

# Migration of Adipose-derived Mesenchymal Stem Cells Stably Expressing Chondroitinase ABC *In vitro*

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## Abstract

**Background:** Several studies have revealed that adipose-derived mesenchymal stem cells (ADSCs) can be used as seed cells for the treatment of spinal cord injury (SCI). Chondroitinase ABC (ChABC) decomposes chondroitin sulfate proteoglycans in the glial scar that forms following SCI, allowing stem cells to penetrate through the scar and promote recovery of nerve function. This study aimed to establish ADSCs that stably express ChABC (ChABC-ADSCs) and evaluate the migratory capability of ChABC-ADSCs *in vitro*.

**Methods:** ADSCs were obtained from Sprague-Dawley rats using secondary collagenase digestion. Their phenotypes were characterized using flow cytometry detection of cell surface antigens and their stem cell properties were confirmed by induction of differentiation. After successful culture, ADSCs were transfected with lentiviral vectors and ChABC-ADSCs were obtained. Proliferation curves of ChABC-ADSCs were determined using the Cell Counting Kit-8 method, ChABC expression was verified using Western blotting, and the migration of ChABC-ADSCs was analyzed using the transwell assay.

**Results:** Secondary collagenase digestion increased the isolation efficiency of primary ADSCs. Following transfection using lentiviral vectors, the proliferation of ChABC-ADSCs was reduced in comparison with control ADSCs at 48 h ( $P < 0.05$ ). And the level of ChABC expression in the ChABC-ADSC group was significantly higher than that of the ADSC group ( $P < 0.05$ ). Moreover, ChABC-ADSC migration in matrigel was significantly enhanced in comparison with the control ( $P < 0.05$ ).

**Conclusions:** Secondary collagenase digestion can be used to effectively isolate ADSCs. ChABC-ADSCs constructed using lentiviral vector transfection stably express ChABC, and ChABC expression significantly enhances the migratory capacity of ADSCs.

**Key words:** Adipose-derived Mesenchymal Stem Cells; Chondroitinase ABC; Spinal Cord Injury

## INTRODUCTION

Spinal cord injury (SCI) seriously affects the survival and quality of life of trauma patients. Although there are many treatments available for SCI, it is still difficult to achieve complete recovery of nerve function.<sup>[1]</sup> However, advances in stem cell therapies have made it possible to restore nerve function following SCI. Stem cells can be induced to form oligodendrocyte progenitor cells (OPCs)<sup>[2]</sup> and neural progenitor cell lines.<sup>[3]</sup> After SCI, OPCs produce a series of neurotrophic immunoregulatory molecules that promote the regeneration of axons.

Survival rates of donor stem cells directly injected in the site of injury in SCI are affected by a number of factors, including ischemia, hypoxia, and inflammation. Therefore, a large quantity of donor stem cells is required for SCI studies. Moreover, a few hours after SCI, activated astrocytes

start to synthesize and secrete large amounts of chondroitin sulfate proteoglycans (CSPGs). CSPGs alter the extracellular matrix, arrest axon regeneration and reconstruction, and inhibit recovery of neural function. In theory, early treatment should offer the best chance of recovery of neural function following SCI.

Adipose-derived mesenchymal stem cells (ADSCs) are mesenchymal stem cells isolated from adipose tissues. ADSCs have attracted extensive attention because they

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**Received:** 18-02-2016 **Edited by:** Qiang Shi  
**How to cite this article:** Wu JH, Li M, Liang Y, Lu T, Duan CY. Migration of Adipose-derived Mesenchymal Stem Cells Stably Expressing Chondroitinase ABC *In vitro*. Chin Med J 2016;129:1592-9.

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10.4103/0366-6999.184464

are easy to obtain<sup>[4,5]</sup> and retain good multidimensional differentiation capacity.<sup>[6,7]</sup> Studies<sup>[8]</sup> have revealed that 2% of adipose tissue comprises ADSCs; consequently, a large number of ADSCs can be obtained from fat, unlike mesenchymal stem cells, which are isolated from bone marrow. Previous investigations have indicated that ADSCs could potentially improve astrocytic inflammatory reactions during SCI. Therefore, it may be possible to treat SCI using ADSCs by promoting neural regeneration and synaptic reconstruction.<sup>[9]</sup>

CSPGs contain glycosaminoglycan (GAG) side chains that play a key role in inhibiting nerve regeneration.<sup>[10]</sup> Removing the GAG side chains or hindering their synthesis can provide favorable conditions to improve axon regeneration and promote the recovery of nerve function. Chondroitinase ABC (ChABC) is an extracellular lyase from *Proteus vulgaris*, which specifically degrades CSPGs by cleaving the GAG side chains.<sup>[11]</sup> ChABC can break down CSPGs to reduce the glial scar, which is conducive to the regeneration of damaged axons, and can restore motor and sensory functions.<sup>[12,13]</sup> Indeed, Sharma *et al.*<sup>[14]</sup> found that SCI patients had increased axon numbers in glial scar tissue following treatment with ChABC. Their results indicated that when ChABC was transplanted onto the surface of the glial scar, OPCs autonomously migrated into the scar and restored function in neurodegenerative disorders.

