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Regeneration of peripheral nerves by transplanted sphere of human mesenchymal stem cells derived from embryonic stem cells

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ABSTRACT

In cell therapy, the most important factor for therapeutic efficacy is the stable supply of cells with best engraftment efficiency. To meet this requirement, we have developed a culture strategy such as three-dimensional sphere of human embryonic stem cell-derived mesenchymal stem cells (hESC-MSCs) in serum-free medium. To investigate the in vivo therapeutic efficacy of hESC-MSC spheres in nerve injury model, we transected the sciatic nerve in athymic nude mice and created a 2-mm gap. Transplantation of hESC-MSC as sphere repaired the injured nerve significantly better than transplantation of hESC-MSC as suspended single cells in regard to 1) nerve conduction (sphere; 28.81 ± 3.55 vs. single cells; 18.04 ± 2.10, $p < 0.05$) and 2) susceptibility of nerve stimulation at low voltage (sphere; 0.38 ± 0.08 vs. single cells; 0.66 ± 0.11, $p < 0.05$) at 8 weeks. Recovery after sphere transplantation was near-complete when compared with the data of normal control (sphere 28.81 ± 3.55 vs normal 32.62 ± 2.85 in nerve conduction : sphere 0.38 ± 0.08 vs normal 0.36 ± 0.67 in susceptibility of nerve stimulation, no significant difference, respectively). Recovery in function of the injured nerve was well corroborated by the histologic evidence of regenerated nerve. In the mechanistic analysis, the supernatant of sphere-forming hESC-MSC contains hepatocyte growth factor and insulin-like growth factor-binding protein-1 significantly more than the supernatant of the single cells of hESC-MSC has, which might be the key factors for the improved engraftment efficiency and greater regeneration of injured peripheral nerve.

1. Introduction

The peripheral nerves can be recovered from injury through regeneration of axons, leading to the reinnervation of end organs [1]. Despite early diagnosis and modern surgical techniques, the outcome after peripheral nerve injury remains relatively poor. In order to recover the injured peripheral nerve to the pre-injury level, investigators tried several experimental approaches, such as, the administration of an electric field and supplementation with stem cells and neurotrophic factors [2,3]. Among these techniques, cell transplantation is considered one of the most promising, because several studies demonstrated that peripheral nerve regeneration could be achieved by neural stem cells, bone marrow stromal cells, and umbilical cord-derived mesenchymal stromal cells through neurotrophic factor production and Schwann cell differentiation [4–6]. However, most adult stem cells have inherent limitations such as the insufficient number of cells and the invasive procedure to obtain them, which limits the applicability of these stem cells to broader clinical fields. We have previously reported the successful derivation of human mesenchymal stem cells (hMSCs) from human embryonic stem cells (hESCs) and demonstrated that hESC-derived MSCs are consistently produced, maintained, and amplified [7]. Furthermore hESC-MSCs were safe in terms of tumorigenesis, effective in repairing the infracted heart, and thus would be useful platform for regenerative medicine [7].

In cell transplantation, another important factor to limit the efficacy is poor engraftment rate after transplantation. Although

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hESC-MSCs may have superior regenerative capability to adult stem cells, they also are not free from the issue of poor engraftment after transplantation and need additional strategy to augment it for the enhancement of therapeutic efficacy [8–10]. Therefore, to improve the poor engraftment, we have developed a sphere from hESC-MSCs which restores cell–cell interactions without the need for additional cytokines, serum or matrixes. Previously [7], we demonstrated that the delivery of cells without the disruption of the extracellular matrix led to higher engraftment efficiency and greater therapeutic efficacy. In this study, we tried to test whether hESC-MSCs have capability to regenerate the injured peripheral nerves, to compare therapeutic efficacy between transplantation of hESC-MSCs as sphere versus as single-cell suspension, and finally to decipher the underlying mechanism for the difference in efficacy between sphere versus single-cell suspension of hESC-MSCs. On the basis of the results of the present study that transplanted hESC-MSC spheres can augment the regeneration of the sciatic nerve, we suggest that these spheres constitute candidate stem cells with potential of the widespread applications in regenerative medicine.

2. Materials and methods

2.1. Derivation of hESC-MSCs

This study was approved by the institutional review board of Seoul National University Hospital (H-0603-029-170). hESC-MSCs were obtained using a previously reported protocol [7]. In brief, SNUhES3 hES cells (Institute of Reproductive Medicine and Population, Medical Research Center, Seoul National University Hospital, Seoul, Korea) were detached from feeder cells and incubated in petri dishes without FGF-2 for 14 days to establish the embryonic body (EB). Round EBs were attached to gelatin-coated dishes for 16 days in low-glucose DMEM (Invitrogen) with 10% FBS (Invitrogen). The derived hESC-MSCs were expanded in EGM-2MV media (Lonza). These cells were already tested for differentiation into myocytes and chondrocytes under guided conditions to evaluate the differentiation potency toward specific cell types [7].

2.2. Sphere formation

After trypsinization (0.025% trypsin; HyClone) of hESC-MSCs in a monolayer culture state, cells were cultured in suspension using a bacterial dish containing sphere medium, namely, bovine serum-free Dulbecco's Modified Eagle's Medium (DMEM)/F12 (Invitrogen, Madason, WI) supplemented with 20% Serum Replacement (SR; GIBCO), in a humidified 37 °C cell incubator with 5% CO_2. hESC-MSC spheres appeared approximately 24 h later (Fig. 1A).

