Schwann cell-like cells derived from human amniotic mesenchymal stem cells promote sciatic nerve repair through an exosome-induced SOX2/FN1 pathway *in vitro*

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Abstract. The unsatisfactory sensory function reconstruction after flap transplantation to repair tissue defects and reconstruct organs results in decreased quality of life. Schwann cells (SCs) can promote sensory function reconstruction, but the underlying mechanism is not completely understood. The aim of the present study was to explore the in-depth mechanism underlying SCs in sensory function reconstruction. Sciatic nerve transection and a repair animal model were performed to evaluate the effect of SC-like cells (iSCs) and a neurotrophin 3 (NT-3) chitosan conduit. SC RNA-seq data indicated that the SOX2/fibronectin 1 (FN1) axis promoted proliferation and migration, which are the cytological bases of nerve regeneration. Subsequently, the effects of SOX2, FN1 and exosomes secreted by iSCs on SC proliferation and migration were assessed using scratch wound and EdU assays, respectively. The RNA-seq of SCs indicated that SOX2 overexpression increased iSC viability and migration. Furthermore, SOX2 increased FN1 expression to promote nerve recovery by fibronectin fibrillogenesis. In addition, exosomes secreted by iSCs increased SC viability and migration. In conclusion, iSCs with an NT-3 chitosan conduit promoted sciatic nerve recovery via the SOX2/FN1 axis and exosomes secreted by iSCs. Therefore, the present study identified potential effective therapeutic approaches for sciatic nerve repair.

Introduction

The gold standard for peripheral nerve repair is autologous nerve grafting. Flap transplantation is commonly used to repair tissue defects and reconstruct organs, achieving a satisfying appearance repair effect (1,2). However, the sensory function reconstruction is often not ideal. Currently, there are still some issues that have not been solved. The main trunk nerve of the cutaneous flap is too short to recipient other nerves (3,4). The aim of the present study was to explore the in-depth mechanism underlying Schwann cells (SCs) in sensory function reconstruction. An established approach in neural tissue engineering involves the fabrication of polymeric scaffolds with nerve cells to produce a three-dimensional functional tissue suitable for implantation (5-7). Neurotrophin 3 (NT-3) reduces motor neuron excitability, maintains sensory and motor neuron survival, promotes nerve cell differentiation, induces axon growth and participates in nerve repair after injury (8-13). Stem cells have great potential for peripheral nerve repair (14,15), and undifferentiated adipose stromal cells have been shown to increase nerve regeneration in conduit-based peripheral nerve repair studies (14,16).

It is well known that SCs promote the repair of peripheral nerve injury (17,18). However, Schwann cells are more difficult to obtain and culture than stem cells (such as adipose stem cells and amniotic mesenchymal stem cells). Therefore, we induced stem cells to differentiate into Schwann-like cells to promote the repair of peripheral nerve injury. After peripheral nerve injury, SCs as the main body of peripheral nerve regeneration, have a critical role in the repair of peripheral nerve injury (19,20). SCs secrete various neurotrophic factors and axon-guiding factor, and maintain the stability of the surrounding microenvironment, prevent neuronal body death and promote neuronal axonal regeneration (21). In addition, SCs can synthesize and secrete extracellular matrix and cell adhesion factors (22). Moreover, SCs serve an important role in maintaining the survival of nerves, guiding the regeneration of axons, and promoting the regeneration and repair of nerves after injury. Spontaneous repair rarely occurs after central nervous system injury, whereas partial or complete repair can occur in the peripheral nervous system. The repair of peripheral nervous system injury primarily depends on the suitable microenvironment for nerve regeneration provided by SCs.

SCs directionally proliferate and migrate to the injury area, forming bands of Bungner to bridge the nerve defect

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area (19). SCs can provide a suitable microenvironment for axonal regeneration and participate in the formation of axon myelin. The proliferation of SCs is regulated by numerous factors. Neurotropic factors secreted by motor and sensory nerves bind to corresponding receptors on SCs to promote proliferation at sites of nerve damage or injury (23). Precise control of cell-matrix adhesion is necessary for cell migration. Extracellular matrix and cell adhesion molecules secreted by SCs promote axonal regeneration (24). Therefore, SCs have a critical role in maintaining peripheral nerve homeostasis targeted therapy of peripheral nerve injury. It is well known that SCs promote the repair of peripheral nerve injury. However, SCs are more difficult to obtain and culture than stem cells, such as adipose stem cells and amniotic mesenchymal stem cells. Human amnion mesenchymal cells (hAMSCs) are obtained from the extraembryonic mesoderm, expressing not only the characteristics of mesenchymal stem cells, but also characteristics of embryonic stem cells (24). hAMSCs can secrete stem cell-specific genes, including OCT4, SOX2 and NANOG, and can differentiate into multiple cell lines under certain conditions (25). Therefore, we speculated that hAMSCs could be induced into SC-like cells (iSCs) to promote nerve regeneration and functional recovery.

Chitosan is a cationic derivative of chitin after deacetylation. As a natural polymer, it is widely used in gene carrier, cell culture and tissue engineering due to its low toxicity, good biodegradability and histocompatibility (26). Further research confirmed that NT-3 chitosan has suitable histocompatibility, which is beneficial to tissue regeneration and reduces adverse reactions, such as inflammation (27). It has been reported that NT-3 chitosan provides an environment to promote nerve repair and transected spinal cord recovery in rats (28). As a biodegradable and biocompatible polymer material, hydrogel can be used as a sustained release carrier for cells or drugs to fill chitosan ducts and form an effective scaffold for axonal regeneration. Modified collagen hydrogel scaffolds can be combined with SCs to repair nerve defect (29). Hydrogel can simulate the tissue-like physical and spatial structure needed for cell growth, and has the advantages of high plasticity, a relatively simple fabrication process and convenient clinical application.

SOX2 is a member of the SRY-related HMG box family of transcription factors involved in the regulation of embryonic development. SOX2 is necessary for stem cells maintained in the nervous system. Thomson *et al* (30) reported that SOX2 is important in neural differentiation, indicating that SOX2 is a neural lineage-poised factor. SOX2 is a transcription factor essential for self-renewal in stem cells and neural progenitor cells (31). Fibronectin 1 (FN1) is a glycoprotein present in a soluble dimeric form in plasma, and in a dimeric or multimeric form at the cell surface and in the extracellular matrix. The encoded preproprotein is proteolytically processed to generate the mature protein. Fibronectin is involved in cell adhesion and migration processes, including embryogenesis, wound healing, blood coagulation, host defense and metastasis. Adachi *et al* (31) proved that SOX2 directly activated fibronectin expression in SCs, leading to an increase in fibrillogenesis and cellular huddling. Loss of fibrillogenesis leads to glial disassembly and disorganized axon regrowth (31). FN1 expression reflects fibronectin fibrillogenesis (32). SOX2 directly controls fibrillogenesis and provides a novel mechanism for the modification of the environmental architecture by glial cells during neuronal repair. Cell proliferation and migration are the cytological basis of fibronectin fibrillogenesis (33,34).

