Stem Cells Translational Medicine, 2025, **14**, szaf005
https://doi.org/10.1093/stcltm/szaf005
Advance access publication 11 April 2025
Original Research

Reprogramming of skin fibroblasts by 3D spheroid culture promotes peripheral nerve regeneration via the ID3/semaphorin7a pathway

Xu Tan[1,†], Zhou Zhang[2,†], Xiaohui Cao[1,†], Langfan Qu[1], Yinchun Xiong[1], Huijuan Li[1], Yu Wang[1,*], Zelin Chen[1,*], Chunmeng Shi[1,*]

[1]Institute of Rocket Force Medicine, State Key Laboratory of Trauma and Chemical Poisoning, College of Preventive Medicine, Army Medical University, Chongqing 400038, People's Republic of China,
[2]Department of Ophthalmology, The Affiliated Hospital of Guizhou Medical University, Guizhou 550004, People's Republic of China.

[*]Corresponding authors. Chunmeng Shi, PhD, Institute of Rocket Force Medicine, State Key Laboratory of Trauma and Chemical Poisoning, College of Preventive Medicine, Army Medical University, Chongqing 400038, People's Republic of China. E-mail: shicm@tmmu.edu.cn, Zelin Chen, PhD, Institute of Rocket Force Medicine, State Key Laboratory of Trauma and Chemical Poisoning, College of Preventive Medicine, Army Medical University, Chongqing 400038, People's Republic of China. E-mail: czlwx1811@163.com; Yu Wang, PhD, Institute of Rocket Force Medicine, State Key Laboratory of Trauma and Chemical Poisoning, College of Preventive Medicine, Army Medical University, Chongqing, 400038, People's Republic of China. E-mail: wangyusmmu@163.com.
[†]These authors contributed equally to this work.

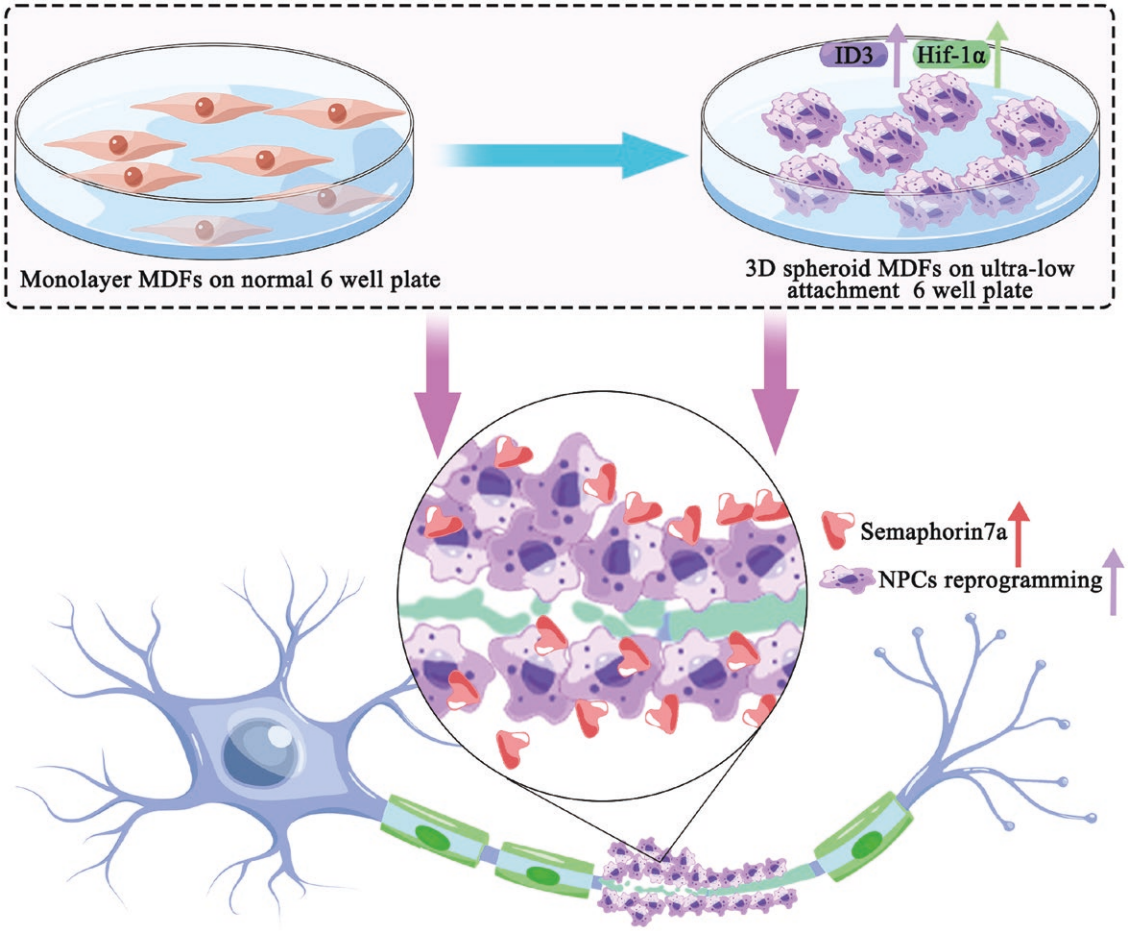
Abstract

Peripheral nerve injury remains an intractable clinical issue with high morbidity, causing an excessive burden on the economy and society. Peripheral nerve tissue engineering combined with nerve conduits and supporting seed cells is considered a promising strategy for treating of long nerve defects. However, supporting seed cell sources that are easily accessible, capable of rapid expansion, and do not require genetic intervention are still urgently needed. This study intended to clarify whether the easily accessible and rapid expansion skin fibroblasts are the ideal supporting seed cells and can be reprogrammed into neural progenitor-like cells (NPCs) by forcing them to grow into a three-dimensional (3D) spheroid morphology. Results showed that 3D spheroid mouse dermal fibroblasts (MDFs) exhibited neural cell-like properties and could efficiently induce dorsal root ganglion neurons to extend the neurites. Transplantation of 3D spheroid MDFs significantly accelerated the regeneration of the sciatic nerve and improved the motor function of rats after transection compared to monolayer MDFs. Mechanism studies revealed that 3D spheroid culture significantly upregulated the expressions of the inhibitor of DNA binding 3 (ID3) and the hypoxia-inducible factor-1α (HIF-1α). The upregulation of the inhibitor of DNA binding 3 in 3D spheroid MDFs plays a critical role in acquiring NPC properties. Meanwhile, the upregulated ID3 and HIF-1α could synergistically upregulate semaphorin7a expression, which finally improved the extending of nerve axon in vitro and in vivo. This study may shed new light on treatments for peripheral nerve injury.

Key words: peripheral nerve injury; fibroblasts; 3D spheroid; reprogram; semaphorin7a.

Received: 7 October 2024; Accepted: 31 January 2025.

Graphical Abstract



Significance statement

This study demonstrated a highly efficient and feasible 3D culture approach to reprogram skin fibroblasts into a neural cell-like state via the upregulation of ID3, which further coordinates with the upregulated HIF-1α to increase the expression of the axonal guidance protein semaphorin7a. Transplantation of neural cell-like fibroblasts reprogrammed by 3D culture significantly promoted the regeneration of transection injured sciatic nerve. The results of this study proved a favorable approach to supply sufficient cell sources for peripheral nerve tissue engineering and might aid the clinical challenge of treating severe peripheral nerve injury and promoting peripheral nerve regeneration.

Introduction

Peripheral nerve injury remains an intractable clinical issue with high morbidity, causing an excessive burden on the economy and society.[1-3] Patients with long nerve defects are often subject to functional loss, such as impaired motor, sensory, and autonomic nerve functions, which require of surgical treatments.[1] Autologous nerve grafting has long been recognized as the golden standard for treating severe peripheral nerve injury, however, it encounters donor nerve sacrifice and nerve mismatch.[4,5] Among the current potential treatments, peripheral nerve tissue engineering combined with nerve guidance materials and nerve supporting cells is considered a promising strategy.[6-9] Schwann cells (SCs) and numerous stem cells have been used as supporting cells to increase the efficacy of engineered nerve grafts and significantly boost peripheral nerve regeneration.[10-13] SCs have been considered the ideal supporting cells with the multifaceted properties of nerve regeneration[14-16]; however, their use will be limited by the surgical need to collect autologous SCs and the difficulty in culturing and expanding SCs to a sufficient amount.[17-19] Stem cells with strong expansion and differentiation capacity exhibit great clinical potential as supporting cells. Nevertheless, there are still some defects. For example, the invasive and painful procedure for autologous harvesting for bone marrow-derived stem cells,[10] the differentiation potential toward adipocytes for adipose-derived stem cells,[20] the difficulty in collecting and high rate of neuroblastoma formation for neural stem cells,[21,22] the potential of teratoma formation and ethical dilemma for embryonic stem cells,[1,23] and the epigenetic memory from original somatic cells, chromosomal aberrations, as well as tumorigenicity for induced pluripotential stem cells.[24,25] Therefore, it is a high priority to develop ideal supporting seed cells in peripheral nerve regeneration research.

Fibroblasts are another main cell type, except for SCs, within the peripheral nerve microenvironment.[26] However, their roles in nerve regeneration have not been extensively studied. Previous studies have demonstrated that fibroblasts could promote nerve regeneration in various ways. Fibroblasts could clear the debris after nerve injury, contributing to the formation of bands of Büngner both by physically interacting with SCs and producing extracellular matrix (ECM) to initiate the regenerative response.[27] More recently, researchers have reported that cocultured adult dorsal root ganglion (DRG) neurons with nerve-derived fibroblasts could promote their neurite outgrowth in-vitro.[28] Furthermore, co-transplantation of nerve fibroblasts and SCs efficiently enhanced peripheral nerve repair.[2] However, transplantation with nerve-derived fibroblasts will also face drawbacks similar to those of SCs.

Skin fibroblasts are easily accessible, capable of rapid expansion in culture, and amenable to stable differentiation or transdifferentiation, which may serve as an optimal alternative supporting seed cells for nerve regeneration. Numerous studies have reported that the overexpression of different combinations of neural lineage-specific transcription factors could reprogram fibroblasts into functional neurons,[29-31] neural progenitor-like cells (NPCs)[32,33] or SCs.[34] Although the above gene edited skin fibroblast cells possess favorable effects to facilitate peripheral nerve repair and regeneration, they cannot bypass the tumorigenic risk brought about by the exogenous introduction or overexpression of relative functional genes. Except for the transgenic approaches, manipulating the cell microenvironment by external signal stimulation could also manipulate the cell fate with minimal genetic intervention.[35,36] In our previous study,[37] we have demonstrated that the skin wound microenvironment could activate local fibroblasts, which exhibit upregulated expression of inhibitor of DNA binding 1 (ID1)/ID3 and could tightly contact with local injured nerves during wound healing. Moreover, ID1/ID3 upregulated fibroblast cells displayed neural cell-like properties. Most importantly, transplantation of ID1/ID3-overexpressing fibroblasts significantly improved nerve regeneration in mouse skin wound and rat sciatic nerve transection models. Could skin fibroblasts be easily induced to upregulate ID1/ID3 without wounding or genetic intervention? It was widely recognized that force stem cells in spheroid or colony morphology can maintain their stemness.[38-40] Djabali et al revealed that force fibroblasts grow into three-dimensional (3D) spheroids in the skin-derived precursor (SKP) medium by shaking the flask vigorously daily to prevent cells to from adhering to the bottom of the flask could efficiently reprogram fibroblasts into multipotent SKPs showing Nestin, TG30, Oct4, and Nanog upregulation.[41] However, whether the 3D culture approach could induce skin fibroblasts to upregulate ID1/ID3 and promote their nerve regenerative properties is still largely unknown.