2.3. Sciatic nerve defect

Male nude mice, each weighing approximately 25–30 g, were anesthetized using intramuscular Zoletil (50 mg/mL, 10 μL per animal) and Rompun 2% (10 μL per animal). Incisions were made on the right hindlimb and dissection was performed to expose the sciatic nerve. The nerves were cut in the central area underneath the gluteus maximus to create a complete 2-mm nerve defect [11]. To maintain nerve's original position without any sutures, there was no adjacent extra dissection of the proximal and distal ends of the nerve.

Animals were divided into 4 groups: sciatic nerve defect with no treatment (Group 1), sciatic nerve defect with matrigel transplantation (Group 2, matrigel group), sciatic nerve defect with matrigel plus transplantation of single-cells suspension of hESC-MSCs (Group 3, single-cells group), and sciatic nerve defect with matrigel plus transplantation of spheres of hESC-MSCs (Group 4, sphere group). Each mouse was transplanted 5×10^4 cells in single-cells or sphere group (Fig. 1B).

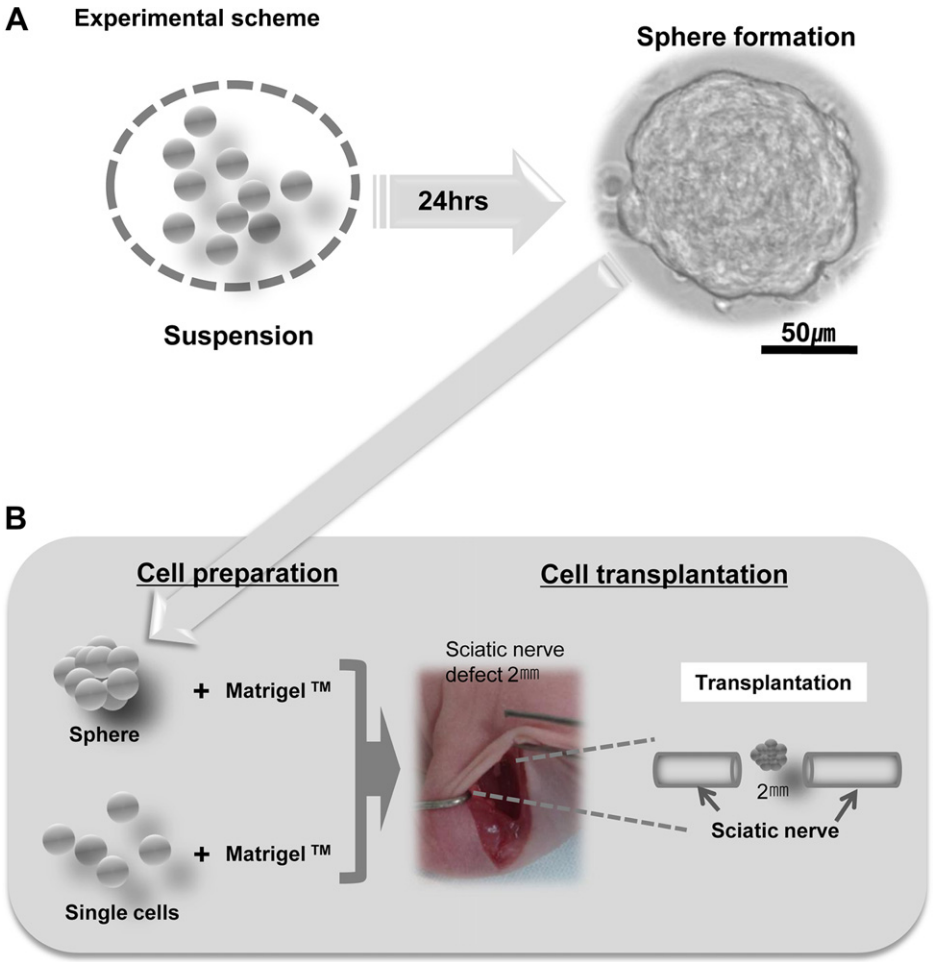

Fig. 1. Experimental scheme. (A) Sphere formation from the single cell suspension culture of hESC-MSCs in a bacterial dish using serum free medium without any additional factors for 24 h. (B) Spheres or single cells (the same 5×10^4 cells in both groups) were transplanted into defect site immediately after sciatic nerve injury. $N > 8$, for each group.

The skin incision was closed with nylon sutures (6–0). Eight weeks after transplantation, the regenerated sciatic nerves were dissected and biopsy was performed under anesthesia.

All animals were maintained in separate cages under controlled temperature (24–26 °C), humidity, and photoperiod conditions. All experiments in this study were performed in accordance with the guidelines for animal research from the National Institutes of Health and were approved by the Institutional Animal Care and Use Committee, institute of review board at Seoul National University Hospital in Seoul, Korea (10-0040, H-1003-002-310). Ethics approval was obtained from the institutional review board of Seoul National University Hospital (H-0603-029-170).

2.4. Nerve conduction test (electrophysiological examination)

At 2, 4, and 8 weeks after injury, compound muscle action potentials (CMAPs) of the sciatic nerve were measured. The proximal part of the nerve near the spine was stimulated using a stimulator constructed in-house with a needle EP electrode. CMAPs were recorded from active and reference electrodes attached to the gastrocnemius muscle (filtering frequency 10 HZ to 10 kHZ, sweep speed 1 ms/division, sensitivity 1 mV/division) using a portable 2-channel electromyography/nerve conduction velocity system (Medelec Synergy; Oxford Instrument Medical Systems, Oxford, UK).

2.5. Walking-foot-print analysis

At 2, 4, and 8 weeks, walking tracks were obtained using a 6×40 cm^2 corridor open at 1 end to a darkened compartment [7]. Paws were soaked in gentian violet, and the mice were walked multiple times on a white paper to obtain measurable prints. Multiple linear regression analysis was performed with factors derived from each of the parameters. Factors were derived for each parameter by subtracting the normal value (normal right side) from the experimental right side and dividing by the normal value [8].