Our previous results demonstrated that iSCs derived from hAMSCs promote peripheral nerve regeneration through the miR-214/c-JUN pathway (35). The present study showed that iSCs derived from hAMSCs promote sciatic nerve repair (a classical peripheral nerve regeneration model) through an exosome-induced SOX2/FN1 pathway. In the present study, the model of sciatic nerve defect in Sprague-Dawley (SD) model rats was used to explore the process and feasibility of inducing hAMSCs into SCs. In addition, iSCs induced by collagen hydrogel combined with an NT-3 chitosan scaffold were used to repair rat nerve defect *in situ*. In the process of sciatic nerve repair, SOX2/FN1 promoted sciatic nerve repair by increasing the vitality of iSCs and promoting cell migration. iSCs are used to promote the recovery of nerve function of the skin flap and difficult wounds, which allowed investigation of its function in promoting peripheral nerve repair and regeneration. Moreover, the results of the present study provide a novel treatment strategy for the clinical promotion of peripheral nerve recovery and regeneration. The results are of clinical significance for the application of stem cells in the field of wound and nerve repair. We hope to explore the mechanism of promoting peripheral nerve repair in many aspects to promote the development of this field.

Materials and methods

Cell lines and culture. Human adipose-derived stem cells (hADSCs) were purchased from Guangzhou Saliai Stem Cell Science and Technology Co. Ltd. and human SCs were purchased from The Cell Bank of the Type Culture Collection of The Chinese Academy of Sciences. The identification method of hADSCs used were as follow: i) cell survival rate ≥80%; ii) microorganism detection (bacteria: negative, Mycoplasma: negative, endotoxin <0.25 EU/ml); iii) special human-derived virus (HIV: negative, HBV: negative, HCV: negative, TP-DNA: negative, HCMV: negative, EBV: negative); iv) cell surface markers (CD73 ≥95%, CD90 ≥95%, CD105 ≥95%, CD45 ≤2%, CD34 ≤2%, HLA-DA ≤2%, CD11b ≤2%, CD19 ≤2%); v) differentiation abilities (Oil red O staining was positive after adipogenic differentiation for 21 days; Alizarin red S staining was positive after osteogenic differentiation for 21 days). hADSCs were maintained in low glucose-DMEM (Gibco; Thermo Fisher Scientific, Inc.) containing 10% FBS (Gibco; Thermo Fisher Scientific, Inc.), 2 mM glutamine (Merck KGaA), 1% non-essential amino acids (Gibco; Thermo Fisher Scientific, Inc.), 55 mM 2-mercaptoethanol (Bio-Rad Laboratories, Inc.), 1 mM sodium pyruvate (Gibco; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin (Beyotime Institute of Biotechnology) and 100 mg/ml streptomycin (Beyotime Institute of Biotechnology). SCs were cultured in SC medium (ScienCell Research Laboratories, Inc.) with 10% FBS. Cells were cultured at 37˚C in a humidified incubator with 5% CO_2. hAMSCs were induced to differentiate into iSCs as previously described (36). Following exosome co-culture for 24 h, the cells were harvested and washed with PBS. In addition, the stem cell research underwent an EMRO process according

to the guidelines of the International Society for Stem Cell Research (37).

Sciatic nerve transection and repair animal models. A total of 24 adult male SD rats (weight, 200-250 g; age, 8-weeks) were anesthetized with pentobarbital (50 mg/kg) as previously described (38). The animals were purchased from Guizhou Laboratory Animal Engineering Technology Center. Animals were kept in an environmentally controlled breeding room (temperature: 20±2˚C; humidity: 60±5%; 12 h dark/light cycle) with sterilized food and clean water. At 1 cm above the bifurcation into the tibial and common fibular nerves, the sciatic nerve was exposed and cut using ophthalmic scissors. The incision was closed with 4-0 nylon sutures. Subsequently, ~1 cm NT-3 chitosan conduit (Guangzhou Beogene Biotech Co., Ltd.) filled with iSC hydrogel (100 μl; 5x10^7 cells) was used to promote sciatic nerve repair. The NT-3 chitosan conduit was sutured at both ends of the disconnected sciatic nerve to connect them. The *in vivo* experiment was divided into the following four experimental groups (n=6): i) normal (sham operation); ii) iSCs (sciatic nerve cut and filled with iSC hydrogel); iii) conduit (sciatic nerve cut and NT-3 chitosan conduit connection); and iv) iSCs + conduit (sciatic nerve cut and NT-3 chitosan conduit connection filled with iSC hydrogel). Sciatic function, step length and stride length (39) were measured at 4, 8 and 12 weeks of treatment. After anesthesia with pentobarbital (100 mg/kg) by intraperitoneal injection, cervical dislocation was used as the euthanasia method, and death was confirmed after complete cardiac arrest and pupil dilation. Human intervention was implemented to prevent or relieve unnecessary pain and disease if considered endpoints were met at any moment during the experiment: i) 20% body weight loss; ii) the occurrence of other complications. All experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee of Zunyi Medical Hospital [(2017)2-035].

Cell transfection. The pcDNA3.1-vector and pcDNA3.1-SOX2 were constructed and purchased from Guangzhou RiboBio Co., Ltd. Small interfering (si)RNAs were purchased from Angon Biotech Co., Ltd. The sequences of the siRNAs are as follows: si-SOX2-1, 5'-UGCAUCAUGCUGUAGCUG CCG-3'; si-SOX2-2, 5'-UGUCCAUGCGCUGGUUCA CGC-3'; si-FN1-1, 5'-ACAAACUGGAGGUUAGUGGGA-3'; si-FN1-2, 5'-UUUAUCUGAUAGUGUUUUCCA-3' and negative control (NC), 5'-UUCUCCGAACGUGUCACG UTT-3'. The cells were transfected with siRNAs or overexpression plasmids using Lipofectamine® 3000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Transfected cells were cultured at 37˚C with 5% CO_2 for 48 h.

Reverse transcription-quantitative PCR (RT-qPCR). RT-qPCR was performed as previously described (40). In brief, total RNA was extracted using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Total RNA (1 μg) was reverse transcribed into cDNA using PrimeScript™ RT Reagent kit (Takara Bio, Inc.). Subsequently, qPCR was performed in triplicate for each sample using TB Green® Fast qPCR Mix (Takara Bio, Inc.) with GAPDH as the internal reference gene. The following reaction conditions were applied: 3 min at 95˚C, 40 cycles of 15 sec at 95˚C and 30 sec at 58˚C and maintenance at 4˚C. The following primers were used: SOX2 forward, 5'-GCC GAGTGGAAACTTTTGTCG-3' and reverse, 5'-GGCAGC GTGTACTTATCCTTCT-3'; FN1 forward, 5'-CGGTGGCTG TCAGTCAAAG-3' and reverse, 5'-AAACCTCGGCTTCCT CCATAA-3'; GAPDH forward, 5'-GGAGCGAGATCCCTC CAAAAT-3' and reverse, 5'-GGCTGTTGTCATACTTCTCA TGG-3'.