Based on the above investigations, this study intended to expand neonatal mouse skin dermal fibroblasts (MDFs) in 3D spheroid morphology and assess their effect on the facilitation of peripheral nerve regeneration in this study. In vitro and in vivo studies demonstrated that 3D spheroid MDFs could significantly promote the axon extension and sciatic nerve regeneration. Mechanism studies found that 3D spheroid MDFs remarkably upregulate the expression of ID3, hypoxia-inducible factor-1α (HIF-1α) and semaphorin7a, which belongs to the semaphorin family, playing an essential role in guiding growing axons and controlling the plasticity of synaptic connections.[42,43] In summary, these results demonstrated a simple and feasible 3D culture approach to reprogram skin fibroblasts to exhibit neural cell-like properties and simultaneously upregulate semaphorin7a expression, which finally improves the extension of nerve axons both in vitro and in vivo. This study may shed new light on treatments for peripheral nerve injury.

Materials and methods

Cell preparation

Mouse dermal fibroblasts cell preparation

The isolation of neonatal mouse dermal fibroblasts (MDFs) was described previously.[44] In brief, after being washed with 75% ethanol and PBS, the dorsal skin tissues from neonatal C57BL/6 mouse were collected. Then the subcutaneous tissues were carefully removed and the remaining parts were cut into 1-2 cm^2 pieces with scissors. Then, they were digested overnight at 4°C with a digestion medium containing 1 mg/mL dispase (Roche). The next day, following epidermal stripping, the dermis was cut up and further digested with 0.25% collagenase 1 (Worthington) at 37°C for 1 hour with shaking. Finally, the digested cells were then passed through a 75μm cell strainer, centrifuged, and resuspended in DMEM with 10% fetal bovine serum (Hyclone) and 1% streptomycin/penicillin (Hyclone) for the subsequent culture. For 3D spheroid culture, 6×10^6 MDFs cells at third passage were seeded to a 6-well ultra-low attachment plate purchased from STEMCELL Technologies without changing the culture media and cultured at 37°C with 5% CO_2. All 3D cultured MDFs used in the present study were at 48h following suspension.

Dorsal root ganglion neuron preparation

As described previously,[28] dorsal root ganglion neurons were prepared from 10-week-old Sprague-Dawley (SD) rats. In brief, bilateral lumbar DRGs were dissected, cut into 1–2 mm pieces with scissor and incubated in the digestion medium consisting of DMEM/Ham's F-12 with 1% collagenase XI (Sigma-Aldrich) for 45min at 37°C. After terminating the digestion with DMEM supplied with 10% FBS, the tissue pieces were resuspended in neuron culture medium consisting of Neurobasal™ Medium (12348017, Gibco) supplemented with 2% B27 (12587010, Gibco), L-glutamine (2mM, Gibco) and 1% P/S. The tissue pieces were gently triturated with a 1 ml pipette to acquire single-cell suspension of DRG neurons and then were inoculated to a 6-well dish for the subsequent culture.

Co-culture the DRG neurons with fibroblasts

1×10^4 monolayer or spheroid mouse fibroblast cells were seeded into 24-well plates with slides precoated with 0.1% gelatin in fibroblast culture media. 24 hours later, the fibroblast culture medium was removed, and 5×10^3 DRG neurons per well were seeded into the 24 well plates with the neuron culture medium described above. 48 hours later, the co-culture cells were fixed with 4% polyoxymethylene, and then stained with Tuj1 and detected with fluorescent microscope. For detecting the effects of Semaphorin7a siRNA inhibiting the enhancement of 3D spheroid MDFs to the axon regrowth, the control or Semaphorin7a siRNA were administrated to the monolayer MDFs 48 hours before 3D spheroid culture. For detecting the effects of Anti-β1 integrin receptor antibodies inhibiting the enhancement of 3D spheroid MDFs to the axon

regrowth, DRG neurons were pretreated with Anti-β1 antibody prior to coculturing with 3D spheroid MDFs. 48 hours later, 1×10^4 3D spheroid MDFs collecting from 3D cultured monolayer MDFs subjected with small interferring RNAs (siRNAs) interference treating were seeded into 24-well plates with slides coated by 0.1% gelatin. 24 hours later, 5×10^3 DRG neurons per well were seeded into the 24 well plates with the neuron culture medium described above. The following staining protocols were the same as above.

Sciatic nerve injury models and cell transplantation

SD rats (8-10 weeks old) were used to establish sciatic nerve injury models as refer to the previous study.[34] Briefly, SD rats were anesthetized with 1% pentobarbital (30 mg/kg), and a longitudinal incision was made at the lower limb of right side. A 5 mm sciatic nerve defect gap was created, and the proximal and distal termini were bridged using a silicon conduit. All the sciatic rats were randomly divided into 3 groups: control group, monolayer MDFs transplantation group and 3D spheroid MDFs transplantation group, and then injected with 50ul PBS alone, 1×10^6 monolayer MDFs or 1×10^6 3D spheroid MDFs dissociated into single-cell state prestaining with Dil (C7001, Invitrogen) suspended in the same volume of PBS into the conduits, respectively. Sham operated rats were also constructed, and they were received the same surgical procedure, however, without the creation of the nerve defect and cells transplantation. Six weeks later, the feet prints were recorded and were using for calculating the sciatic function index (SFI) values as referring our previous study.[37] The gross morphological of feet, gastrocnemius muscles and the sciatic nerves were collected, and additionally the gastrocnemius muscles and the sciatic nerves were harvested for further histological evaluation.

RNA sequencing and quantitative RT-PCR testing

Total RNA of monolayer or 3D spheroid MDFs was isolated with RNAiso Plus reagent (TaKaRa). RNA sequencing of monolayer and 3D spheroid MDFs were delegated to Shanghai Majorbio Bio-pharm Biotechnology Co., Ltd.. RT-PCR testing was performed with a SYBR Premix Ex TaqII (TliRnaseH Plus) real-time PCR kit (TaKaRa) according to the manufacturer's protocol. The relative expression of objective genes was normalized to the expression of Gapdh or β-actin. The primers sequences are presented in Supplementary Table S1.

Western blot

Total protein was extracted from monolayer or 3D spheroid MDFs using RIPA buffer (Beyotime). Proteins were separated on SDS-PAGE and transferred to PVDF membranes (Millipore) after measuring their concentrations with Protein Assay against BCA standards. Then membranes were blocked in blocking buffer (Beyotime) for 45 minutes and incubated with different primary antibodies (1:1000) at 4°C overnight, the primary antibodies were presented as follows: rabbit anti-ID3 (ab41834, Abcam), rabbit anti-Nestin (ab22035, Abcam), rabbit anti-α-SMA (ab124964, Abcam), rabbit anti-Anti-Collagen 1A1 (ab260043, Abcam), mouse anti-β-actin (AF5001, Beyotime), rabbit anti-Semaphorin7a (ab308254, Abcam). After washing with TBST buffer, membranes were incubated with HRP-conjugated goat anti-mouse or anti-rabbit secondary antibody (1:2000; Beyotime) for 2 hours at room temperature and finally visualized using chemiluminescence detection system (Bio-Rad Laboratories).

RNA Interference

Knockdown of Semaphorin7a or ID3 were administrated with Semaphorin 7a siRNA or ID3 siRNA, respectively (Gene-Pharma). The sequences of siRNA were presented below: mouse Semaphorin7a siRNA (5′-3′): S, GCUGCAUCCUGUCAUUGATT; AS, UCAAUGAACAGGAUGCAGCTT. Mouse ID3 siRNA (5′-3′): S, UUAGCCUCUUGGACGACAUTT; AS, AUGUCGUCCAAGAGGCUAATT. siRNA transfection was conducted by using Lipofectamine 3000 (L3000008, Invitrogen) according to the manufactures' protocol.

Immunofluorescence

For Immunofluorescence staining, monolayer or 3D spheroid MDFs pre-seeded onto slides were fixed with 4% paraformaldehyde for 15 minutes. After washing with PBS, cells were permeabilized with 0.25% TritonX-100 for 30 minutes, and then were incubated with blocking buffer containing 10% normal goat serum and 0.3% Triton X-100 for another 30 minutes at room temperature. Next, cells were incubated overnight at 4°C with different primary antibodies including rabbit anti-Sox2 (1:100, ab97959, Abcam), rabbit anti-Gfap (1:100, 16825-1-AP, Proteintech), rabbit anti-Nestin (1:100, ab22035, Abcam), rabbit anti-Tuj1 (1:250, ab18207, Abcam), rabbit anti-S100β (1:100, ab52642, Abcam), rabbit anti-ID3 (ab41834, Abcam), rabbit anti-Semaphorin7a (1:200, ab308254, Abcam), rabbit anti- Hif-1α (1:100, ab216842, Abcam). The next day, secondary antibodies included Alexa Fluor-647–conjugated goat anti-rabbit IgG (1:300, WL333739, Invitrogen) and Alexa Fluor 555 goat anti-rabbit IgG (1:300, YF374178, Invitrogen) were added to cells after 3 times PBS washing. Finally, slides were sealed with anti-fluorescence quenching reagent containing DAPI (P0131, Beyotime), and detected with Leica confocal microscope.

Histology

To detected the transplanted Dil labeled fibroblasts, the regenerated sciatic nerve was processed as cryosections (10 μm) for Tuj1 immunofluorescence staining, the specific staining protocols were the same as the above cell climbing slices staining. Dewaxed paraffin sections of regenerated sciatic nerve or innervated gastrocnemius muscles were undergone H&E staining to test their histological changes. Regenerative sciatic nerves in all groups were harvested 6 weeks later and were undergone transmission electron microscopy detection. The area of myelinated axons and the thickness of the myelin sheath were measured by the Image J software. The specific protocols of H&E staining and transmission electron microscopy detection refer to our previous study.[37]

Statistical analysis

Statistical analysis was conducted using SPSS 13.0 software. Data were expressed as the mean ± SD. Comparisons of multiple groups were performed using a one-way analysis of variance. $P < 0.05$ was considered to be statistically significant.

