolfi A, Todorova TI, Okoye-Okafor UC, Stanley RF. Satb1 regulates the self-renewal of hematopoietic stem cells by promoting quiescence and repressing differentiation commitment. Nat Immunol 2013; 14: 437-445.

19) Savarese F, Dávila A, Nechanitzky R, De LR-VI, Pereira CF, Engelke R, Takahashi K, Jenuwein T, Kohwi-Shigematsu T, Fisher AG. Satb1 and Satb2 regulate embryonic stem cell differentiation and Nanog expression. Genes Dev 2009; 23: 2625-2638.

20) Meng WJ, Yan H, Zhou B, Zhang W, Kong XH, Wang R, Zhan L, Li Y, Zhou ZG, Sun XF. Correlation of SATB1 overexpression with the progression of human rectal cancer. Int J Colorectal Dis 2012; 27: 143-150.

21) Lu X, Cheng C, Zhu S, Yang Y, Zheng L, Wang G, Shu X, Wu K, Liu K, Tong Q. SATB1 is an independent prognostic marker for gastric cancer in a Chinese population. Oncol Rep 2010; 24: 981-987.

22) Han HJ, Russo J, Kohwi Y, Kohwi-Shigematsu T. SATB1 reprogrammes gene expression to promote breast tumour growth and metastasis. Nature 2008; 452: 187-193.

23) Mao L, Yang C, Wang J, Li W, Wen R, Chen J, Zheng J. SATB1 is overexpressed in metastatic prostate cancer and promotes prostate cancer cell growth and invasion. J Transl Med 2013; 11: 111.

24) Guo C, Mi J, Brautigan DL, Larner JM. ATM regulates ionizing radiation-induced disruption of HDAC1:PP1:Rb complexes. Cell Signal 2007; 19: 504-510.

25) Huang J, Ding CH, Li ZY, Zhang XB, You ZS, Zhou CQ, Xu YW. Epigenetic changes of histone deacetylation in murine oocytes matured in vitro versus in vivo. Eur Rev Med Pharmacol Sci 2017; 21: 2039-2044.

26) Boer AK, Drayer AL, Vellenga E. Effects of overexpression of the SH2-containing inositol phosphatase SHIP on proliferation and apoptosis of erythroid AS-E2 cells. Leukemia 2001; 15: 1750-1757.

27) Liu Q, Sasaki T, Kozieradzki I, Wakeham A, Itie A, Dumont DJ, Penninger JM. SHIP is a negative regulator of growth factor receptor-mediated PKB/Akt activation and myeloid cell survival. Genes Dev 1999; 13: 786.

28) Ward SG, Westwick J, Harris S. Sat-Nav for T cells: Role of PI3K isoforms and lipid phosphatases in migration of T lymphocytes. Immunol Lett 2011; 138: 15-18.

29) Uddin S, Ahmed M, Hussain A, Assad L, Al-Dayel F, Bavi P, Al-Kuraya KS, Munkarah A. Cyclooxygenase-2 inhibition inhibits PI3K/AKT kinase activity in epithelial ovarian cancer. Int J Cancer 2010; 126: 382-394.

30) Gao N, Zhang Z, Jiang BH, Shi X. Role of PI3K/AKT/mTOR signaling in the cell cycle progression of human prostate cancer. Biochem Biophys Res Commun 2003; 310: 1124.

31) Tokunaga E, Kimura Y, Mashino K, Oki E, Kataoka A, Ohno S, Morita M, Kakeji Y, Baba H, Maehara Y. Activation of PI3K/Akt signaling and hormone resistance in breast cancer. Breast Cancer 2006; 13: 137-144.

32) Geng W, Zhang HY. Research on the mechanism of HP mediated PI3K/AKT/GSK3beta pathways in gastric cancer. Eur Rev Med Pharmacol Sci 2017; 21: 33-37.

33) Lioubin MN, Algate PA, Tsai S, Carlberg K, Aebersold A, Rohrschneider LR. p150Ship, a signal transduction molecule with inositol polyphosphate-5-phosphatase activity. Genes Dev 1996; 10: 1084-1095.

34) Damen JE, Liu L, Rosten P, Humphries RK, Jefferson AB, Majerus PW, Krystal G. The 145-kDa protein induced to associate with Shc by multiple cytokines is an inositol tetraphosphate and phosphatidylinositol 3,4,5-triphosphate 5-phosphatase. Proc Natl Acad Sci U S A 1996; 93: 1689-1693.

35) Pesesse X, Moreau C, Drayer AL, Woscholski R, Parker P, Erneux C. The SH2 domain containing inositol 5-phosphatase SHIP2 displays phosphatidylinositol 3,4,5-trisphosphate and inositol 1,3,4,5-tetrakisphosphate 5-phosphatase activity. FEBS Lett 1998; 437: 301-303.