Western blotting. Western blotting was performed as previously described (40). In brief, after washing with PBS, cells and tissues were lysed in RIPA lysis buffer (Beyotime Institute of Biotechnology). Protein quantification was performed using a BCA assay (Beyotime Institute of Biotechnology). Proteins were mixed with 5X Loading buffer (Beyotime Institute of Biotechnology). The following primary antibodies were used: NF160 (1:1,000; cat. no. ab254348; Abcam), GFAP (1:5,000; cat. no. ab7260; Abcam), CD31 (1:1,000; cat. no. ab9498; Abcam), SOX2 (1:1,500; cat. no. ab92494; Abcam), FN1 (1:1,000; cat. no. 15613-1-AP; ProteinTech Group, Inc.) and GAPDH (1:15,000; cat. no. 60004-1-Ig; ProteinTech Group, Inc.). The membranes were incubated with anti-rabbit IgG HRP-conjugated (1:2,000; cat. no. 7074S; Cell Signaling Technology, Inc.) and anti-mouse IgG HRP-conjugated (1:2,000; cat. no. 7076S; Cell Signaling Technology, Inc.) secondary antibodies for 2 h. Protein bands were then visualized using an ECL chemiluminescent kit (Thermo Fisher Scientific, Inc.) and semi-quantified using ImageJ v1.8 software (National Institutes of Health). The numbers under the band of western blotting were the semi-quantitative results measured using ImageJ v1.8 software.

Scratch wound assay. Cells were seeded into 6-well plates and cultured to the sub-confluent state. After starvation in serum-free SC medium for 24 h, a straight wound was scratched at the bottom of the plate with a 200-μl sterile pipette tip. After gently rinsing with PBS, cells were cultured in serum-free medium for 24 or 48 h. Cell migration was observed and calculated at 0, 24 and 48 h using an inverted light microscope. Scratch-healing was calculated as follows: Scratch healing (%)=(initial scratch area-final scratch area)/initial scratch area x100.

Transwell assay. Transwell assays were performed using Transwell chambers (Corning, Inc.). Cells were seeded (7.5x10^3) into the upper compartment in SC medium with 1% FBS, whereas the lower chamber was filled with SC medium with 10% FBS. Invading cells were fixed and calculated after 24 h.

Cell Counting Kit-8 (CCK-8) assay. Cell viability was assessed using the CCK-8 assay. Cells were seeded (5x10^3 cells/well) into a 96-well plate. After 24 h, the cells were treated. After 48 h, the cells were incubated with 100 μl SC medium containing 10% CCK-8 reagent (Dojindo Molecular Technologies, Inc.) for 1.5 h at 37˚C. Then absorbance was measured at a wavelength of 450 nm using a microplate reader (Tecan Group, Ltd.). Cell viability was calculated as follows: Cell

viability (%)=[optical density (OD) treatment-OD blank)/(OD control-OD blank) x100.

5-Ethynyl-2'-deoxyuridine (EdU) assay. Cell proliferation was determined using an EdU assay. Cells were seeded into a 24-well cell culture plate and then 10 μM EdU was added to each well. After incubation for 2 h at 37˚C, the cells were fixed with 4% paraformaldehyde for 30 min at room temperature. After washing with PBS, the incorporated EdU was detected using a EdU kit (Beijing Solarbio Science & Technology Co., Ltd.) for 30 min at room temperature. Subsequently, the cells were stained with DAPI for 5 min at room temperature and then visualized using a fluorescence microscope (Olympus Corporation).

Bioinformatics analysis. SCs with SOX2 overexpression RNA-seq data (GSE94590) were obtained from the Gene Expression Omnibus database (41). Then, the values in the Sox2 overexpression group was compared with the normal group to obtain the differentially expressed genes, respectively. The analysis was performed using limma package (http://bioinf.wehi.edu.au/limma/) in R. The threshold for significant was set at 1.2 or 0.83 fold change and P<0.05. A Venn diagram (http://bioinformatics.psb.ugent.be/webtools/Venn/) was used to identify the common differential genes in Sox2 clone1 and Sox2 clone2 group. Then, the common differential genes were used for Kyoto Encyclopedia of Genes and Genome (KEGG) analysis in the David database (https://david.ncifcrf.gov/).

Exosome isolation and identification. iSCs were cultured in iSC induction medium containing 10% exosome-depleted FBS. Exosomes were extracted using Ribo Exosome Isolation Reagent (for cell culture media) (Guangzhou RiboBio Co., Ltd.) according to the manufacturer's protocol. A Nanosight LM10 HS-BF (Nanosight Ltd.) was used to measure the size and concentration of the purified exosomes (42). CD81 and CD63 (43), as exosome markers, were used to identified the exosomes using flow cytometry. The following monoclonal antibodies were used: CD63 (1:50; cat. no. GTX41877; GeneTex, Inc.) and CD81 (1:50; cat. no. GTX34568; GeneTex, Inc.). Exosome pellets were resuspended in PBS and frozen at -80˚C. The exosome pellet was used for subsequent experiments. The exosomes were added to the medium with exosome-free FBS and then co-cultured with SCs for 24 h.

Statistical analysis. Data are presented as the mean ± SD of three independent experiments. Statistical analyses were performed using SPSS software (version 19.0; IBM Corp.). All cell experiments were independently repeated at least in triplicate. Comparisons between two groups were analyzed using an unpaired Student's t-test. One-way *ANOVA* was adopted to compare multiple groups. Post hoc multiple comparisons were made using Tukey's post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

Rat model of sciatic nerve transection and repair. The rat sciatic nerve injury model was used to simulate clinical peripheral nerve injury. hAMSCs were induced into iSCs *in vitro*. At 1 cm above the bifurcation into the tibial and common fibular nerves, the sciatic nerve was exposed and cut using ophthalmic scissors. An NT-3 chitosan conduit filled with iSC hydrogel was used to promote sciatic nerve repair (Fig. 1A). The *in vivo* experiment was divided into the following four groups: i) normal (sham operation); ii) iSCs (sciatic nerve cut and filled with iSC hydrogel); iii) conduit (sciatic nerve cut and NT-3 chitosan conduit connection); iv) iSCs+conduit (sciatic nerve cut and NT-3 chitosan conduit connection filled with iSC hydrogel). The maximum percentage of body weight loss was 16% throughout the whole experiment. To investigate the repair effect of the sciatic nerve, the gastrocnemius muscle weight was measured. As shown in Fig. 1B, the weight of the gastrocnemius muscle in the iSCs+conduit group was significantly heavier compared with that in the conduit or iSCs groups. The sciatic function index, step length and stride length data demonstrated recovery in nerve function. At 4, 8 and 12 weeks, measurements were taken. The results indicated that iSCs with the NT-3 conduit promoted sciatic nerve recovery. Compared with the iSCs or conduit groups, the iSCs+conduit group effectively promoted the repair of the sciatic nerve (Fig. 1C). The results indicated that iSCs with the NT-3 chitosan conduit promoted sciatic nerve recovery.