In this study, ADSCs were first isolated and cultured. After several repeated attempts, we decided to isolate ADSCs using secondary collagenase digestion. Using this method, large quantities of good quality ADSCs were reproducibly achieved, and the isolation and cultivation periods of ADSCs were reduced. Next, lentiviral vectors were used to establish ADSCs that stably expressed ChABC. We investigated the expression of the *P. vulgaris* ChABC gene because of its known ability to degrade CSPGs by cleaving its GAG side chains, reduce glial scar formation, inhibit astrocyte secretions, and promote ADSC migration into glial scars. Following verification of ChABC expression in ADSCs, the proliferation, migration, and other properties of ADSCs that stably express ChABC (ChABC-ADSC) lines were explored.

## METHODS

### Cell isolation and culture

Sprague-Dawley (SD) rats were obtained from the Animal Experiment Department at Central South University, and the experimental methods were approved by the Ethics Committee of Xiangya Hospital, Central South University. ADSCs were isolated and cultured using the method proposed by Zuk *et al.*<sup>[4]</sup> Six-week-old male SD rats were anesthetized with lidocaine and their inguinal fat pads removed. Fat pads were rinsed several times with phosphate buffered saline (PBS), after which blood vessels and fibrous tissues were excised under a dissecting microscope and discarded. The remaining adipose tissues were cut into fine pieces, digested with 0.1% collagenase (17018-029, Gibco, USA)

for 10 min, and centrifuged at 800 ×g for 10 min to remove precipitates. The uppermost white layer (comprising adipose tissue) was collected following centrifugation and digested a second time with an equal volume of 0.1% collagenase at 37°C for 50 min. An equal volume of a complete medium comprising Dulbecco's modified Eagle medium (DMEM/F12; Hyclone, USA) and 10% fetal bovine serum (Gibco, Australia) was added, and the mixture centrifuged again at 800 ×g for 10 min. The precipitate was washed a second time with basal medium (DMEM/F12) and centrifuged at 800 ×g for 10 min. Washed cells were transferred into a 25 cm<sup>2</sup> culture flask containing complete medium and incubated at 37°C with 5% carbon dioxide (CO<sub>2</sub>). The medium was replaced after 24 h, and the suspended cells were washed with PBS when replaced the medium. When confluence reached 70–80%, cells were detached from the culture flask by incubating with 0.25% trypsin (Sigma, USA) at 37°C for 1 min. Complete medium was added and cells were harvested by centrifugation at 800 ×g for 5 min and resuspended in basal culture medium to remove the trypsin. Cells were seeded in a large petri dish, and primary cells were obtained after 4–5 days. The medium was replaced every 2<sup>nd</sup> day in subsequent passages. Cells that had undergone 2–5 passages were used for experiments.

### Identification of surface antigens

Third-generation ADSCs were treated separately using the following antibodies: fluorescein isothiocyanate (FITC)-conjugated anti-CD90, FITC-conjugated anti-CD45, FITC-conjugated anti-CD31, phycoerythrin (PE)-conjugated anti-CD34, and PE/Cy7-conjugated anti-CD29 PE-conjugated anti-CD-11b/c (Cyagen Biosciences, USA). Aliquots of cells were incubated with 2 μg/ml of antibody at 4°C in the dark for 30 min, washed twice with 350 μl of fluorescence-activated cell sorting (FACS) buffer, and centrifugation at 1000 ×g for 5 min. Cell pellets were gently resuspended in 100 μl of buffer and transferred to FACS tubes for analysis using single-channel flow cytometry (BD, USA); after which the percentage of positive cells were calculated for each antibody marker.

### In vitro adipocyte differentiation

Third-generation ADSCs were seeded into a six-well plate (2 × 10<sup>4</sup> cells/ml) with 2 ml of complete medium. Cells were incubated at 37°C with 5% CO<sub>2</sub> and the medium was changed every 48 h. When the cells reached confluence, the medium was carefully removed and 2 ml of adipogenic differentiation medium (Cyagen Biosciences) was added and the cells incubated as before, with medium replacement every 48 h. After 9 days, cells were fixed to the culture plate with 4% neutral formaldehyde solution for 30 min, rinsed twice with PBS, stained with 1 ml of oil red O for 30 min, rinsed with PBS two to three times, and observed under a microscope (Leica, Germany DMI 3000 B).

### In vitro osteoblast differentiation

ADSCs were seeded into a six-well plate (2 × 10<sup>4</sup> cells/ml) that was precoated with 0.1% gelatin, and cultured in

complete medium at 37°C with 5% CO<sub>2</sub>. When confluence reached 60–70%, the medium was replaced with 2 ml of osteogenic differentiation medium (Cyagen Biosciences) and the medium was replaced every 3 days thereafter. After 3 weeks, cells were fixed to the culture plate with 4% neutral formaldehyde solution for 30 min, rinsed twice with PBS, stained with alizarin red S for 3–5 min, rinsed with PBS two to three times, and observed under a microscope (Leica, Germany).