2.6. Nerve stimulation test

The proximal part of the sciatic nerve (proximal 1 cm to the injured lesion) was mounted on a bipolar silver electrode (Grass SD9; Grass Instrument, Quincy, Massachusetts, USA) at 8 weeks after the injury. The nerve was then stimulated using different volt pulses at an amplitude of 0.05 mA at 10 Hz, with pulse widths of 0.5 ms. We calculated the voltage that induce the contraction of the gastrocnemius muscle.

2.7. Immunohistochemical analysis

Mouse sciatic nerves were fixed for 24 h in 10% buffered formalin at room temperature. They were washed in distilled water, dehydrated in graded alcohol, embedded in paraffin, and sectioned at 5 μm thickness. The unstained sections were deparaffinized and rehydrated by xylene, absolute alcohol, and 90%, 80% and 70% alcohol, and then washed with 0.05% TBS-T for 10 min. Antigen retrieval was performed using Retrieval solution (DAKO) for 30 min at 95 °C and then cooling. To block endogenous peroxidase activity, the slides were treated with 3.0% hydrogen peroxidase in distilled water for 10 min. After blocking non specific antigen with 1% BSA solution for 30 min, the sections were incubated for 60 min at room temperature with monoclonal anti-HLA Class 1 ABC antibody (clone EMR8-5, 1:200 dilution, Abcam Inc., Cambridge, MA), anti-Myelin Basic Protein antibody (clone BDI221, 1:200 dilution, Abcam Inc., Cambridge, MA), anti-Neurofilament antibody (clone NF-05, 1:200 dilution, Abcam Inc., Cambridge, MA) were applied. Anti-mouse IgG1 HRP conjugated antibody (Promega), Anti-mouse IgG2b HRP conjugated antibody (Promega) were used for signal detection.

2.8. Toluidine blue (TB) staining and transmission electron microscopy (TEM)

The regenerated nerves were harvested and immersion-fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) overnight at 4 °C. The specimens were postfixed in 1% OsO4 in 0.1 M phosphate buffer (pH 7.4) for 3–4 h at 4 °C, dehydrated with an ascending series of ethanol, longitudinally oriented, and embedded in epoxy resin. Transverse semithin sections (1–1.5 μm) were cut, stained with 0.5% toluidine blue in 0.5% borate, and viewed with an Olympus B51 light microscope (Olympus Optical Co.). Thin (70–90 nm) nerve cross-sections for transmission electron microscopy were placed on Glider thin bar 300-mesh grids and stained with uranyl acetate and lead citrate.

2.9. Assays of human growth factors

Single cells or sphere hESC-MSCs were replaced with DMEM/F12 (Gibco), 20% serum replacement (Gibco). After 24 h, the media were collected and then centrifuged at 1200 rpm for 5 min to remove cell debris. The conditioned media were analyzed using human growth factor arrays (RayBiotech, Norcross, GA). Targets; AR|bFGF|b-NGF|EGF|EGFR|FGF-4|FGF-6|FGF-7|GCSF|GDNF|GM-CSF|HB-EGF|HGF|IGFBP-1|IGFBP-2|IGFBP-3|IGFBP-4|IGFBP-6|IGF-I|IGF-I SR|IGF-II|M-CSF|M-CSF R|NT-3|NT-4|PDGFRa|PDGFRb|PDGF-AA|PDGF-AB|PDGF-BB|PlGF|SCF|SCF R|TGF-a|TGF-b|TGF-beta 2|TGF-beta 3|VEGF|VEGF R2|VEGF R3|VEGF-D. The array membranes were blocked with a blocking buffer and incubated with 1 ml of conditioned medium at 4 °C overnight. The membranes were then washed three times with wash buffer I and twice with wash buffer II and assayed by chemiluminescence.

2.10. Statistical analysis

Continuous variables are expressed as mean ± standard error (SE). Differences between continuous variables were analyzed by the unpaired Student *t*-test. Group differences of continuous variables were tested by analysis of variance (ANOVA) followed by Scheffe's post-hoc test. A probability value of <0.05 was considered significant. Statistical analysis was performed using the SPSS 13.0 statistical package (SPSS Inc., Illinois).

3. Results

3.1. Functional analysis of hESC-MSC spheres

In two weeks after the injury and treatment with vehicle or cell, no significant differences were observed between groups. At 4 weeks, however, the hESC-MSC sphere group showed a slight augmentation of compound muscle action potentials (CMAPs), compared with the other groups. A period of 4 weeks after injury can be considered as mainly degenerative phase, although slight reinnervation occurred in the denervated muscles. At 8 weeks, augmentation of CMAPs in the hESC-MSC sphere group was significantly greater than in the other groups (sphere 28.81 ± 3.55 mV vs. group 1, 2, 3; 11.75 ± 1.48 mV, 14.72 ± 1.54 mV, 18.04 ± 2.11 mV respectively, $p < 0.05$) (Fig. 2A). Such a recovery by sphere transplantation was near-complete and comparable to the data of normal controls (sphere 28.81 ± 3.55 mV vs. normal 32.62 ± 2.85 mV, $p =$ ns.)