Analysis of the role of SOX2 in SC nerve repair. SCs with SOX2 overexpression RNA-seq data (GSE94590) were obtained from the Gene Expression Omnibus database. Differential genes identified by Venn analysis are presented in Fig. 2A. There were 501 different genes in both SOX2 clone 1 and SOX2 clone 2 SCs. The differential genes were then analyzed using Kyoto Encyclopedia of Genes and Genomes enrichment (KEGG) analysis. The results identified that SOX2 was related to 'focal adhesion process', 'PI3K-AKT signaling pathway' and 'ECM-receptor interaction' (Fig. 2B). It is critical for cell proliferation and migration. In addition, cell proliferation and migration are the cytological basis of fibronectin fibrillogenesis. SOX2 and FN1 protein expression in gastrocnemius muscle tissues was measured. The results indicated that SOX2 and FN1 levels were decreased in the iSCs or conduit groups compared with those in the normal group. However, the expression levels were increased in the iSCs+conduit group compared with those in the iSCs or conduit groups (Fig. 2C). In addition, SOX2 and FN1 protein expression levels were more abundant in iSCs compared with those in hAMSCs (Fig. 2D). These results suggest that SOX2 participates in SC proliferation and migration.

Effect of SOX2 on the migratory ability and cell viability of iSCs. SOX2 was overexpressed and knocked down using the SOX2 overexpression plasmid (pcDNA3.1-SOX2) and si-SOX2, respectively. SOX2 mRNA expression was increased following SOX2 overexpression plasmid transfection, but decreased after si-SOX2 transfection (Fig. 3A). hAMSCs were induced to differentiate into iSCs as previously described. The transfection efficiency was confirmed for subsequent experiments. Scratch wound assays were conducted to evaluate cell migration after SOX2 transfection. The distance between iSCs cells induced by stem cells is larger than that of ordinary cells, consistent with a study by Feng *et al* (44). The migration rate was significantly increased after SOX2 overexpression, but

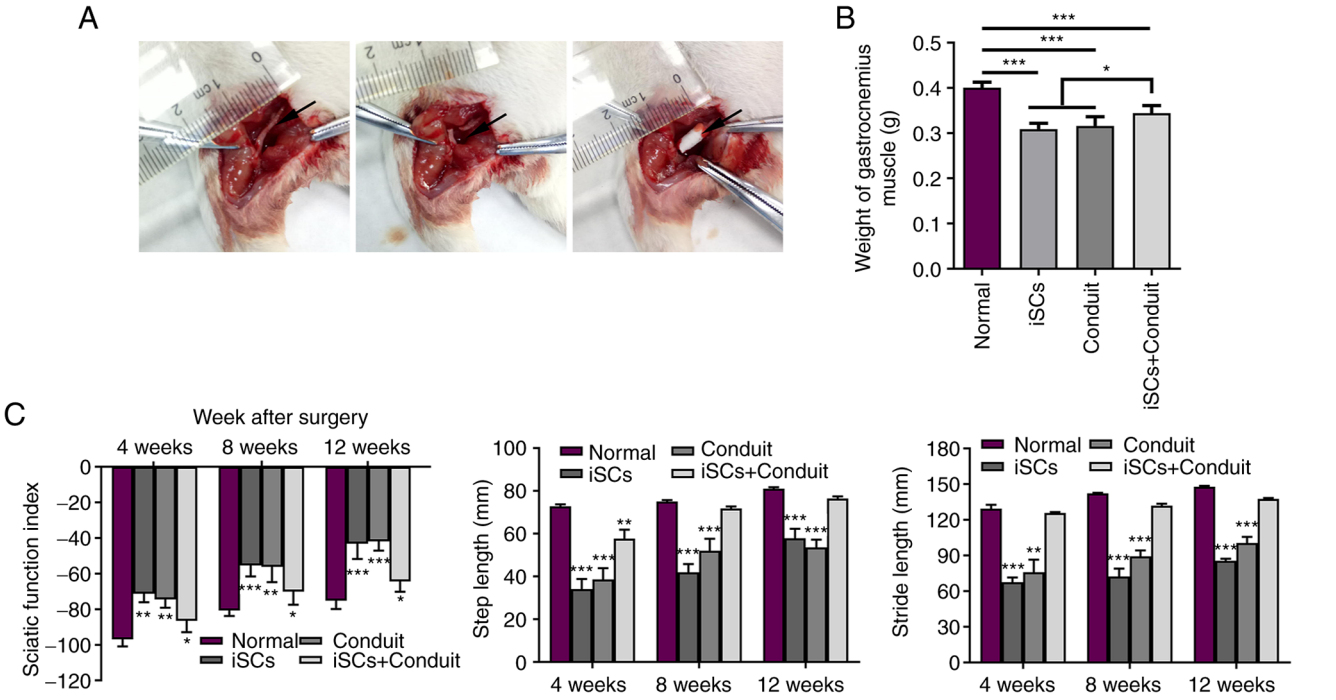

Figure 1. Rat model of sciatic nerve transection and repair. (A) Representative images of the sciatic nerve transection and repair. (B) Statistical analysis of the gastrocnemius muscle weight (g). (C) Statistical analysis of sciatic function, step length and stride length at 4, 8, and 12 w (weeks) post-surgery. Data represent the mean ± SD. *P<0.05, **P<0.01 and ***P<0.001, compared to the normal group. iSCs, Swann cell-like cells; conduit, neurotrophin 3 (NT-3) chitosan conduit.

significantly decreased after SOX2 knockdown in the iSCs (Fig. 3B). In addition, the migratory ability was analyzed using Transwell assays. After 24 h of seeding, the number of migratory SOX2-overexpression or -knockdown iSCs in the lower chamber was counted. The number of cells in the lower chamber was significantly increased with SOX2 overexpression, but significantly decreased with si-SOX2 transfection (Fig. 3C). The aforementioned results indicated that SOX2 promoted the migratory ability of iSCs. In addition to cell migration, cell proliferation was assessed to evaluate nerve repair. The CCK-8 assay was performed to evaluate the effect of SOX2 on cell viability in iSCs. The results showed that SOX2 overexpression significantly increased iSC viability (Fig. 3D). The protein expression levels of neurofilament 160 (NF-160), glial fibrillary acidic protein (GFAP) and CD31 were measured to evaluate nerve cell activity. The protein expression levels of NF-160, GFAP and CD31 were increased with SOX2 overexpression. However, the opposite trend was observed with si-SOX2 transfection (Fig. 3F). Cell proliferation was examined by performing EdU assays. EdU labelling is indicated by green fluorescence and the cell nucleus is shown in blue. The results indicated that SOX2 promoted iSC proliferation (Fig. 3E). Taken together, these results indicated that SOX2 increased the migration and proliferation of iSCs.