Results

NPC properties of 3D spheroid MDFs through low-attachment culture

To force cells to acquire 3D spheroid morphology, MDFs at the third passage were cultured on a 6-well ultra-low attachment plate (purchased from STEMCELL Technologies) in DMEM

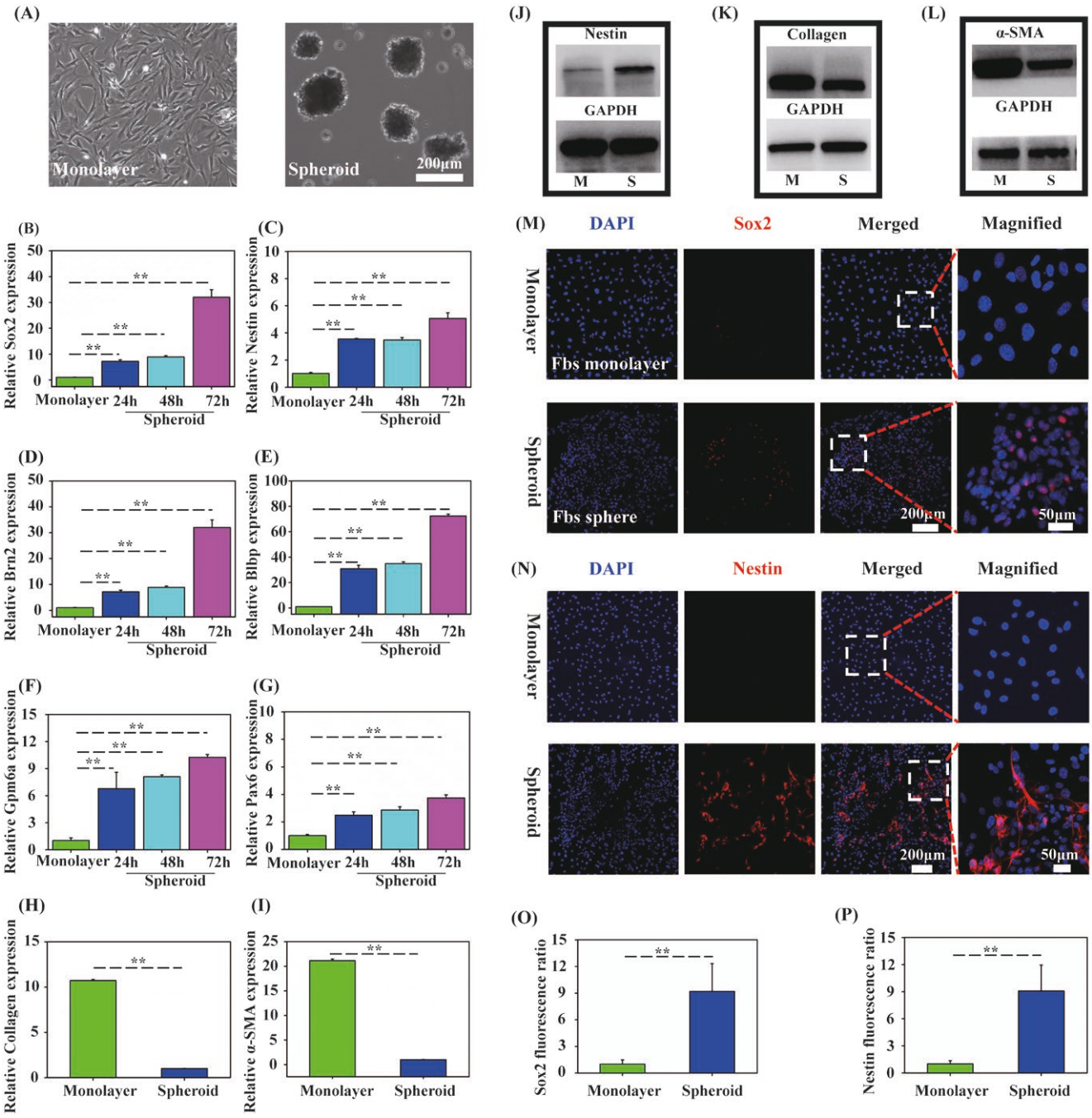


Figure 1. Characterization of 3D spheroid MDFs through low attachment culture. (A), Phase-contrast images of monolayer MDFs and 3D spheroid MDFs. (B-G), RT-PCR testing for NPC-specific genes including Sox2, Nestin, Brn2, Blbp, Gpm6a and Pax6 in monolayer MDFs and 3D spheroid MDFs ($n = 3$). (H, I), RT-PCR testing for Collagen 1A1 and α-SMA genes in monolayer MDFs and 3D spheroid MDFs ($n = 3$). (J-L), Western blotting for Nestin, Collagen 1A1 and α-SMA in monolayer MDFs and 3D spheroid MDFs. Immunostaining (M, N) and the corresponding fluorescence intensity quantification (O, P) ($n = 6$) of Sox2 and Nestin of monolayer MDFs and 3D spheroid MDFs. Data are mean ± SD. $^{**}P < 0.01$. M means monolayer MDFs; S means spheroid MDFs.

expansion medium supplemented with 10% FBS and 1% penicillin/streptomycin without further adding of other supplements. MDFs formed a 3D spheroid in low attachment dish within 24 hours (Figure 1A), and maintained the morphology for at least 72 hours as our longest observation time point. Because spheroid or colony morphology has been reported to help maintain the stemness of stem cells.[38-40] This study intended to test whether maintaining 3D spheroid morphology could reendow MDFs with the characteristics of neural progenitor cells (NPCs). Gene expression results showed that NPC-related genes, including Sox2, Nestin, Brn2, Blbp, Gpm6a, and Pax6, were significantly upregulated in 3D spheroid MDFs (Figure 1B-G), whereas mesoderm genes, such as collagen 1A1 and α-SMA, were remarkably downregulated (Figure 1H and I). Further WB testing (Figure 1J-L), immunofluorescence staining (Figure 1M and N) and the corresponding fluorescence intensity quantification (Figure 1O and P) revealed that Nestin and Sox2 expression of 3D spheroid MDFs were increased; however, collagen

1A1 and α-SMA expression decreased compared to monolayer MDFs. Moreover, the transcription and expression of the SC marker S100β (Supplementary Figure S1A-C) and the astrocyte marker Gfap (Supplementary Figure S1D-F) were obviously upregulated in 3D spheroid MDFs. These results suggested that 3D spheroid MDFs may acquire NPC properties and lose mesoderm properties.

ID3 regulates the NPC properties of 3D spheroid MDFs

ID proteins have been found to play important roles in neurogenesis[45-47] and stem cell maintenance,[48-50] and could enhance cell reprogramming.[51,52] The roles of ID proteins in the spheroid MDFs were further tested. RNA sequencing of monolayer MDFs and 3D spheroid MDFs showed that ID proteins (ID1/ID2/ID3) were significantly upregulated in 3D spheroid MDFs. Among them, ID3 showed the highest expression (Supplementary Table S2). It was further verified that ID3 expression was remarkably upregulated in 3D spheroid MDFs by RT-PCR (Figure 2A), WB (Figure 2B), immunofluorescence staining (Figure 2C) and the corresponding fluorescence intensity quantification (Figure 2E). Our previous study showed that overexpressed ID1 or ID3 in skin dermal fibroblasts significantly increased the proliferation and migration of fibroblasts and reprogrammed fibroblasts into a neural cell-like state. Thus, in this study, MDFs were pretreated with siRNAs targeting ID3 before the 3D spheroid culture to investigate the effect of ID3 on the NPC properties accumulating for 3D spheroid MDFs. Immunostaining and the corresponding fluorescence intensity quantification, as well as WB results showed that ID3 siRNAs sharply decreased Nestin expression (Figure 2D, F and G; Supplementary Figure S2A and C). These above results implied that ID3 may play a critical role in acquiring NPC properties for 3D spheroid MDFs. In addition, low oxygen has been verified to enhance primitive and definitive neural stem cell colony formation by inhibiting distinct cell death pathways.[53] At lower O_2 tensions, HIF-1α facilitates signal transduction pathways that promote self-renewal and inhibits pathways that promote NSC differentiation or apoptosis and vice versa.[54] In this study, the lower O_2 tensions of 3D spheroid MDFs were identified using the Hypoxyprobe-1 Plus Kit. Compared to monolayer MDFs, 3D spheroid MDFs showed potent immunostaining (Figure 2H), indicating significant lower O_2 tensions. Further immunofluorescence staining of HIF-1α also revealed the upregulated expression in 3D spheroid MDFs (Supplementary Figure S2B and D). Moreover, the 3D spheroid MDFs were treated with the HIF-1a inhibitor KC7F2, and then their Sox 2 and Nestin expressions were tested by RT-PCR (Supplementary Figure S3A and B), WB (Supplementary Figure S3C), immunofluorescence staining (Supplementary Figure S3D and F) and the corresponding fluorescence intensity quantification (Supplementary Figure S3E and G), respectively. It is surprising that all the 3 kinds of tested results of KC7F2 treated 3D spheroid MDFs showed no significant differences comparing to the control 3D spheroid MDFs, which means HIF-1a does not play the critical role in the reprogramming of 3D MDFs into neural progenitor-like cells in our experimental system.

3D spheroid MDFs enhance nerve axon extension and promote the regeneration of injured sciatic nerves

Encouraged by the favorable NPC properties of 3D spheroid MDFs, this study aimed to investigate their effects on enhancing the neurite extension of nerve axon and promoting the regeneration of injured sciatic nerves. Thus, rat DRG neurons were firstly prepared and cocultured with monolayer or 3D spheroid MDFs, and neurite elongation was detected by Tuj1 immunostaining. Fluorescent images showed that neurites were significantly elongated when cocultured with 3D spheroid MDFs compared to monolayer MDFs (Figure 3A and B).

Given that 3D spheroid MDFs exerted remarkable promotion of neurites extending in vitro, their therapeutic potential in peripheral nerve injury was secondly verified by inoculating 3D spheroid MDFs into nerve conduits after the sciatic transection operation. At 7 days after the sciatic transection injury, regenerating axons were observed to be traversing the injury site in 3D spheroid and the monolayer MDFs transplanted rats; however, many more survived MDFs were observed at the injury site in 3D spheroid MDFs transplanted rats than monolayer MDFs transplanted rats (Figure 3C). The hydrogen peroxide incubation assay also revealed that 3D spheroid MDFs could tolerate higher concentrations of stimulation (Supplementary Figure S2E). More importantly, Tuj1 staining showed that 3D spheroid MDFs, but not monolayer MDFs, were in close contacted with regenerating nerve axons (Figure 3C). Moreover, S100β staining revealed that spheroid MDFs did not convert into SCs at the injured site of the sciatic nerve (Figure 3D). Tuj1 and S100β staining suggests the possibility that 3D spheroid MDFs are involved in axon regeneration through direct contact with injured nerves.