The results of the walking-foot-print analysis were well correlated with the results of CMAPs (Fig. 2B). In foot print, fine shape of digits cannot be observed when sciatic nerve was injured. However, athymic nude mice transplanted with sphere of hESC-MSCs showed distinctive print of digits from 4 weeks and near-normal data at 8 weeks. Next, to demonstrate the regeneration of injured peripheral nerves, we calculated the voltage that induced the contraction of the gastrocnemius muscle at 8 weeks. In the hESC-MSC sphere group, the required voltage to induce muscle contraction was very low, which was comparable to the voltage in normal mice (sphere 0.38 ± 0.08 V vs normal 0.36 ± 0.67 V) and significantly lower than the voltage in the animals with nerve injury treated by the other measures (sphere 0.38 ± 0.08 vs. group 1, 2, 3; 1.5 ± 0.25, 0.8 ± 0.14, 0.66 ± 0.16 respectively, $p < 0.05$) (Fig. 2C). These data indicate that transplantation of hESC-MSCs as spheres can regenerate the injured nerve to almost normal level.

3.2. Morphologic analysis of transplanted hESC-MSC spheres

At gross examination of the sciatic nerves in five different groups of mice, the injured nerves in nude mice treated with spheres of hESC-MSCs near-completely regenerated, closely resembled normal nerves, and showed the regular rejoining of the resected parts. Such a remarkable recovery was significantly different from the other animals with nerve injury (Fig. 3A). Next at microscopic examination of the sciatic nerves after H&E staining, transplantation of spheres regenerated nerves as the mature circular bundles that were very similar to normal nerves bundle, whereas transplantation of single-cells regenerated nerves as the incomplete bundles (Fig. 3B). Vehicle treatment did not regenerate the nerves as the circular bundles. To evaluate the inside structure of circular nerve bundles, we examined cross-sections of nerves with toluidine blue to stain myelinated axons (Fig. 3C). The normal

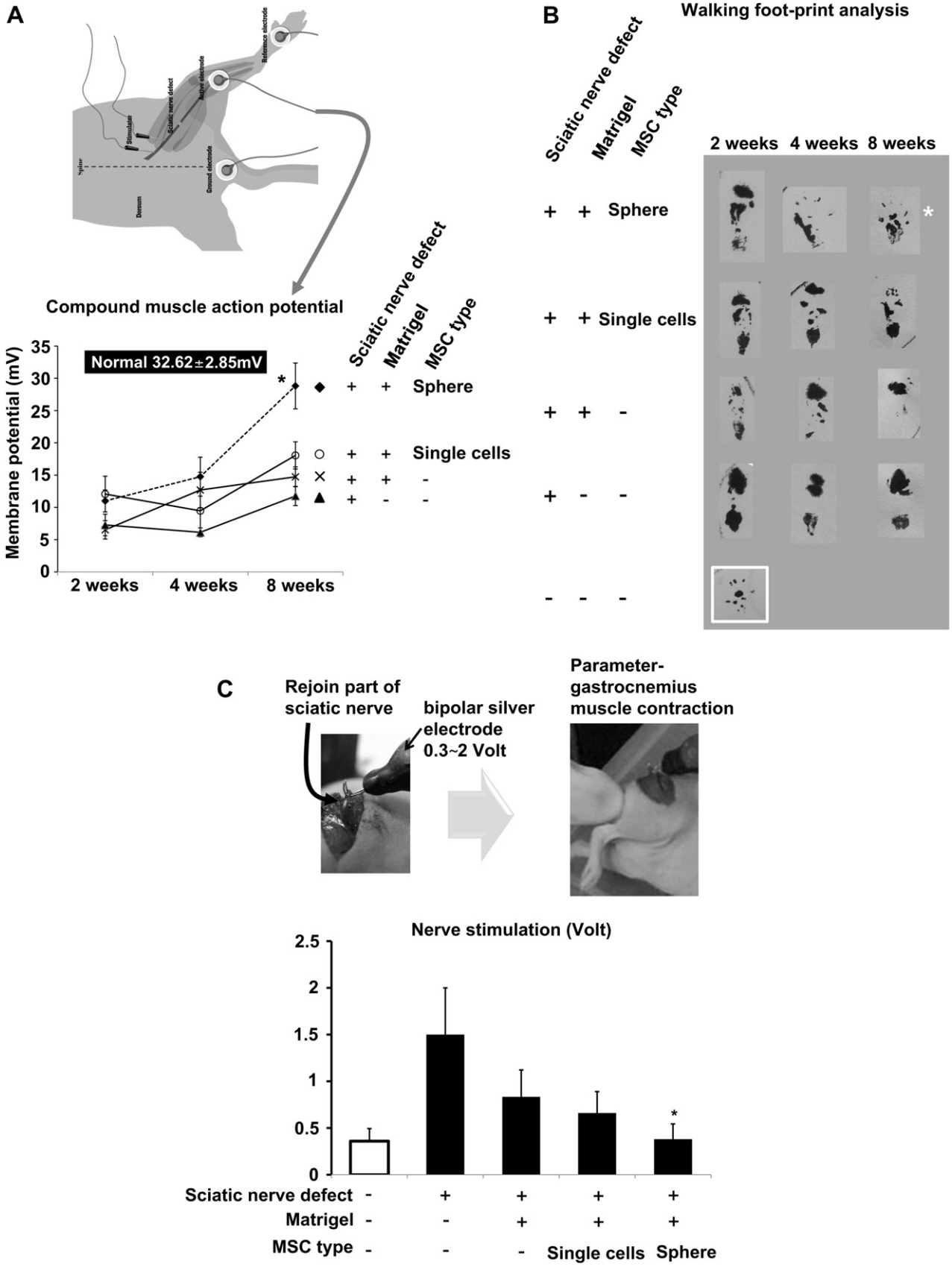

Fig. 2. Functional analysis of the recovery of the injured sciatic nerves in the athymic nude mice treated with hESC-MSCs as spheres or single-cell suspension, or with vehicle. (A) Compound muscle action potential (CMAP) test. In the hESC-MSC sphere group, significant augmentation was detected at 8 weeks when compare with the other groups (sphere 28.81 ± 3.55 mV vs. group 1, 2, 3; 11.75 ± 1.48 mV, 14.72 ± 1.54 mV, 18.04 ± 2.11 mV respectively, $p < 0.05$). Such a recovery by sphere transplantation was near-complete and