SOX2 upregulates FN1 expression to promote cell migration and viability in iSCs. SOX2 was overexpressed and knocked down using the SOX2 overexpression plasmid and si-SOX2 in the iSCs, respectively. FN1 mRNA and protein expression levels were increased following SOX2 overexpression, but decreased following SOX2 knockdown (Fig. 4A and B). Therefore, the results indicated that FN1 expression may be regulated by SOX2. FN1 was knocked down in SOX2-overexpressing iSCs. The protein expression levels of NF-160, GFAP and CD31 were detected using western blotting. The protein expression levels of NF-160, GFAP and CD31 were decreased after FN1 knockdown in SOX2-overexpression iSCs. The result indicated that FN1 inhibited SOX2 overexpression-mediated improvements in nerve cell viability (Fig. 4C). In addition, cell migration was measured by performing Transwell assays. The number of migratory cells was significantly increased with SOX2 overexpression, whereas the number was significantly decreased with si-SOX2 transfection. Compared with the SOX2 overexpression group, the number of migratory cells was decreased in the SOX2-overexpression iSCs with FN1 interference (Fig. 4D). Furthermore, scratch wound assays were performed to detected the migratory ability of SOX2-overexpression iSCs with FN1 interference. The results were consistent with the aforementioned results, in which FN1 interference inhibited SOX2 overexpression-mediated improvements in the migration ability of iSCs (Fig. 4E). Cell proliferation was measured by performing EdU assays under the same treatment. As presented in Fig. 4F, cell proliferation was elevated by SOX2 overexpression. However, FN1 interference decreased SOX2 overexpression-mediated improvements in iSC proliferation. Taken together, these results indicated that SOX2 upregulated FN1 expression to promote iSC migration and viability.

Exosomes secreted by iSCs promote cell migration and viability. Exosomes were isolated from iSCs using ultracentrifugation. Next, the effect of the exosomes isolated from iSCs on SC proliferation and migration was investigated. From Genecards database (https://www.genecards.org/), we know that CD81 and CD63 are located not only in the plasma membrane, but also in other organelles, such as the endosome, cytosol, and lysosome. Western blot analysis can be used to

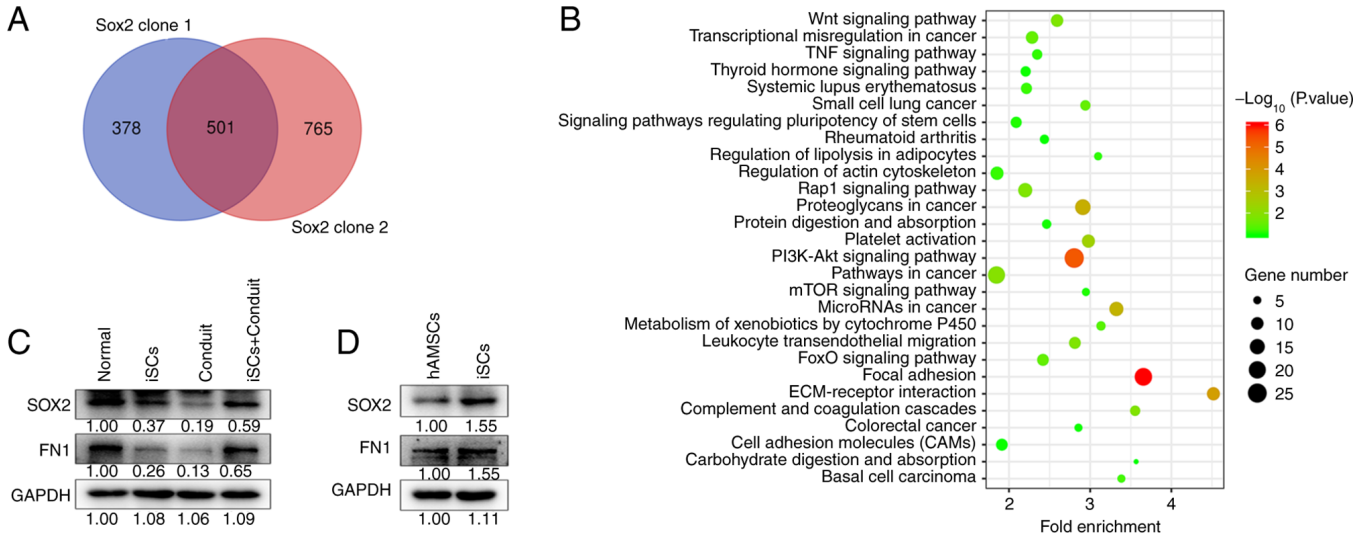

Figure 2. Analysis of the role of SOX2 in Schwann cell (SC) nerve repair. (A) Venn diagram analysis of differentially expressed genes in SOX2-positive clone 1 SCs and SOX2-positive clone 2 SCs. (B) KEGG enrichment analysis of differentially expressed genes. (C) The SOX2 and FN1 protein expression were determined in gastrocnemius muscle. (D) The SOX2 and FN1 protein expression were determined in hAMSCs and iSCs cells. FN1, fibronectin 1; hAMSCs, human amnion mesenchymal cells; iSCs, Swann cell-like cells; conduit, neurotrophin 3 (NT-3) chitosan conduit.

detect CD81/CD63 in all cells. However, flow cytometry can detect CD81/CD63 in the cell membrane to identify the type of cell. The surface markers (CD63 and CD81) of iSC exosomes were assessed using flow cytometry to confirm the successful isolation of exosomes from iSCs. The percentages of CD63 and CD81 were increased from 50% to 83.5 and 86.8%, respectively (Fig. 5A). The protein expression levels of NF-160, GFAP and CD31 were elevated in the SCs with exosome treatment or iSC co-culture (Fig. 5B). The exosome treatment group and iSC co-culture group displayed decreased wound gap widths (Fig. 5C). Taken together, the results indicated that the effect of exosomes isolated from iSCs was similar to that of iSC co-culture on cell migration. SCs cells were treated with si-SOX2 or si-NC alone with exosomes isolated from iSCs, and migration ability was measured by wound scratch assay. The functional rescue results indicated that SOX2 and exosomes isolated from iSCs promoted migration in the SCs. In some sense, SOX2 promotes SC migration via exosomes (Fig. 5D). In addition, the CCK-8 assay was performed to assess the effect of exosomes isolated from iSCs on SC viability. Exosomes secreted by iSCs significantly elevated the viability of SCs (Fig. 5E). Furthermore, cell proliferation was enhanced in SCs treated with exosomes (Fig. 5F). These results indicated that exosomes secreted by iSCs promote SC viability and migration.