Six weeks after the transection of sciatic nerve, the footprints of rats in different groups were collected to quantify their functional recovery, and the nerve tissues and their innervated gastrocnemius muscles were harvested for histological evaluations. The SFI values of 3D spheroid MDFs rats were obviously higher than those of the monolayer MDFs and PBS control groups (Supplementary Figure S4A and B). Gross images of the injured lateral foot also revealed much better recovery in 3D spheroid MDFs transplanted rats (Supplementary Figure S4C). Gross images of the sciatic nerves and corresponding H&E staining showed a larger diameter of regenerated nerves in rats transplanted with 3D spheroid MDFs (Supplementary Figure 4A-C). TEM results showed that the diameter and thickness of regenerated myelinated nerve fibers of rats administrated 3D spheroid MDFs were much thicker than those administrated monolayer MDFs and PBS (Figure 4D; Supplementary Figure S4D-F). Moreover, the gross images of innervated gastrocnemius muscles (Figure 4E) and quantification of the relative gastrocnemius muscle weight (RGMW) among the different groups (Figure 4F) verified the more conspicuous promotion of regenerated nerves by 3D spheroid MDF transplantation. H&E staining of the cross-sectional area of the muscle fibers (Figure 4G) further confirmed more successful reinnervation and less muscular atrophy in 3D spheroid MDF transplantation rats after peripheral nerve injury.

3D spheroid MDFs promote peripheral nerve regeneration by upregulating semaphorin7a

To study the mechanism of the promotion in peripheral nerve regeneration, this study investigated the differences between monolayer and 3D spheroid MDFs in greater depth through RNA sequencing. The gene expression of the 2 groups was significantly segregated based on principal component analyses shown in Supplementary Figure S5A. As shown in

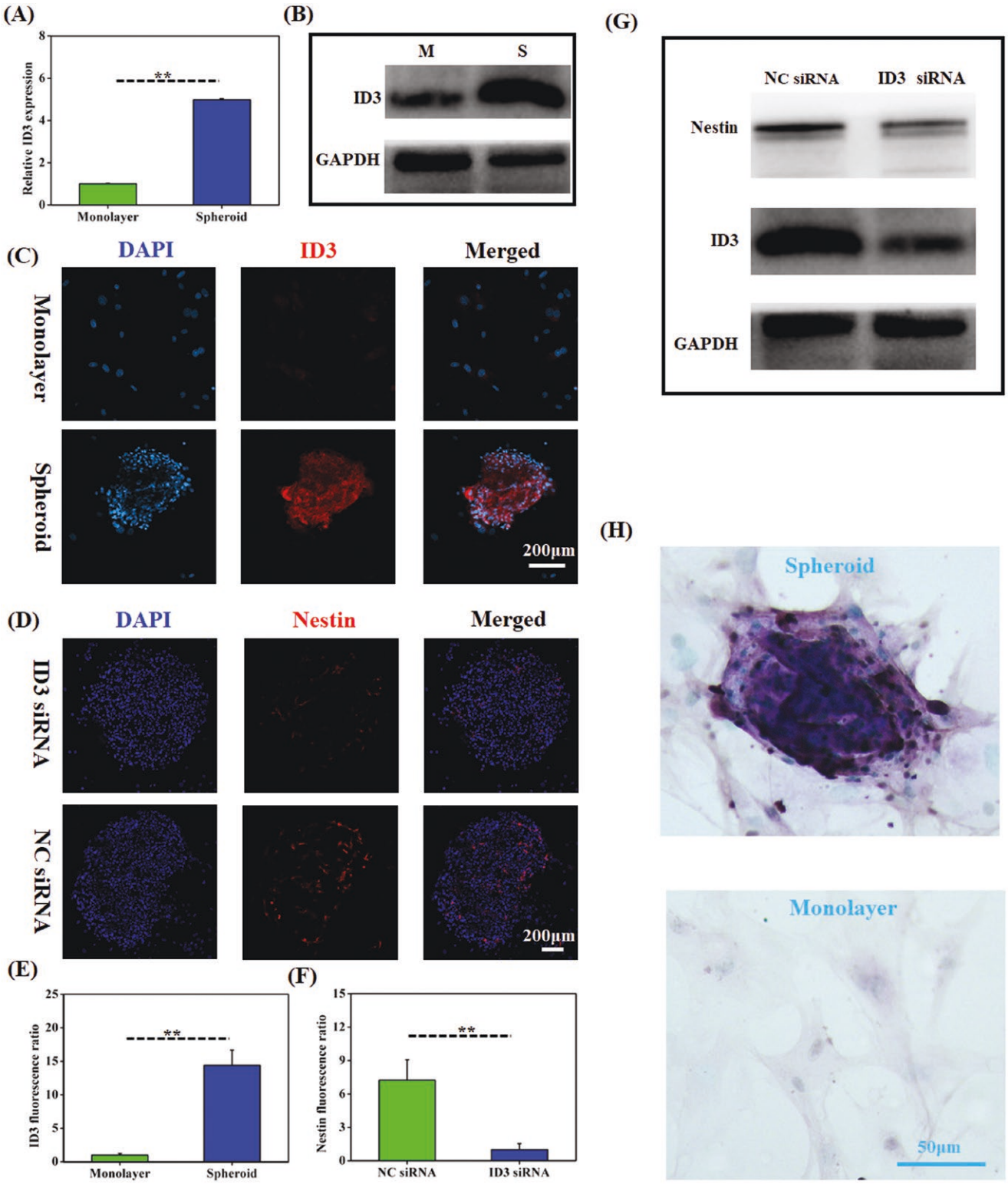


Figure 2. ID3 regulates the NPC properties of 3D spheroid MDFs. (A-C), RT-PCR testing (A) ($n = 3$), Western blotting (B) immunostaining (C) and the corresponding fluorescence intensity quantification (E) ($n = 8$) for ID3 in monolayer MDFs and 3D spheroid MDFs. Immunostaining (D) and the corresponding fluorescence intensity quantification (F) ($n = 5$), and Western blotting (G) of Nestin in 3D spheroid MDFs post ID3 siRNA interfering treatment. (H), The O_2 tensions of 3D spheroid MDFs identified by HypoxyprobeTM-1 Plus Kit. Data are mean ± SD. $^{**}P < 0.01$. M means monolayer MDFs; S means spheroid MDFs.

the volcano map, 1345 upregulated and 1181 downregulated genes with highly significant expression patterns were identified (Figure 5A). About 554 genes in monolayer MDFs and 771 genes in 3D spheroid MDFs showed significant differential expression (Figure 5B and C). Kyoto Encyclopedia of Genes and Genomes pathways enrichment analysis showed

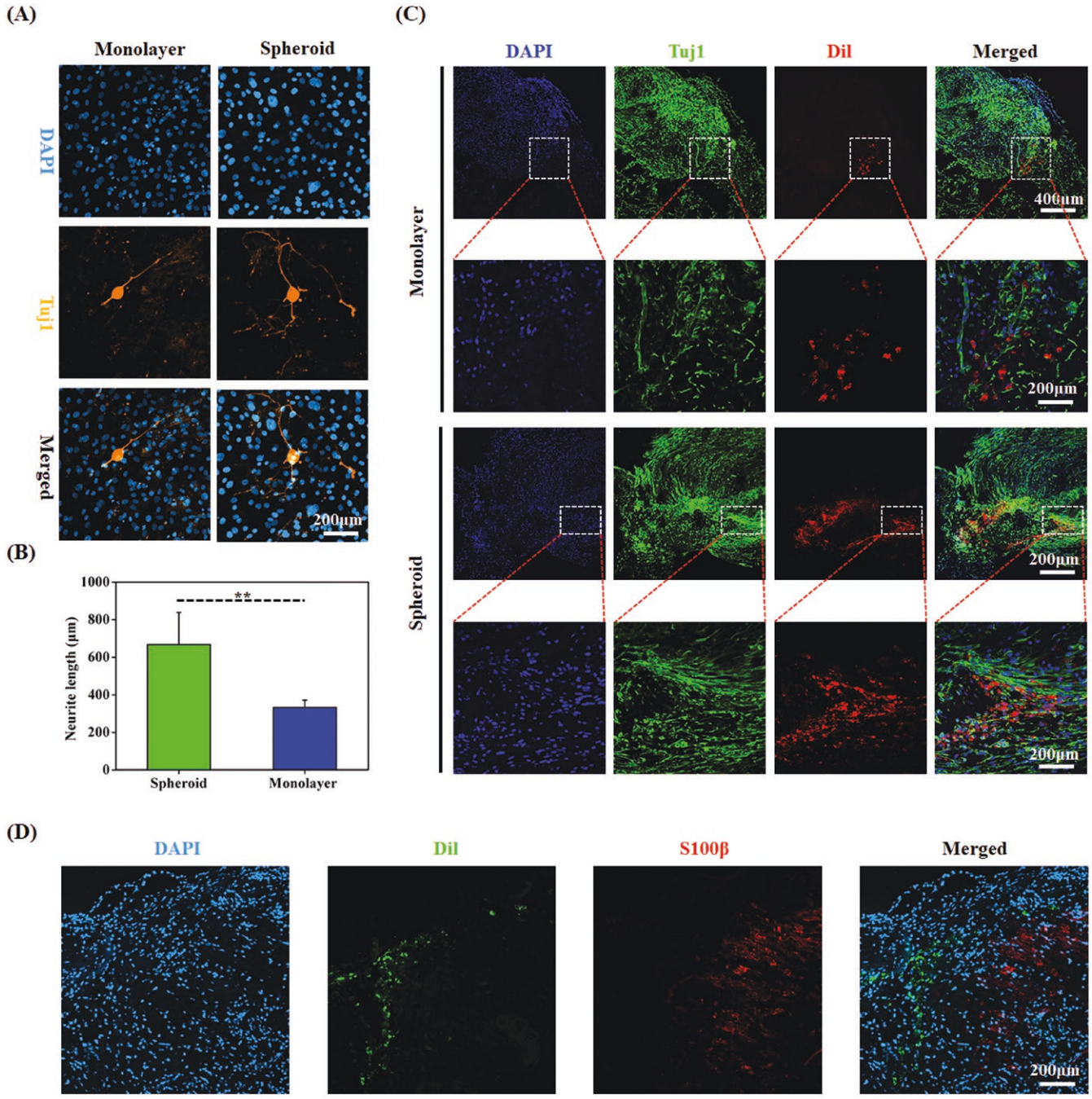


Figure 3. The neurite extending enhancement effects of 3D spheroid MDFs. (A, B) The neurite lengths of DRG neurons tested by Tuj1 staining after coculturing with monolayer MDFs or 3D spheroid MDFs ($n = 10$). (C) Tuj1 immunostaining and the DiI labeled monolayer MDFs or 3D spheroid MDFs in the regenerated axons of rat sciatic nerve 7 days post transplantation. (D) Tuj1 immunostaining and the DiI labeled 3D spheroid MDFs in the regenerated axons of rat sciatic nerve 7 days post transplantation. Data are mean ± SD. $^{**}P < 0.01$.