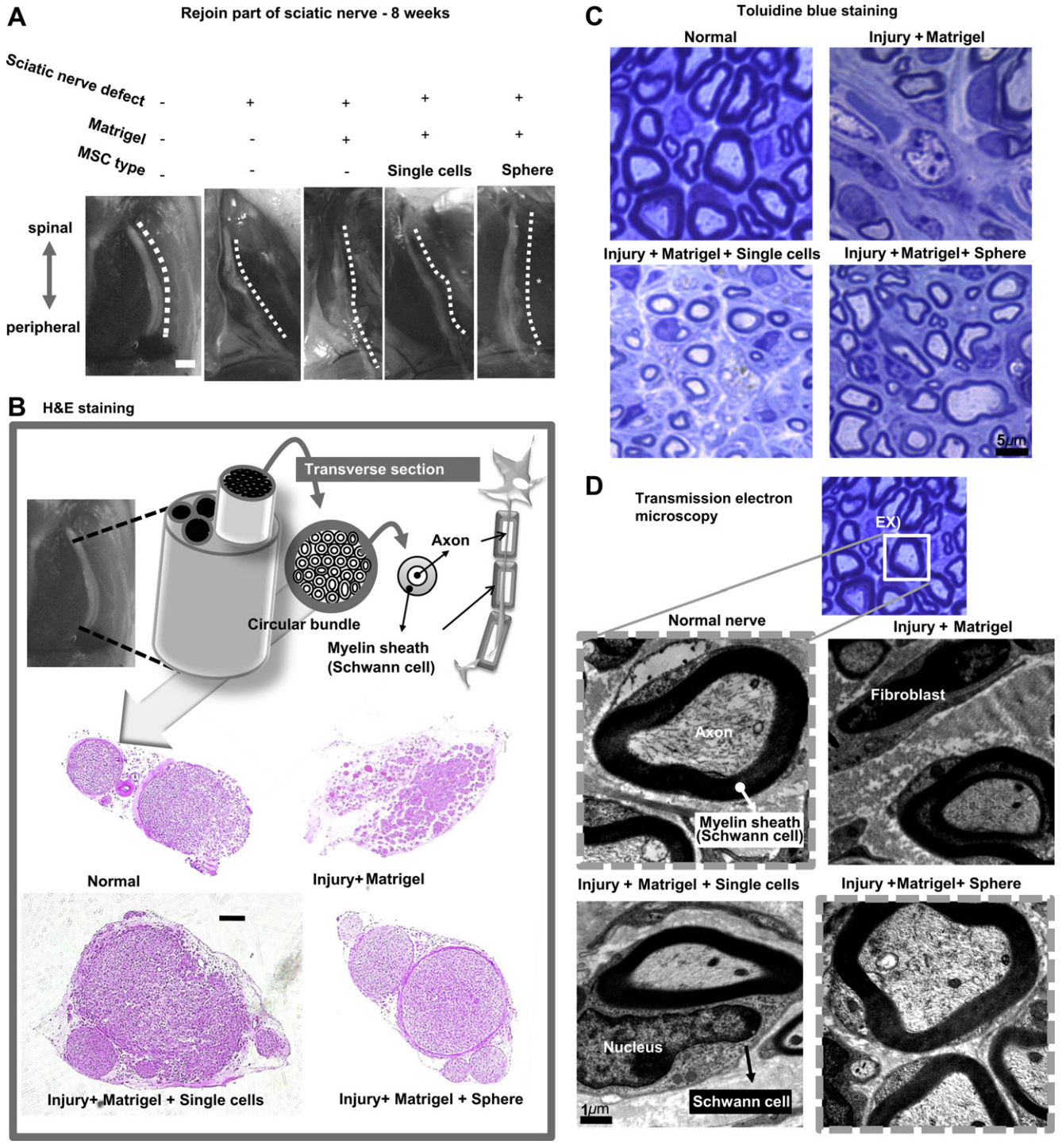

Fig. 3. Histological examination of the recovery of the injured sciatic nerves in the athymic nude mice treated with hESC-MSCs as spheres or single-cell suspension, or with vehicle. (A) At gross examination of the sciatic nerves in five different groups of mice, the injured nerves in nude mice treated with spheres of hESC-MSCs near-completely regenerated, closely resembled normal nerves, and showed the regular rejoining of the resected parts. Such a remarkable recovery was significantly different from the other animals with nerve injury. (B) In microscopic examination of the sciatic nerves after H&E staining, transplantation of spheres regenerated nerves as the mature circular bundles that were very similar to normal nerves bundle, whereas transplantation of single-cells regenerated nerves as the incomplete bundles. Vehicle treatment did not regenerate the nerves as circular bundles. (C) In Toluidine blue (TB) staining, the normal nerve showed numerous large well-myelinated axons. In vehicle-treatment group (matrigel without cell transplantation), axons are rare and not myelinated with abundant collagen bundles and fibroblasts. In single-cell treatment group, myelinated axons were observed even though the numbers were less than in sphere group. In sphere-treatment group, axons are numerous and myelinated with least fibrosis. (D) In transmission electron microscopic examination (TEM), transplantation of the hESC-MSC as spheres enhanced structural maturation and organization of the injured sciatic nerve characterized by better myelination and less fibrosis, which corroborates the findings of toluidine blue staining. In the other groups with nerve injury, invasion of fibroblast or poorly-myelinated axon was observed.