Discussion

In the present study, the rat sciatic nerve injury model was established to simulate clinical peripheral nerve injury. A neurotrophin 3 (NT-3) chitosan conduit filled with Swann cell-like cell (iSC) hydrogel promoted the repair of damaged sciatic nerve *in vivo* and improved the recovery of motor function. Furthermore, SOX2 overexpression RNA-seq data were analyzed, which indicated that SOX2 participates in iSC proliferation and migration. Subsequently, SOX2 was overexpressed and knocked down in iSCs to assess the effect of SOX2 on cell viability and migration. This experiment indicated that SOX2 elevated cell viability and promoted cell migration in the iSCs. SOX2 was also found to promote the generation of fibronectin in the process of nerve repair. Fibronectin 1 (FN1) interference suppressed SOX2 overexpression-induced increases in iSC viability and migration. Exosomes were then isolated from iSCs and added to SCs. The results indicated that exosomes isolated from iSCs promoted cell viability and migration. Taken together, the results demonstrated that exosomes isolated from iSCs activated the SOX2/FN1 axis to promote SC viability and migration.

SCs are the key cells in the process of peripheral nerve regeneration (45). Fibrous biodegradable polymer conduits are an effective strategy for guided nerve regeneration. Clements *et al* (46) reported that chitosan fibers displayed 90% success in bridging a 12-mm gap in the sciatic nerve after 1 month. Mohammadi *et al* (47) reported that silicone conduit neurorrhaphy promoted nerve regeneration at the time of sciatic nerve repair. In the present study, NT-3 chitosan conduits were filled with iSC hydrogel to promote the repair of damaged sciatic nerve. Guided by NT-3 chitosan conduits, iSCs migrated directionally and promoted the repair of damage sciatic nerve.

Transcription factor SOX2 is involved in the stability of the nervous system, and is necessary for the function and maintenance of neural progenitor cells in the nervous system (48,49). Gaete *et al* (50) indicated that SOX2 was upregulated during spinal cord regeneration. Inhibition of SOX2 limited nerve regeneration in a dose-dependent manner (50). Moreover, SOX2 loss-of-function was found to impair adult neurogenesis (51,52). In the present study, SOX2 increased iSC viability and migration to promote cell regeneration after sciatic nerve injury, enhancing iSC proliferation and migration along the NT-3 chitosan conduit.

FN1 is a ubiquitous extracellular matrix glycoprotein. It has been reported that SOX2 positively regulates FN1 expression, and enhances the process of epithelial-mesenchymal transition