that the axon guidance signaling pathway was prominently upregulated in 3D spheroid MDFs (Figure 5D). Moreover, Gene Ontology enrichment analysis of axon guidance-related genes revealed peripheral nervous system axon regeneration, axon development, and response to axon injury as the 3 highest enrichments of differentially expressed genes. (Figure 5E; Supplementary Figure S5B). Among the upregulated genes, semaphorin7a was the most abundantly expressed gene (Supplementary Table S3), which has been well recognized as having an essential role in extending and guiding axons, as well as controlling the plasticity of synaptic connections.[42,43] Immunofluorescence staining and the corresponding fluorescence intensity quantification (Figure 6A and B), RT-PCR (Figure 6C), and WB (Figure 6D) results further confirmed semaphorin7a upregulation in 3D spheroid MDFs. Next, the mechanism of semaphorin7a upregulation was explored in 3D spheroid MDFs. According to the above data that 3D spheroid MDFs showed upregulated ID3 and HIF-1α, the regulatory roles of ID3 and HIF-1α on semaphorin7a with ID3 small interfering RNA (ID3 siRNA) or HIF-1α inhibitor KC7F2 were further verified. Immunofluorescence staining, the corresponding fluorescence intensity quantification and

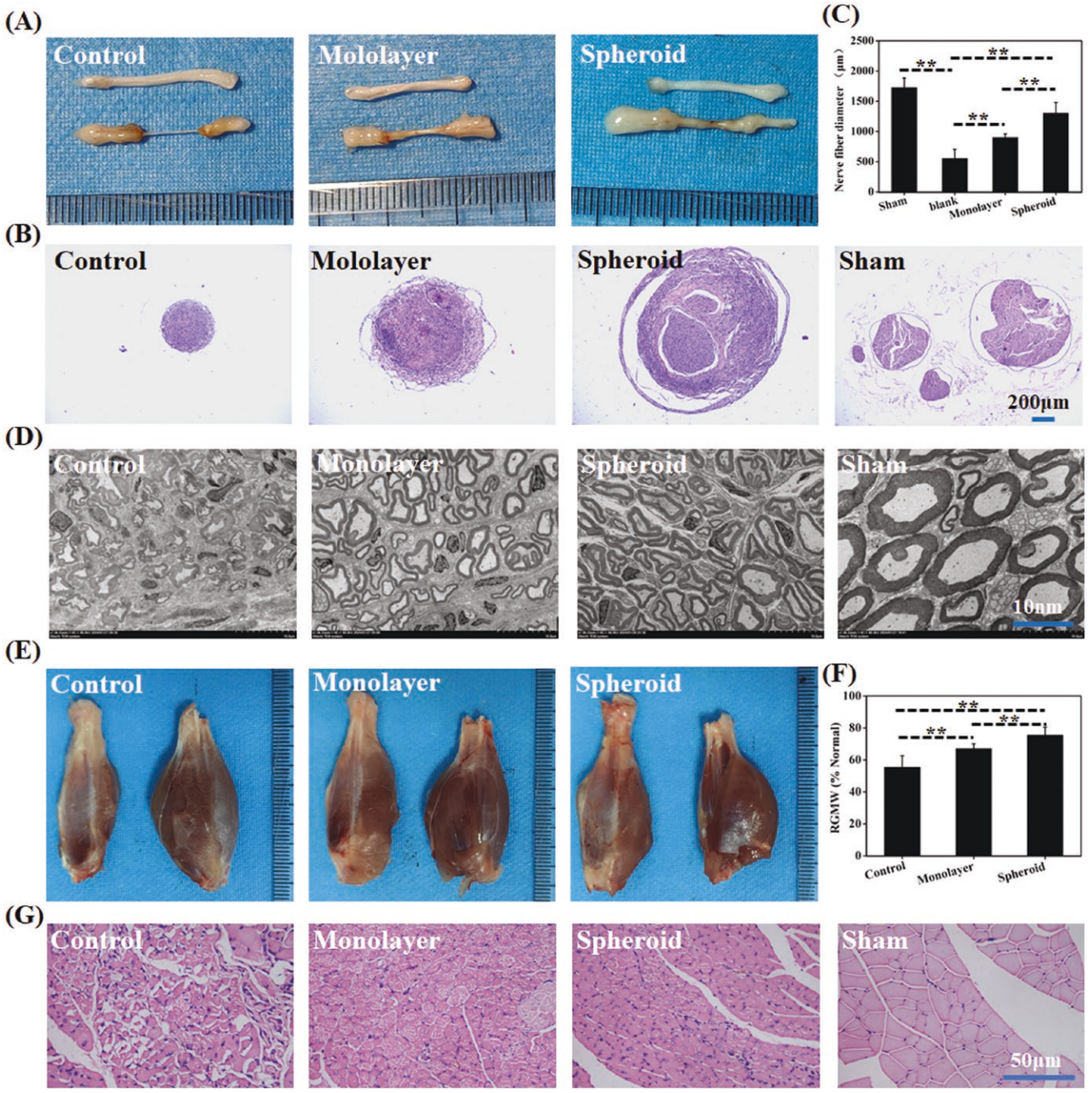


Figure 4. 3D spheroid MDFs promotes the regeneration of the transected sciatic nerves. (A) Images of gross morphology, (B) H&E images, (C) Quantitative analysis of diameter ($n = 6$), and (D) TEM images of regenerated rat sciatic nerve 6 weeks following injury in different treatment groups. (E) Images of gross morphology of gastrocnemius muscle, (F) quantitative analysis of RGMW, and (G) H&E images in different treatment groups ($n = 6$). Data are mean ± SD. $^{**}P < .01$. Abbreviations: HE, hematoxylin–eosin; TEM, transmission electron microscopy; Sham means rats without sciatic transection injury operation; Control means without MDFs transplantation.

WB results showed that ID3 siRNA (Figure 6E-G) and KC7F2 (Supplementary Figure S3H, J, and L) significantly decreased semaphorin7a expression in 3D spheroid MDFs. Moreover, immunofluorescence staining and the corresponding fluorescence intensity quantification (Supplementary Figure S3I and K) of ID3 showed that HIF-1α did not affect ID3 expression. Those aforementioned results verified that except for the enrichment of NPCs related properties, 3D spheroid culture also upregulated semaphorin7a expression in MDFs through ID3 and HIF-1α overexpression.

Next, the promotion effect of semaphorin7a in peripheral nerve regeneration in vitro and in vivo was investigated. Tuj1 immunostaining of DRG neurons showed that neurite length was dramatically decreased when 3D spheroid MDFs were transfected with siRNAs targeting semaphorin7a before coculture (Figure 7A-C). Since β1 integrin receptor-dependent signaling plays a central role in mediating the enhancement of axon outgrowth by semaphorin7a,[42] DRG neurons were treated with anti-β1 integrin antibodies. The neurite promotion effects of 3D spheroid MDFs were

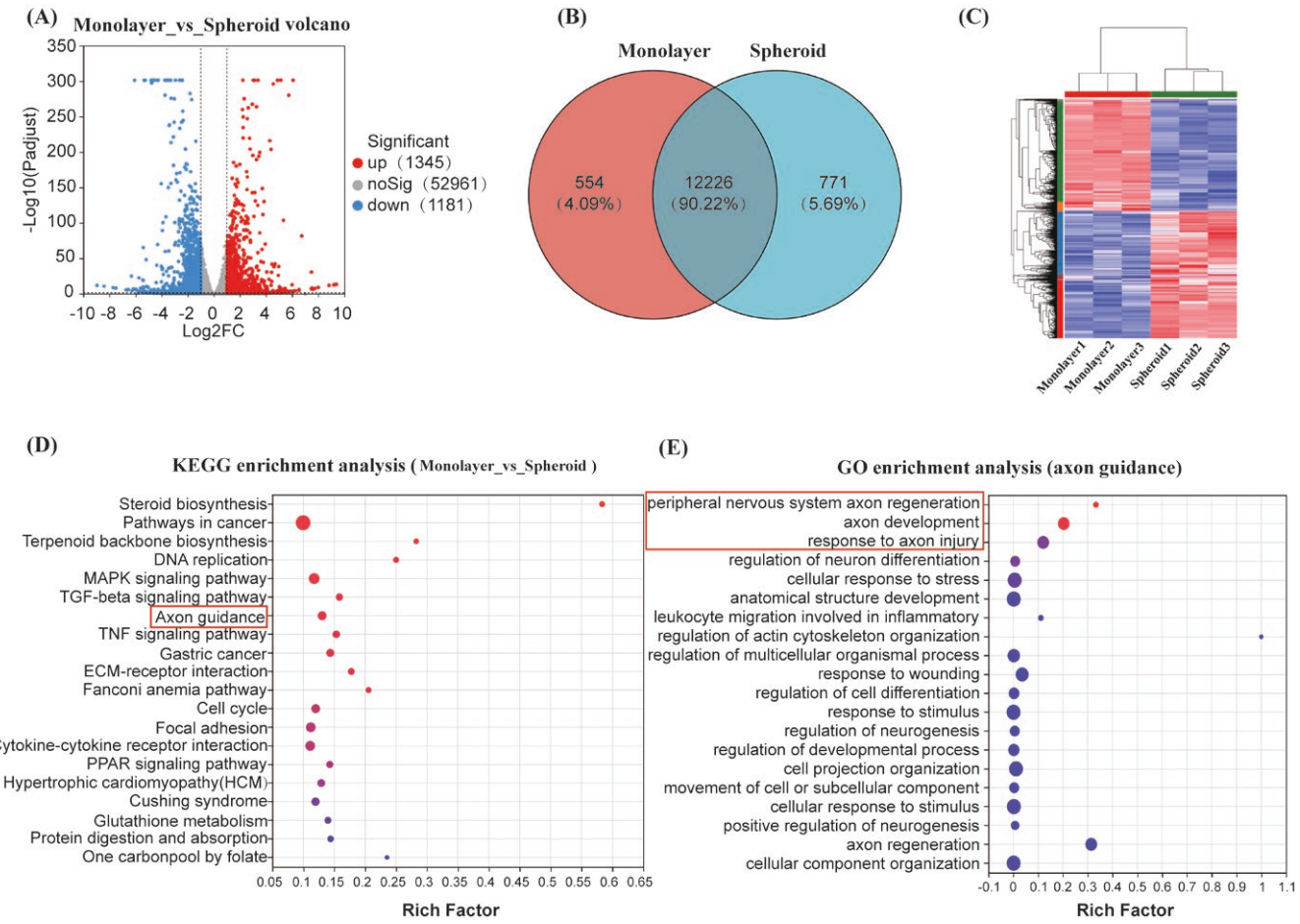


Figure 5. The whole gene expression profile comparing analysis of monolayer and 3D spheroid MDFs. (A) Whole-transcript expression profiling of monolayer and 3D spheroid MDFs. (B) The overlap gene numbers between monolayer and 3D spheroid MDFs. (C) Heatmap showing differential expressing genes in monolayer and 3D spheroid MDFs. (D) KEGG pathways enrichment analysis of monolayer and 3D spheroid MDFs. (E) Gene ontology (GO) enrichment analysis of axon guidance related genes of monolayer and 3D spheroid MDFs.

significantly diminished (Supplementary Figure S6A and B). Furthermore, 3D spheroid MDFs were pretreated with anti-semaphorin7a antibodies and transplanted into rats with sciatic nerve transection injury, as mentioned above. As expected, anti-semaphorin7a blocking treatment remarkably abolished the therapeutic potential in peripheral nerve injury, as shown by gross images of sciatic nerves (Figure 7D) and corresponding H&E staining (Figure 7E, and F), as well as gross images of innervated gastrocnemius muscles (Figure 7G) and quantification of RGMW among different groups (Figure 7H).