comparable to the data of normal controls (sphere 28.81 $\pm$ 3.55 mV vs normal control 32.62 $\pm$ 2.85 mV). (B) Walking-foot-print analysis. Distinctive digits were observed in sphere group at 4 weeks and the near-normalized digital prints were at 8 weeks (white asterisk). (C) Nerve stimulation test. Gastrocnemius muscle contracted even with very low voltage like 0.38 V in the hESC-MSC sphere group, which was comparable to normal mice (sphere 0.38 $\pm$ 0.08 V vs normal 0.36 $\pm$ 0.67 V). In the remaining groups, the muscle contracted only with much higher voltage (1.5 $\pm$ 0.25, 0.8 $\pm$ 0.14, 0.66 $\pm$ 0.16, vs 0.38 $\pm$ 0.08, $p < 0.05$ respectively).

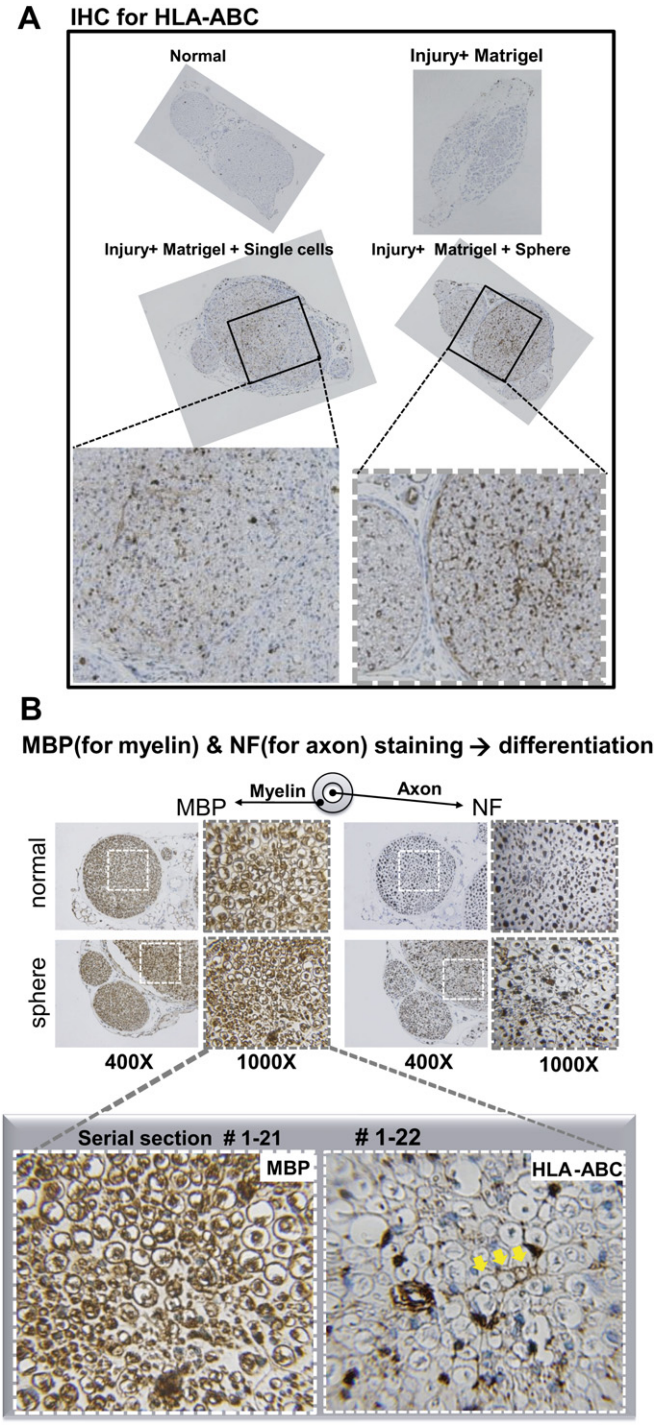

Fig. 4. Tracking the fate of the transplanted hESC-MSC as sphere to the injured sciatic nerve in athymic nude mice. (A) Immunohistochemical staining for HLA-ABC. To trace engrafted cells, the human-specific antibody HLA-ABC was used. A large number of stained cells were detected in the cell transplantation groups (brown color), but not in the control group. Especially, in the sphere group, an organized structure of nerve bundle that are composed of the transplanted human cells, was observed like normal nerve bundle. (B) Immunohistochemical staining for myelin basic protein (MBP) and Neurofilament (NF). To test the possibility of transdifferentiation of hESC-MSCs to Schwann cells or neurons, we performed MBP staining for myelin from Schwann cells and NF for axons from neuron. In serial sections (number 1–21 and 1–22 means sequential slide), we observed HLA-ABC staining (yellow arrow) colocalized with MBP but not with NF. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

nerve showed numerous large well-myelinated axons. In vehicle-treatment group (matrigel without cell transplantation), axons are rare and not myelinated with abundant collagen bundles and fibroblasts. However, in sphere-treatment group, axons are myelinated and numerous with least fibrosis. In single-cell treatment group, myelinated axons were observed even though the numbers were less than in sphere group (Fig. 3C). Finally, we performed transmission electron microscopic examination and found that transplantation of the hESC-MSC as spheres enhanced structural maturation and organization of the injured sciatic nerve characterized by better myelination and less fibrosis, which corroborates the findings of toluidine blue staining (Fig. 3D). In the other groups with nerve injury, invasion of fibroblast or poorly-myelinated axon was observed (Fig. 3D).