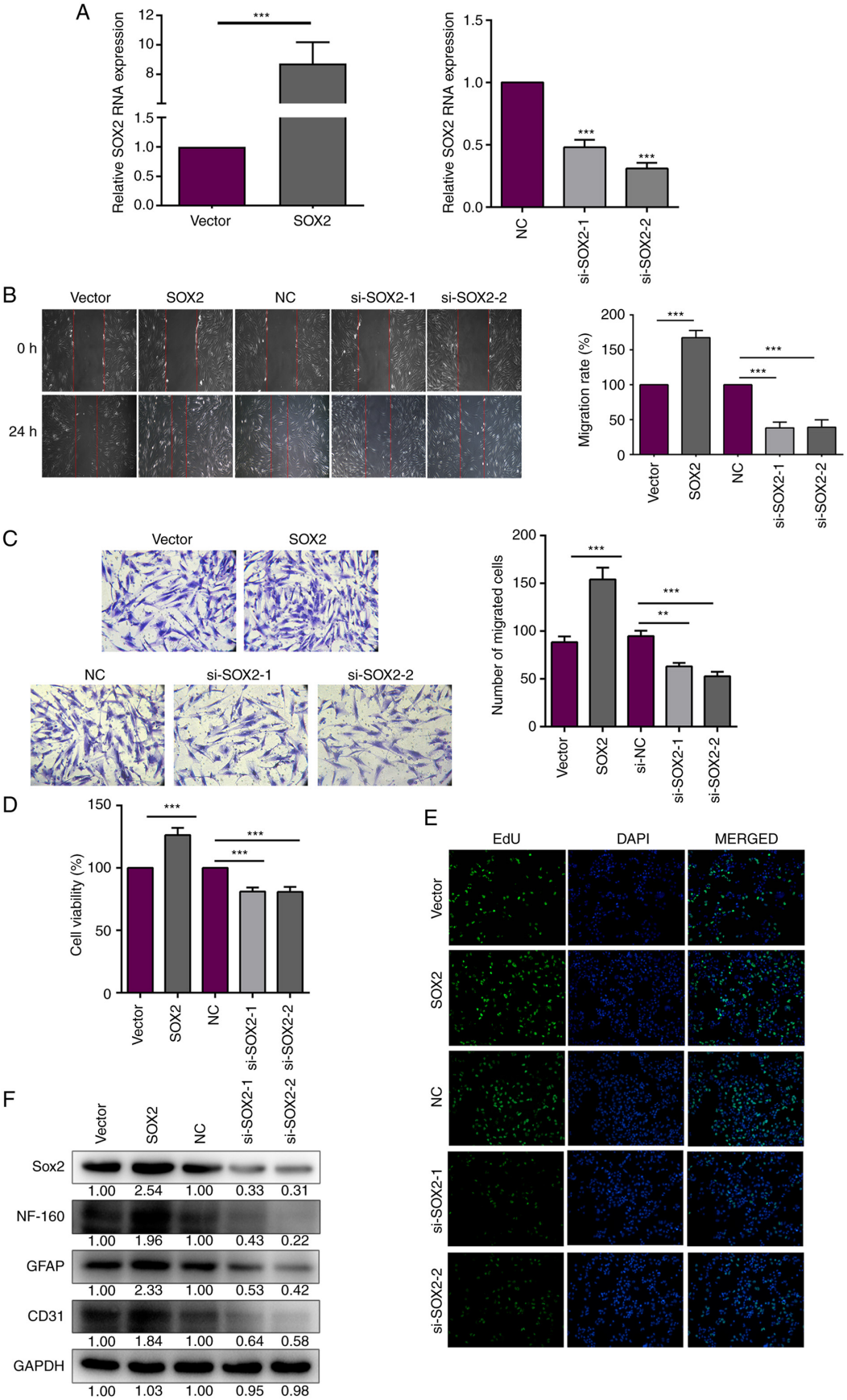

Figure 3. Effect of SOX2 on migratory ability and cell viability in the iSCs. (A) The mRNA expression of SOX2 in iSCs after SOX2 overexpression (SOX2) or silencing (si-SOX2-1 and -2). (B) Migration ability was measured by wound scratch assay in iSCs after SOX2 overexpression or silencing. (C) Migration ability was measured by Transwell assay in iSCs after SOX2 overexpression or silencing. (D) Cell viability was determined by CCK-8 assay in iSCs after SOX2 overexpression or silencing. (E) Cell proliferation was determined by EdU staining in iSCs after SOX2 overexpression or silencing. (F) The SOX2, NF-160, GFAP and CD31 protein levels were determined in iSCs with SOX2 overexpression or silencing by western blot analysis. All experiments were conducted three times, and data represent the mean ± SD. **P<0.01, ***P<0.001. iSCs, Swann cell-like cells; NF-160, neurofilament 160; GFAP, glial fibrillary acidic protein.

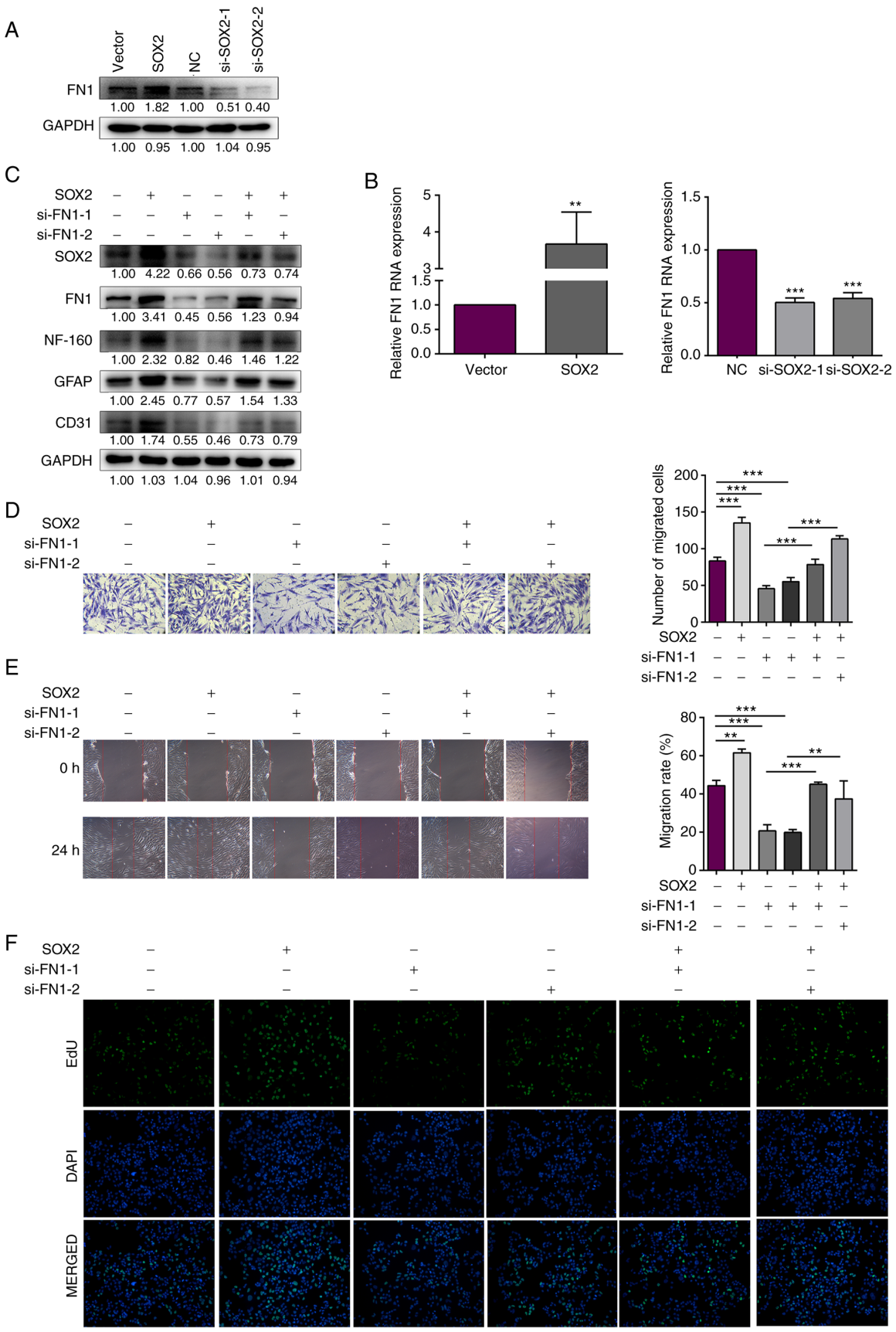

Figure 4. SOX2 upregulates FN1 expression to promote the cell migration and viability ability in iSCs. (A) The FN1 protein expression level was detected in iSCs with SOX2 overexpression (SOX2) or silencing (si-SOX2-1 and -2). (B) The FN1 mRNA expression level was detected in iSCs with SOX2 overexpression or silencing. (C) iSCs were transfected with SOX2 overexpression plasmid or pcDNA3.1 plasmid alone with si-FN1. NF-160, GFAP and CD31 protein expression levels were determined by western blot analysis. (D and E) iSCs were transfected with SOX2 overexpression plasmid or pcDNA3.1 plasmid alone with si-FN1, and cell migration ability were measured by Transwell and wound scratch assay. (F) iSCs were transfected with SOX2 overexpression plasmid or pcDNA3.1 plasmid alone with si-FN1, and cell proliferation and viability ability were measured by EdU assay. All experiments were conducted three times, and data represent the mean ± SD. **P<0.01 and ***P<0.001. FN1, fibronectin 1; iSCs, Swann cell-like cells; NF-160, neurofilament 160; GFAP, glial fibrillary acidic protein.