Discussion

Peripheral nerve tissue engineering combined with nerve conduits and supporting seed cells is considered a promising strategy for peripheral nerve repair and regeneration.[6-9] Previous studies have applied SCs, numerous stem cells, and nerve fibroblasts as supporting seed cells to increase the efficacy of engineered nerve grafts and significantly boost peripheral nerve regeneration. However, many drawbacks limit their potential application, such as the surgical need for collecting autologous SCs or nerve fibroblasts, the difficulty in obtaining sufficient SCs for transplantation, and the ethical uncertainty and tumorigenic properties in applying stem cells. Thus, cell sources that are more easily accessible and capable of rapid expansion in culture are urgently needed.

This study developed a new methodology that forced the skin fibroblasts to suspend and form a 3D spheroid, which could easily reprogram the skin fibroblasts into neural-like cells without genetic intervention. Dai's group previously revealed that force mouse embryonic fibroblasts (MEFs) and mouse tail-tip fibroblasts grow into 3D spheroids on a 0.5% agarose-pretreated cell dish and successfully reprogram them into NPCs.[55] Different from the cell preparation methods reported in Dai's group, 3D spheroid MDFs were obtained by simply maintaining them on a 6-well low attachment dish (STEMCELL Technologies) in DMEM expansion medium supplemented with 10% FBS and 1% penicillin/streptomycin without further adding other supplements. Consistent with the previous study,[55] results showed that 3D spheroid MDFs exhibit neural cell-like properties with significant upregulation of multiple NPC-specific genes, including Sox2, Nestin, Gpm6a, Brn2, Blbp, and Pax6, while downregulating mesoderm genes, including α-SMA and collagen 1A1. Sox2 is a key regulator for maintaining NPC characteristics and inhibiting differentiation and is also the most widely used gene to induce fibroblast transdifferentiation into NPCs.[56,57] In numerous previous studies, ID proteins have been found to play an important role in neurogenesis,[45-47] and stem cell maintenance[48-50] and could

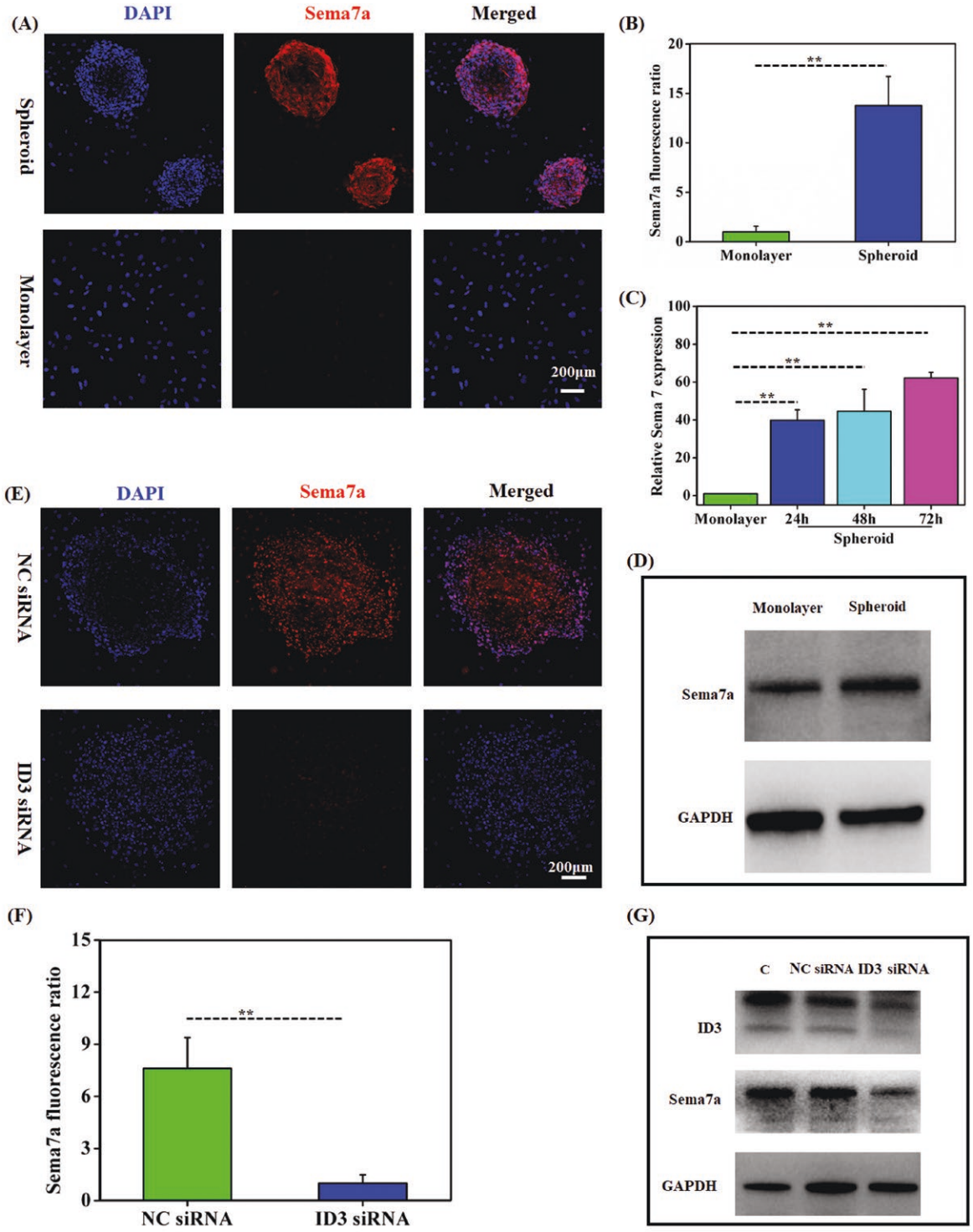


Figure 6. 3D spheroid culture upregulates the expression of Semaphorin7a through overexpressing of ID3. Immunocytochemistry staining and the corresponding fluorescence intensity quantification (A, B) ($n = 8$), RT-PCR (C) ($n = 3$), and western blotting (D) for Semaphorin7a in monolayer MDFs and 3D spheroid MDFs. Immunofluorescence staining and the corresponding fluorescence intensity quantification (E, F) ($n = 6$), and Western blotting (G) for ID3 and Semaphorin7a in 3D spheroid MDFs post ID3 siRNA treatment. Data are mean ± SD. $^{**}P < .01$.

enhance cell reprogramming.[51,52] Hayashi et al revealed that ID proteins could suppress the P16/INK4A-dependent senescence of fibroblasts and further improve the efficiency of pluripotency reprogramming.[51] Moon et al's study also showed that ID3 transduction in MEFs successfully transdifferentiate them into neural stem cell-like cells.[52] Our previous study also verified that overexpressed ID1 or ID3 in skin dermal fibroblasts significantly reprogrammed fibroblasts into a neural cell-like state.[37] Interestingly, this study found that ID3 was intensely upregulated in 3D spheroid MDFs; however, downregulating ID3 expression by pretreating with targeted siRNAs significantly decreased the expression of the classical marker protein of NPCs. This kind of result implies that ID3 may play critical role in acquiring the NPC properties for 3D spheroid MDFs. It

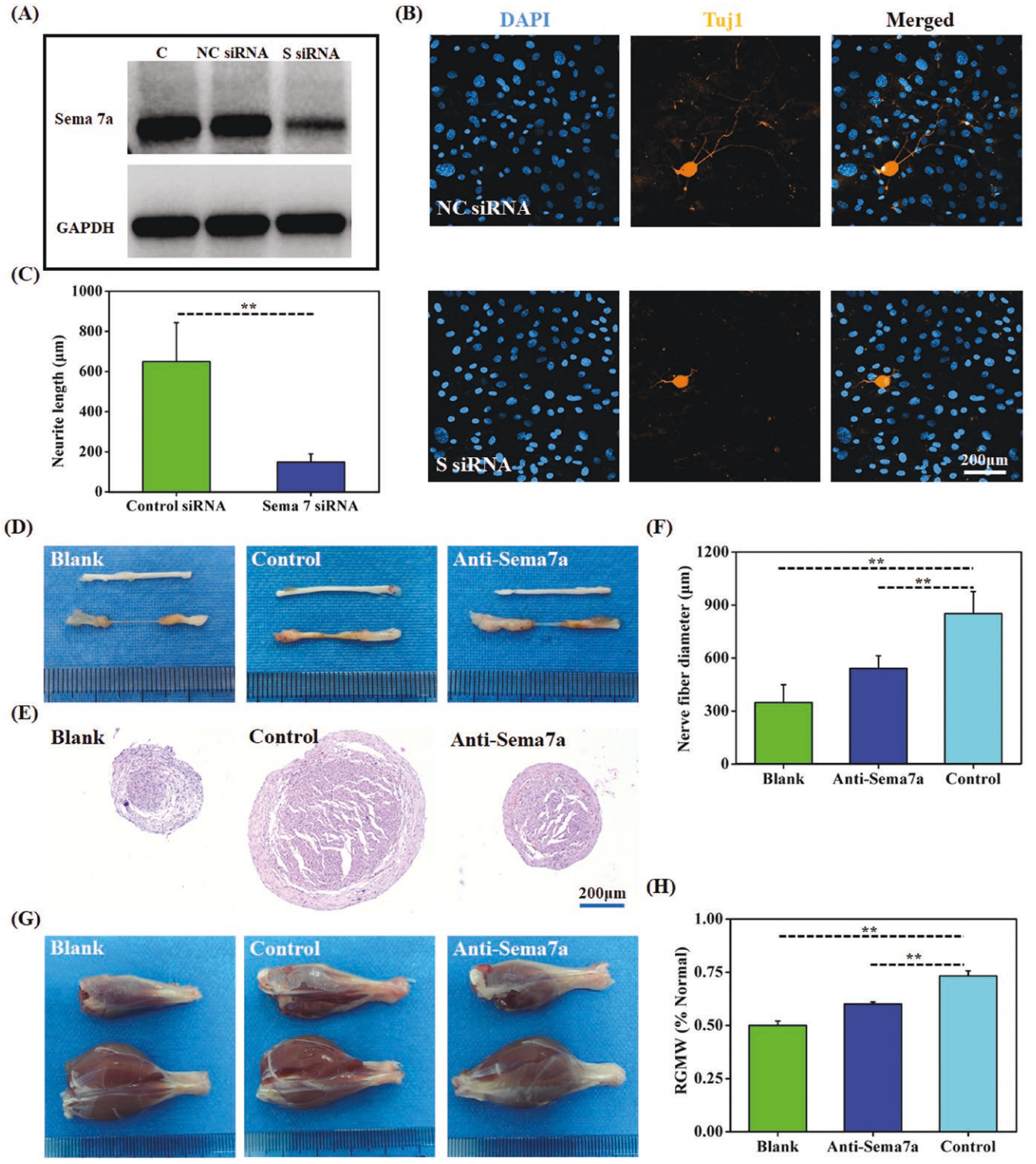


Figure 7. The promotion effect of Semaphorin7a in peripheral nerve regeneration in vitro and in vivo. (A) Western blotting for Semaphorin7a in 3D spheroid MDFs with or without siRNA treatment. (B, C) The neurite lengths of DRG neurons tested by Tuj1 staining after coculturing with Semaphorin7a siRNA pretreated 3D spheroid MDFs ($n = 10$). (D) Images of gross morphology of regenerated sciatic nerve, (E) H&E images, and (F) Quantitative analysis of diameter of regenerated rat sciatic nerve 6 weeks following injury in different treatment groups ($n = 6$). (G) Images of gross morphology of gastrocnemius muscle and quantitative analysis of RGMW (H) in different treatment groups ($n = 6$). Data are mean ± SD. $^{**}P < .01$. Abbreviaition: HE, hematoxylin–eosin; Blank means sciatic transection injured rats without MDFs transplantation; Control means sciatic transection injured rats transplanted with 3D spheroid MDFs; Anti-Sema7a means sciatic transection injured rats transplanted with anti-Semaphorin7a antibody blocking pretreated 3D spheroid MDFs.