3.3. Mechanism of transplanted hESC-MSC spheres

We hypothesized that transplantation as spheres might enhance the efficiency of engraftment and thus tracked down the transplanted human cells in athymic nude mice by IHC using the human-specific antibody for HLA-ABC at 8 weeks after nerve injury and cell therapy. We observed numerous HLA-ABC-positive cells in sphere-treated animals, less positive cells in single-cell suspension-treated ones, and no positive cells in vehicle-treated ones (Fig. 4A). Part of these HLA-ABC–positive cells differentiated into myelinated neurons that were positive for myelin basic proteins (MBP) (Fig. 4B).

In order to seek the plausible explanation of the enhanced engraftment efficiency and the better regenerative efficacy of hESC-MSCs spheres, we analyzed the paracrine capability between sphere versus single-cell suspension of hESC-MSCs. We compared conditioned supernatants from sphere- and monolayer-cultured hESC-MSCs by 41 cytokines array. Interestingly, among of them the secreted amount of HGF and IGFBP-1 were significantly higher in the supernatant of sphere than single cells suspension (Fig. 5).

4. Discussion

4.1. Sequence after peripheral nerve injury

The peripheral nerve regeneration after injury can be divided into the following major events: Wallerian degeneration, axon regeneration/growth, and nerve reinnervation. Wallerian degeneration is a process that starts when a nerve fiber is cut or crushed, in which the part of the axon distal to the injury is separated from the neuron's cell body and degenerates. This is also known as anterograde or orthograde degeneration [12]. After injury, axonal degeneration that is characterized by destruction of axonal skeleton and membrane is followed by degradation of the myelin sheath and infiltration by macrophages. The macrophages, accompanied by Schwann cells, serve to clear the debris from the degeneration [13]. The nerve fiber's neurolemma does not degenerate and remains as a hollow tube. Within 96 h of the injury, the distal end of nerve fiber proximal to the lesion sends out sprouts towards those tubes, which is controlled by growth factors produced from Schwann cells in the tubes. If a sprout reaches the tube, it grows into it and advances about 1 mm per day, eventually reaching and reinnervating the target tissue. In treatment of peripheral nerve defect, the primary aim is a recreating continuity of the nerve to allow axonal regrowth into the nerve tube distal to the lesion [3]. However, despite recent advancement of diagnosis and surgical techniques, the functional recovery of peripheral nerve after injury remains relatively imperfect, requiring new strategy to improve it.