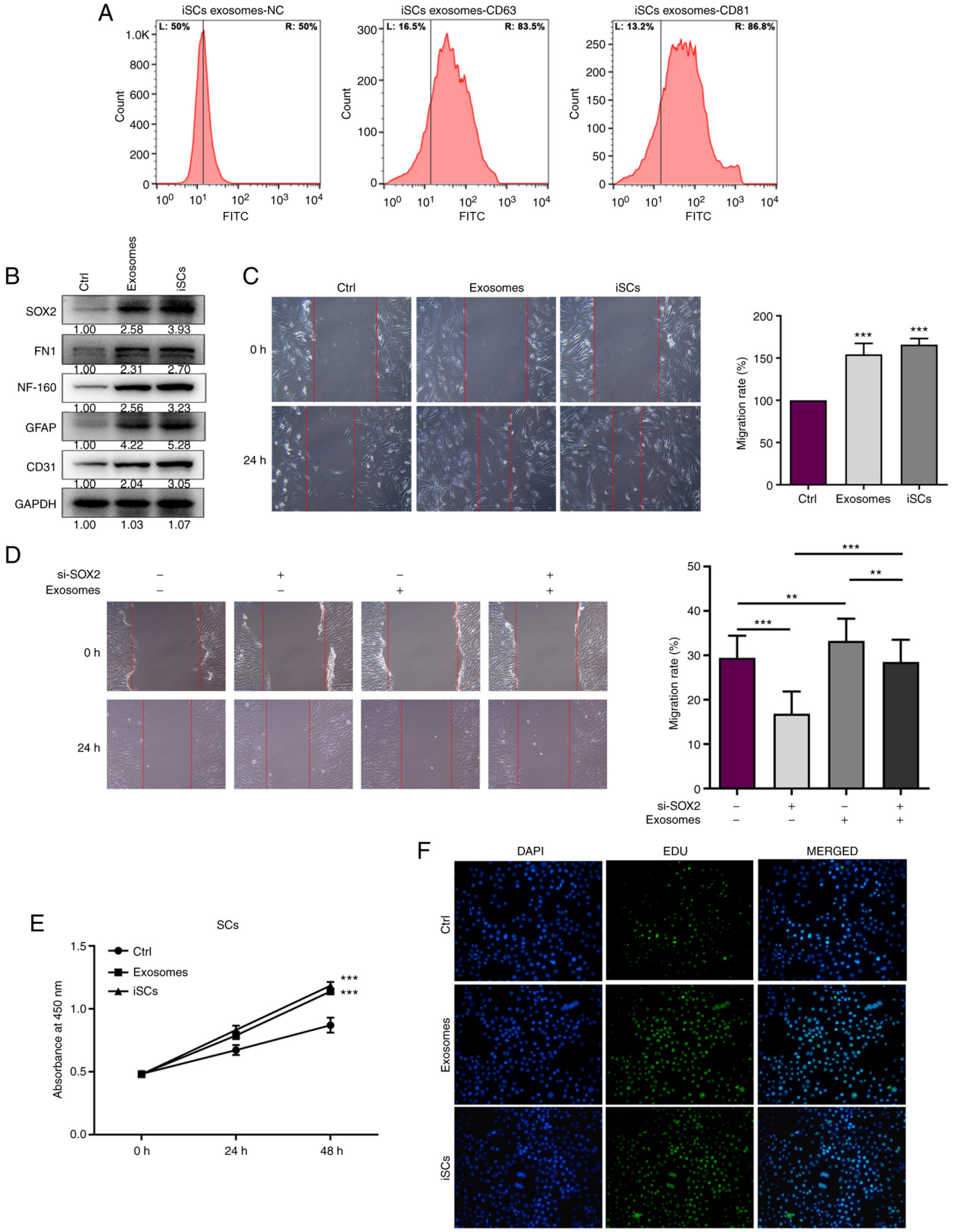

Figure 5. The exosomes secreted by iSCs promote the cell migration and viability ability in Schwann cells (SCs). (A) Flow cytometry of surface markers (CD63 and CD81) on iSC-exosomes. (B) The SOX2, FN1, NF-160, GFAP and CD31 protein expression levels were measured in SCs treated with exosomes secreted by iSCs or iSC co-culture for 48 h. (C) Migration ability was measured by wound scratch Transwell assay in SCs treated with exosomes secreted by iSCs or iSC co-culture for 48 h. (D) SCs were treated with si-SOX2 or si-NC alone with exosomes isolated from iSCs, and the functional rescue assay was used to detect the migration ability. (E) Cell viability was determined by CCK-8 assay in SCs treated with exosomes secreted by iSCs for 48 h. (F) Cell proliferation was determined by EdU staining in SCs treated with exosomes secreted by iSCs for 48 h. All experiments were conducted three times, and data represent the mean ± SD. **P<0.01, ***P<0.001, compared to the control (Ctrl) or as indicated with brackets. iSCs, Swann cell-like cells; FN1, fibronectin 1; NF-160, neurofilament 160; GFAP, glial fibrillary acidic protein.

and self-renewal capacity (53). SOX2-induced fibrillogenesis is involved in the directional collective migration of SCs (41). FN1 is the key molecule involved in integrin-mediated cell extracellular matrix adhesion, which is required for SC migration and axonal regrowth (54,55). SOX2 overexpression resulted in increased FN1 expression, which promoted iSC proliferation and migration, suggesting that sciatic nerve repair was accelerated via the SOX2/FN1 axis. However, the absence of images showing the morphology of the damaged sciatic nerve before and after repair is a limitation of the study. In the follow-up study, we will confirm the repair effect of the SOX2/FN1 axis and exosomes on sciatic nerve by *in vivo* experiments.

In addition, exosomes are important in the repair of sciatic nerve and are used for the treatment of diseases, particularly in regenerative medicine. Exosomes promote nerve cell proliferation and migration in the repair process. Xin *et al* (56) reported that exosome-mediated transfer of miR-133b from multipotent mesenchymal stromal cells to neural cells contributes to neurite outgrowth. Exosomes from iSCs were found to reduce the apoptosis and promote the proliferation of SCs in peripheral nerve injury (57). In the present study, exosomes were isolated from iSCs and co-cultured with SCs, which resulted in increased cell migration and viability.

Taken together, the present study demonstrated that the NT-3 chitosan conduit filled with iSC hydrogel promoted the repair of the damaged sciatic nerve *in vivo*. SOX2 participated in iSC proliferation and migration, increasing the generation of fibronectin in the process of nerve repair. FN1 interference suppressed SOX2 overexpression-mediated increases in iSC viability and migration. Furthermore, exosomes isolated from iSCs promoted SC viability and migration. In conclusion, the present study demonstrated that the SOX2/FN1 axis and exosomes isolated from iSCs promoted sciatic nerve repair using an NT-3 chitosan conduit filled with iSC hydrogel. Therefore, the present study may be used to identify potentially effective therapeutic approaches for sciatic nerve repair.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ZW conceived and designed the study, wrote-review and editing, provided funding support. WC conceived and designed the study, performed the experiments, processed the data and wrote the paper. SC and CY performed the experiments and processed the data. JZ, HZ and KN contributed to the study design, processed the data, and reviewed and edited the drafts of the paper. All authors provided help during the research. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of the data and any other part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

The experimental procedures involving the sciatic nerve transection and repair animal models were approved by the Institutional Animal Care and Use Committee of Zunyi Medical Hospital [(2017) 2-035] (China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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