was speculated that the special cell morphology, oxygen levels, ECM secretion and concentration gradients of signaling factors and mechanical inputs, and different spatial presentation of regulatory cues together promote the NPC reprogramming of fibroblasts.[53,58] The anoxia situation of 3D spheroid MDFs was identified using the Hypoxyprobe-1 Plus Kit, and 3D spheroid MDFs showed much lower O_2 tensions. Therefore, it is believed that the significant upregulation of ID3 and lower oxygen levels may play a role in MDF reprogramming; however, the specific mechanism requires additional investigations.

Cocultured DRG neurons with 3D spheroid MDFs efficiently promoted neurites extension. Moreover, transplantation of 3D spheroid MDFs more significantly accelerated the regeneration of the sciatic nerve and improved the motor function of rats after transection compared to monolayer MDFs. Further experimental findings verified that spheroid MDFs remarkably upregulated the expression of semaphorin7a, which is the only GPI-anchored member of semaphorin family playing a dual role in neural and immune functions.[59,60] Semaphorin7a is prominently expressed in the embryo, lymphoid organs, and the nervous system.[60] However, it has long been regarded as an immunomodulator until one of the critical studies reported by Pasterkamp et al,[42] in which they showed that semaphorin7a can also mediate neuronal functions, and its effects on neuronal development had been widely investigated. Unlike many other repulsive guidance semaphorins, semaphorin7a enhances central and peripheral axon growth and is required for proper axon tract formation during embryonic development. They also revealed that β_1 integrin receptors and activation of focal adhesion kinase and extracellular signal-regulated kinase mitogen-activated protein kinase signaling pathways play important roles in semaphorin7a's enhancement of axon outgrowth. Accumulating evidence supports the roles of semaphorin7a in neuron migration and neurite growth in addition to immune cell regulation. Fourneau and Bareyre reported an essential role of semaphorin7a in guiding the growth of axons and controlling the plasticity of synaptic connections.[43] Studies also found that semaphorin7a could regulate neuroglial plasticity in the adult hypothalamic median eminence and hippocampal neurogenesis.[61,62] Recently, researchers identified semaphorin7a as a critical determinant of serotonergic circuit formation in healthy or spinal cord injured mice.[43] Semaphorin7a-deficient mice showed specific ectopic targeting of serotonin fibers in the lumbar spinal cord after being challenged with a spinal lesion, and injured semaphorin7a-deficient mice exhibited a worsening of their postinjury locomotor abilities. Numerous investigations have identified that semaphorin7a is expressed in different neuronal and glial cells in healthy and regenerating central nervous systems[42,63] and is involved in the maturation of the glial scar after spinal cord injury. Semaphorin7a expression in injured neurons and reactive astrocytes surrounding the glial scar is immediately upregulated after the induction of a lesion.[63] More interestingly, this temporal expression pattern matched the time course of tissue regeneration. The above existing findings highlighted the importance of semaphorin7a in the adult nervous system; however, there is still a lack of investigation focusing on its role in peripheral nerve regeneration. This study demonstrated that 3D spheroid MDFs promote axon extension of DRG neurons depending on semaphorin7a expression and its downstream β_1 integrin receptor-dependent signaling. Moreover, transplantation of 3D spheroid MDFs significantly accelerated sciatic nerve regeneration after transection injury; however, this kind of acceleration was remarkably diminished by the semaphorin7a monoclonal antibody. Furthermore, the mechanism study of semaphorin7a upregulation in 3D spheroid MDFs identified that ID3 and HIF-1α play important roles.

Conclusion

In summary, these results demonstrated a simple and feasible 3D culture approach to reprogram skin fibroblasts to exhibit neural cell-like properties by the upregulation of ID3, which further coordinates with the upregulated HIF-1α to increase semaphorin7a expression and finally improves the extension of nerve axon in vitro and in vivo. This study might shed new light on treatments for peripheral nerve injury.

Acknowledgments

We thank Qing Zhou for histopathologic analysis support. This work was supported by the National Natural Science Foundation General Program of China (Grant No. 82172237 and 82072190), National Key Research and Development Program (2016YFC1000805), National Natural Science Foundation Key Program of China (Grant No. 82030056).

Author contributions

Xu Tan (Conceptualization, Data curation, Funding acquisition, Writing—original draft). Zhou Zhang (Conceptualization, Data curation, Writing—review & editing). Xiaohui Cao (Formal Analysis, Data curation). Langfan Qu, Yinchun Xiong, Huijuan Li (Data curation, Investigation). Chunmeng Shi, Zelin Chen and Yu Wang (Conceptualization, Project administration, Funding acquisition, Writing—review & editing). All authors (Writing—review & editing).

Conflicts of interest

The authors declare that they have no competing interests.

Data availability

All data included in this study are available upon reasonable request by contact with the corresponding author.

Supplementary material

Supplementary material is available at *Stem Cells Translational Medicine* online.

References

1. Yi S, Zhang Y, Gu X, et al. Application of stem cells in peripheral nerve regeneration. *Burns Trauma* 2020;8:tkaa002. https://doi.org/10.1093/burnst/tkaa002
2. Wang Y, Li D, Wang G, et al. The effect of co-transplantation of nerve fibroblasts and Schwann cells on peripheral nerve repair. *Int J Biol Sci*. 2017;13:1507-1519. https://doi.org/10.7150/ijbs.21976
3. Tian L, Prabhakaran MP, Ramakrishna S. Strategies for regeneration of components of nervous system: scaffolds, cells and biomolecules. *Regener Biomater*. 2015;2:31-45. https://doi.org/10.1093/rb/rbu017