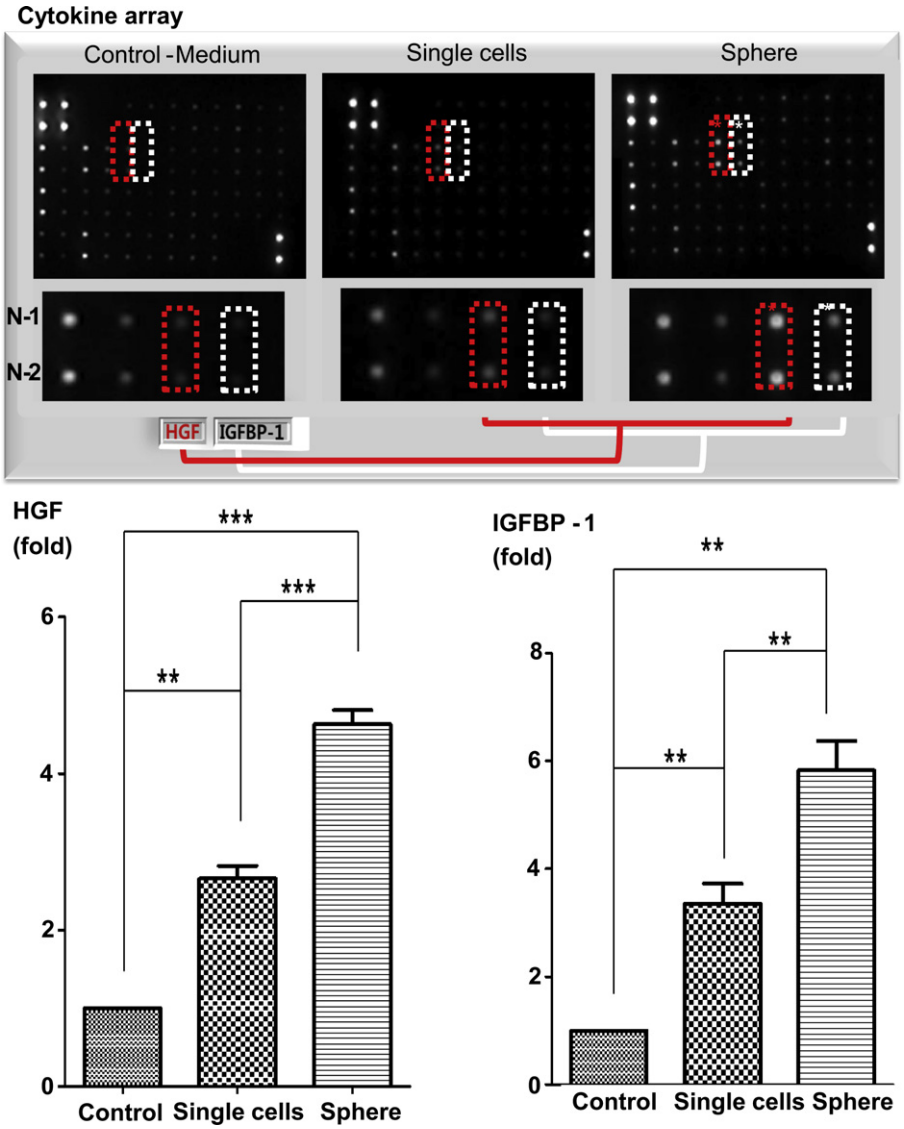

Fig. 5. HGF and IGFBP-1 as key mediator for the therapeutic efficacy of hESC-MSC spheres. In order to seek the plausible explanation of the enhanced engraftment efficiency and the better regenerative efficacy of hESC-MSCs spheres, we analyzed the paracrine capability between sphere versus single-cell suspension of hESC-MSCs. To examine the secretion of growth factors by spheres, we screened the representative 41 cytokines using array (Targets; AR|bFGF|b-NGF|EGF|EGFR|FGF-4|FGF-6|FGF-7|GCSF|GDNF|GM-CSF|HB-EGF|HGF|IGFBP-1|IGFBP-2|IGFBP-3|IGFBP-4|IGFBP-6|IGF-I|IGF-ISR|IGF-II|M-CSF|M-CSFR|NT-3|NT-4|PDGFRa|PDGFRb|PDGF-AA|PDGF-AB|PDGF-B|PlGF|SCF|SCFR|TGF-a|TGF-b|TGF-beta2|TGF-beta3|VEGF|VEGFR2|VEGFR3|VEGF-D). Among them, only HGF and IGFBP-1 secretion were significantly increased by sphere formation than single cell suspension (above 2-fold). $^{**}p < 0.05$, $^{***}p < 0.005$.

4.2. *hESC-MSCs as a promising cell source for nerve regeneration*

Recently, stem cell therapy has been highlighted as a promising option in addition to the present measures to achieve the better efficacy to repair the injured nerves. One possible candidate cell source is adult stem cells. But they have several inherent limitations: insufficient number, inconvenient process to harvest, dysfunctional cells derived from patients, individual variability in their biological characteristics and functionality requiring validation in each donor [14]. To overcome these problems, we have developed a method to derive hMSCs from hESCs [7] and believed that this method may provide a more effective cell source for regenerative medicine. First, they are stable and efficacious cell source because they are easily expanded and maintained. Second, hESC-MSCs have higher regenerative potential because they are derived from hESC. But they are MSCSs, don't produce tumor, thus are safe, and are not rejected even in allogenic transplantation. Third, they are MSCs and have high paracrine actions that would be critical, for example, to induce the regeneration of the resected axons. In addition to the factors mentioned above, we thought that engraftment efficiency was also very important one to determine the therapeutic efficacy. In order to improve the engraftment efficiency, we tried to make spheres from hESC-MSCs, because the maintenance of cell–cell interaction in sphere would help cells survive in the transplanted target tissues. To our expectation, transplantation of hESC-MSCs as spheres was significantly more effective in regenerating the injured sciatic nerves than transplantation of the same cells as a single-cell suspension. The improved therapeutic efficacy was based on the better engraftment rate and higher paracrine activity. Another beauty of hESC-MSC spheres is that preparation process does not require exogenous matrix or cytokines, which may increase the clinical application.

4.3. HGF and IGFBP-1 as a possible mediators in the cell therapy

Interestingly, we detected a significant increase in the secretion of hepatocyte growth factor (HGF) and insulin-like growth factor-binding protein-1 (IGFBP-1) from hESC-MSCs sphere. It has been reported that Schwann cells exhibit an autocrine loop of HGF production, which is necessary for their proliferation [15–17]. Kitamura et al. [18] suggested that the administration of HGF promotes repair and functional recovery after spinal cord injury. Several researchers have suggested that the neurotrophic properties of MSC might induce trans-differentiation to neuron also resulting in nerve repair [19,20].

IGFBP-1 has been reported to be present in the sciatic nerve and to be produced from the activated Schwann cells [21]. In a recent paper, IGFBP-1 regulates human Schwannoma cell proliferation, adhesion, and survival [22]. The role of IGFBP-1 in sciatic nerve defects has not been clearly defined yet. Based on our data in this study, we suggest that IGFBP-1 is also an important neurotrophic factor secreted from the transplanted hESC-MSCs which facilitates repair of Schwann cells and the injured nerves. Next, we plan to assess the sequential change of IGFBP-1 during regeneration of peripheral nerves and also examine its effect on engraftment and trans-differentiation of the transplanted cells. The transplanted hESC-MSCs may differentiate to Schwann cells which help the phagocytosis of myelin and recruitment of macrophages to continue the phagocytosis of myelin in Wallerian degeneration. In addition, trans-differentiation of hESC-MSCs to Schwann cells might also produce myelin sheaths and secrete many neurotrophic factors such as HGF and IGFBP-1 in axon regeneration/growth period.

5. Conclusion

In summary, the injured sciatic nerves in athymic nude mice can be repaired by transplantation of hESC-derived MSCs. The therapeutic efficacy as assessed by several functional tests was better when cells were transplanted as spheres rather than as single-cell suspension. Functional recovery was well corroborated by the consistent histologic findings such as higher engraftment rate as well as greater regeneration of myelinated axons and mature nerve bundles with less replacement fibrosis after transplantation of hESC-MSC as spheres. In the mechanistic analysis, we found two possible neurotrophic factors, HGF and IGFBP-1, that may mediate the therapeutic effect of hESC-MSCs in regeneration of the injured peripheral nerves. Our three-dimensional sphere of hESC-MSCs, that are strong in repair activity and can be supplied consistently and stably, would be an ideal system to overcome the critical limitations of adult stem cells for cell therapy.

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Disclosure

None.

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