4. Han GH, Peng J, Liu P, et al. Therapeutic strategies for peripheral nerve injury: decellularized nerve conduits and Schwann cell transplantation. *Neural Regener Res*. 2019;14:1343-1351. https://doi.org/10.4103/1673-5374.253511
5. Gu X, Ding F, Yang Y, Liu J. Construction of tissue engineered nerve grafts and their application in peripheral nerve regeneration. *Prog Neurobiol*. 2011;93:204-230. https://doi.org/10.1016/j.pneurobio.2010.11.002
6. Fan L, Yu Z, Li J, Dang X, Wang K. Schwann-like cells seeded in acellular nerve grafts improve nerve regeneration. *BMC Musculoskelet Disord*. 2014;15:165. https://doi.org/10.1186/1471-2474-15-165
7. Pan M, Wang X, Chen Y, et al. Tissue engineering with peripheral blood-derived mesenchymal stem cells promotes the regeneration of injured peripheral nerves. *Exp Neurol*. 2017;292:92-101. https://doi.org/10.1016/j.expneurol.2017.03.005
8. Wang Y, Zhao Y, Sun C, et al. Chitosan Degradation Products Promote Nerve Regeneration by Stimulating Schwann Cell Proliferation via miR-27a/FOXO1 Axis. *Mol Neurobiol*. 2016;53:28-39. https://doi.org/10.1007/s12035-014-8968-2
9. Hu Y, Wu Y, Gou Z, et al. 3D-engineering of Cellularized Conduits for Peripheral Nerve Regeneration. *Sci Rep*. 2016;6:32184. https://doi.org/10.1038/srep32184
10. Jiang L, Jones S, Jia X. Stem Cell Transplantation for Peripheral Nerve Regeneration: Current Options and Opportunities. *Int J Mol Sci* . 2017;18:94. https://doi.org/10.3390/ijms18010094
11. Hussain G, Wang J, Rasul A, et al. Current Status of Therapeutic Approaches against Peripheral Nerve Injuries: A Detailed Story from Injury to Recovery. *Int J Biol Sci*. 2020;16:116-134. https://doi.org/10.7150/ijbs.35653
12. Ren Z, Wang Y, Peng J, Zhao Q, Lu S. Role of stem cells in the regeneration and repair of peripheral nerves. *Rev Neurosci*. 2012;23:135-143. https://doi.org/10.1515/revneuro-2011-0069
13. Kornfeld T, Vogt PM, Bucan V, et al. Characterization and Schwann cell seeding of up to 15.0 cm long spider silk nerve conduits for reconstruction of peripheral nerve defects. *J Funct Biomater*. 2016;7:30. https://doi.org/10.3390/jfb7040030
14. Bosch-Queralt M, Fledrich R, Stassart RM. Schwann cell functions in peripheral nerve development and repair. *Neurobiol Dis*. 2023;176:105952. https://doi.org/10.1016/j.nbd.2022.105952
15. Painter MW. Aging Schwann cells: mechanisms, implications, future directions. *Curr Opin Neurobiol*. 2017;47:203-208. https://doi.org/10.1016/j.conb.2017.10.022
16. Madduri S, Gander B. Schwann cell delivery of neurotrophic factors for peripheral nerve regeneration. *J Peripheral Nerv Syst*. 2010;15:93-103. https://doi.org/10.1111/j.1529-8027.2010.00257.x
17. Dilwali S, Patel PB, Roberts DS, et al. Primary culture of human Schwann and schwannoma cells: improved and simplified protocol. *Hear Res*. 2014;315:25-33. https://doi.org/10.1016/j.heares.2014.05.006
18. Zhu C, Huang J, Xue C, et al. Skin derived precursor Schwann cell-generated acellular matrix modified chitosan/silk scaffolds for bridging rat sciatic nerve gap. *Neurosci Res*. 2018;135:21-31. https://doi.org/10.1016/j.neures.2017.12.007
19. Mosahebi A, Fuller P, Wiberg M, Terenghi G. Effect of allogeneic Schwann cell transplantation on peripheral nerve regeneration. *Exp Neurol*. 2002;173:213-223. https://doi.org/10.1006/exnr.2001.7846
20. Faroni A, Smith RJ, Lu L, Reid AJ. Human Schwann-like cells derived from adipose-derived mesenchymal stem cells rapidly de-differentiate in the absence of stimulating medium. *Eur J Neurosci*. 2016;43:417-430. https://doi.org/10.1111/ejn.13055
21. Johnson TS, O'Neill AC, Motarjem PM, et al. Tumor formation following murine neural precursor cell transplantation in a rat peripheral nerve injury model. *J Reconstr Microsurg*. 2008;24:545-550. https://doi.org/10.1055/s-0028-1088228
22. Ernst A, Alkass K, Bernard S, et al. Neurogenesis in the striatum of the adult human brain. *Cell*. 2014;156:1072-1083. https://doi.org/10.1016/j.cell.2014.01.044
23. Rippon HJ, Bishop AE. Embryonic stem cells. *Cell Prolif*. 2004;37:23-34. https://doi.org/10.1111/j.1365-2184.2004.00298.x
24. Ben-David U, Benvenisty N. The tumorigenicity of human embryonic and induced pluripotent stem cells. *Nat Rev Cancer*. 2011;11:268-277. https://doi.org/10.1038/nrc3034
25. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. *Nature*. 2007;448:313-317. https://doi.org/10.1038/nature05934
26. Qu WR, Zhu Z, Liu J, et al. Interaction between Schwann cells and other cells during repair of peripheral nerve injury. *Neural Regener Res*. 2021;16:93-98. https://doi.org/10.4103/1673-5374.286956
27. Zhang Z, Yu B, Gu Y, et al. Fibroblast-derived tenascin-C promotes Schwann cell migration through β1-integrin dependent pathway during peripheral nerve regeneration. *Glia*. 2016;64:374-385. https://doi.org/10.1002/glia.22934
28. Hara M, Kadoya K, Endo T, Iwasaki N. Peripheral nerve-derived fibroblasts promote neurite outgrowth in adult dorsal root ganglion neurons more effectively than skin-derived fibroblasts. *Exp Physiol*. 2023;108:621-635. https://doi.org/10.1113/EP090751
29. Pang ZP, Yang N, Vierbuchen T, et al. Induction of human neuronal cells by defined transcription factors. *Nature*. 2011;476:220-223. https://doi.org/10.1038/nature10202
30. Caiazzo M, Dell'Anno MT, Dvoretskova E, et al. Direct generation of functional dopaminergic neurons from mouse and human fibroblasts. *Nature*. 2011;476:224-227. https://doi.org/10.1038/nature10284
31. Vierbuchen T, Ostermeier A, Pang ZP, et al. Direct conversion of fibroblasts to functional neurons by defined factors. *Nature*. 2010;463:1035-1041. https://doi.org/10.1038/nature08797
32. Lujan E, Chanda S, Ahlenius H, Südhof TC, Wernig M. Direct conversion of mouse fibroblasts to self-renewing, tripotent neural precursor cells. *Proc Natl Acad Sci USA*. 2012;109:2527-2532. https://doi.org/10.1073/pnas.1121003109
33. Han DW, Tapia N, Hermann A, et al. Direct reprogramming of fibroblasts into neural stem cells by defined factors. *Cell Stem Cell*. 2012;10:465-472. https://doi.org/10.1016/j.stem.2012.02.021
34. Sowa Y, Kishida T, Tomita K, et al. Direct Conversion of Human Fibroblasts into Schwann Cells that Facilitate Regeneration of Injured Peripheral Nerve In Vivo. *Stem Cells Transl Med*. 2017;6:1207-1216. https://doi.org/10.1002/sctm.16-0122
35. Eshghi S, Schaffer DV: Engineering microenvironments to control stem cell fate and function. In: *StemBook*. edn. Cambridge (MA): Harvard Stem Cell Institute Copyright: © 2008 Shawdee Eshghi and David V. Schaffer.; 2008.
36. Tavazoie M, Van der Veken L, Silva-Vargas V, et al. A specialized vascular niche for adult neural stem cells. *Cell Stem Cell*. 2008;3:279-288. https://doi.org/10.1016/j.stem.2008.07.025
37. Chen Z, Shen G, Tan X, et al. ID1/ID3 mediate the contribution of skin fibroblasts to local nerve regeneration through Itga6 in wound repair. *Stem Cells Transl Med*.. 2021;10:1637-1649. https://doi.org/10.1002/sctm.21-0093
38. Huang GS, Dai LG, Yen BL, Hsu SH. Spheroid formation of mesenchymal stem cells on chitosan and chitosan-hyaluronan membranes. *Biomaterials*. 2011;32:6929-6945. https://doi.org/10.1016/j.biomaterials.2011.05.092
39. Cheng NC, Wang S, Young TH. The influence of spheroid formation of human adipose-derived stem cells on chitosan films on stemness and differentiation capabilities. *Biomaterials*. 2012;33:1748-1758. https://doi.org/10.1016/j.biomaterials.2011.11.049
40. Sarig R, Baruchi Z, Fuchs O, Nudel U, Yaffe D. Regeneration and transdifferentiation potential of muscle-derived stem cells propagated as myospheres. *Stem Cells (Dayton, Ohio)*. 2006;24:1769-1778. https://doi.org/10.1634/stemcells.2005-0547
41. Wenzel V, Roedl D, Ring J, Djabali K. Naïve adult stem cells isolation from primary human fibroblast cultures. *J Visual Exp*. 2013;3:e50185. https://doi.org/10.3791/50185
42. Pasterkamp RJ, Peschon JJ, Spriggs MK, Kolodkin AL. Semaphorin 7A promotes axon outgrowth through integrins and MAPKs. *Nature*. 2003;424:398-405. https://doi.org/10.1038/nature01790

43. Fourneau J, Bareyre FM. Semaphorin7A: its role in the control of serotonergic circuits and functional recovery following spinal cord injury. *Neural Regener Res*. 2022;17:959-962. https://doi.org/10.4103/1673-5374.324828
44. Chen Z, Wang Z, Jin T, et al. Fibrogenic fibroblast-selective near-infrared phototherapy to control scarring. *Theranostics*. 2019;9:6797-6808. https://doi.org/10.7150/thno.36375
45. Nagata Y, Todokoro K. Activation of helix-loop-helix proteins Id1, Id2 and Id3 during neural differentiation. *Biochem Biophys Res Commun*. 1994;199:1355-1362. https://doi.org/10.1006/bbrc.1994.1380
46. Light W, Vernon AE, Lasorella A, Iavarone A, LaBonne C. Xenopus Id3 is required downstream of Myc for the formation of multipotent neural crest progenitor cells. *Development (Cambridge, England)*. 2005;132:1831-1841. https://doi.org/10.1242/dev.01734
47. Tzeng SF, de Vellis J. Id1, Id2, and Id3 gene expression in neural cells during development. *Glia*. 1998;24:372-381. https://doi.org/10.1002/(sici)1098-1136(199812)24:4<372::aid-glia2>3.0.co;2-b
48. Williams SA, Maecker HL, French DM, et al. USP1 deubiquitinates ID proteins to preserve a mesenchymal stem cell program in osteosarcoma. *Cell*. 2011;146:918-930. https://doi.org/10.1016/j.cell.2011.07.040
49. Bohrer C, Pfurr S, Mammadzada K, et al. The balance of Id3 and E47 determines neural stem/precursor cell differentiation into astrocytes. *EMBO J*. 2015;34:2804-2819. https://doi.org/10.15252/embj.201591118
50. Jung S, Park RH, Kim S, et al. Id proteins facilitate self-renewal and proliferation of neural stem cells. *Stem Cells Dev*. 2010;19:831-841. https://doi.org/10.1089/scd.2009.0093
51. Hayashi Y, Hsiao EC, Sami S, et al. BMP-SMAD-ID promotes reprogramming to pluripotency by inhibiting p16/INK4A-dependent senescence. *Proc Natl Acad Sci USA*. 2016;113:13057-13062. https://doi.org/10.1073/pnas.1603668113
52. Moon JH, Heo JS, Kwon S, et al. Two-step generation of induced pluripotent stem cells from mouse fibroblasts using Id3 and Oct4. *J Mol Cell Biol*. 2012;4:59-62. https://doi.org/10.1093/jmcb/mjr038
53. Clarke L, van der Kooy D. Low oxygen enhances primitive and definitive neural stem cell colony formation by inhibiting distinct cell death pathways. *Stem Cells (Dayton, Ohio)* 2009;27:1879-1886. https://doi.org/10.1002/stem.96
54. Panchision DM. The role of oxygen in regulating neural stem cells in development and disease. *J Cell Physiol*. 2009;220:562-568. https://doi.org/10.1002/jcp.21812
55. Su G, Zhao Y, Wei J, et al. Direct conversion of fibroblasts into neural progenitor-like cells by forced growth into 3D spheres on low attachment surfaces. *Biomaterials*. 2013;34:5897-5906. https://doi.org/10.1016/j.biomaterials.2013.04.040
56. Ring KL, Tong LM, Balestra ME, et al. Direct reprogramming of mouse and human fibroblasts into multipotent neural stem cells with a single factor. *Cell Stem Cell*. 2012;11:100-109. https://doi.org/10.1016/j.stem.2012.05.018
57. Graham V, Khudyakov J, Ellis P, Pevny L. SOX2 functions to maintain neural progenitor identity. *Neuron*. 2003;39:749-765. https://doi.org/10.1016/s0896-6273(03)00497-5
58. Keung AJ, Kumar S, Schaffer DV. Presentation counts: microenvironmental regulation of stem cells by biophysical and material cues. *Annu Rev Cell Dev Biol*. 2010;26:533-556. https://doi.org/10.1146/annurev-cellbio-100109-104042
59. Suzuki K, Okuno T, Yamamoto M, et al. Semaphorin 7A initiates T-cell-mediated inflammatory responses through alpha1beta1 integrin. *Nature*. 2007;446:680-684. https://doi.org/10.1038/nature05652
60. Czopik AK, Bynoe MS, Palm N, Raine CS, Medzhitov R. Semaphorin 7A is a negative regulator of T cell responses. *Immunity*. 2006;24:591-600. https://doi.org/10.1016/j.immuni.2006.03.013
61. Parkash J, Messina A, Langlet F, et al. Semaphorin7A regulates neuroglial plasticity in the adult hypothalamic median eminence. *Nat Commun*. 2015;6:6385.
62. Jongbloets BC, Lemstra S, Schellino R, et al. Stage-specific functions of Semaphorin7A during adult hippocampal neurogenesis rely on distinct receptors. *Nat Commun*. 2017;8:14666. https://doi.org/10.1038/ncomms14666
63. Kopp MA, Brommer B, Gatzemeier N, Schwab JM, Prüss H. Spinal cord injury induces differential expression of the profibrotic semaphorin 7A in the developing and mature glial scar. *Glia* 2010; 58(14):1748–1756.