### **In vitro chondrocyte differentiation**

ADSCs were counted and transferred to a 15 ml centrifuge tube. ADSCs were washed with PBS, centrifuged at 800 ×g for 5 min at room temperature (RT), and the supernatant removed. ADSCs were washed a second time in premixed solution at a density of 7.5 × 10<sup>5</sup> cells/ml, centrifuged at 800 ×g for 5 min at RT, and resuspended in complete medium for chondrocyte differentiation of mesenchymal stem cells (Cyagen Biosciences) at a density of 5.0 × 10<sup>5</sup> cells/ml. An aliquot of 0.5 ml of cell suspension (2.5 × 10<sup>5</sup> cells) was transferred to a 15 ml polypropylene centrifuge tube and centrifuged at 800 ×g for 5 min at RT. The lid was loosened to allow air exchange and the cells cultured at 37°C with 5% CO<sub>2</sub>. Every 2 days the medium was replaced, and the bottom of the tube was tapped to float the chondrocyte balls. After continuous differentiation for 26 days, chondrocyte balls were fixed with formalin, paraffin-embedded and stained with alcian blue, then observed under a microscope (Leica, Germany).

### **Transfection of adipose-derived mesenchymal stem cells with lentiviral vectors**

The *ChABC* gene (GenBank No. GQ996964.1) was integrated into LV5-CMV-green fluorescent protein (GFP)-EF1a-Puro to produce the LV5-CMV-GFP-EF1a-Puro-*ChABC* lentiviral vector (GenePharma, Shanghai, China). Third-generation ADSCs were collected, digested into a single cell suspension, seeded into a 12-well plate (2 × 10<sup>4</sup> cells per well), and cultured in 1 ml of complete medium at 37°C with 5% CO<sub>2</sub> overnight. The virus solution was then diluted with serum-free culture medium, an appropriate amount of LV5-CMV-GFP-EF1a-Puro-*ChABC* lentiviral vector and a final concentration of 5 μg/ml polybrene. A negative control was also prepared using the LV5-CMV-GFP-EF1a-Puro lentiviral vector. After ADSCs were exposed to lentivirus for 24 h, medium was replaced with fresh complete medium containing 2% puromycin for a further 24 h to select for transfected cells. At 72 h, GFP expression was visualized using a fluorescent microscope (CKX31, Olympus, Japan) and the optimal multiplicity of infection (MOI) determined.

### **Cell proliferation curves**

*ChABC*-ADSCs and ADSCs were seeded into a 96-well culture plate in two groups using 100 μl of cell suspension in each well (10<sup>3</sup> cells). The culture plate was incubated at 37°C with 5% CO<sub>2</sub>, and 10 μl of Cell Counting Kit-8 (CCK-8) (RiboBio, China) solution was added into each well at appropriate time points (0, 24, 36, 48, 60, and

72 h). After each addition, cells were incubated at 37°C for 3 h and their absorbance at 450 nm was measured using a microplate reader (synergy 2, Beckman, USA). Finally, cell proliferation curves were obtained by plotting time on the horizontal axis against absorbance on the vertical axis.

### **Western blotting analysis**

Whole protein was extracted from passaged *ChABC*-ADSCs and quantified using the bicinchoninic acid method and spectrophotometry (BD, USA). Total protein was electrophoresed on a 10% resolving gel and a 4% polyacrylamide gel and electrically transferred to nitrocellulose membranes. Membranes were blocked by incubating with 5% nonfat milk and 0.05% Tween in Tris-buffered saline on a shaker at RT for 2 h, rinsed three times with PBS, and incubated with a 1:500 dilution of mouse anti-*ChABC* antibody (Novus Biologicals, USA) at 4°C overnight. The next day the membrane was rinsed three times with PBS and incubated with secondary antibody (Proteintech, USA) at 37°C for 1 h. Protein levels were quantified using micro-imaging software and β-actin levels were used as internal reference controls.

### **Transwell assay**

*ChABC*-ADSCs and ADSCs were collected, rinsed with PBS one to two times, resuspended with serum-free culture medium containing bovine serum albumin, and the cell density adjusted to 5 × 10<sup>4</sup> cells/ml. The transwell chamber (Matrigel Invasion Chamber, BD BioCoat, USA) was placed into a culture dish and 300 μl of prewarmed serum-free medium added to the upper chamber. To rehydrate the matrigel, the chamber was allowed to stand at RT for 15–30 min until the medium was absorbed. Then, 200 μl of cell suspension was added to the chamber, 500 μl of complete medium added to the lower chamber, and the apparatus was incubated for 24 h. After incubation, cells in the upper side of transwell membrane in the chamber were wiped off and stained with crystal violet. Stained cells were observed under a microscope, five fields of view with an equal area were selected to count cells, and their mean values calculated.

### **Statistical analysis**

Statistical analyses were performed using SPSS for Windows, version 16.0 (SPSS Inc., IL, USA). All Data were expressed as the mean ± standard deviation (SD) and comparisons between cell lines were performed using a paired *t*-test. A value of *P* < 0.05 was considered significantly different.

## **RESULTS**

### **Morphological characteristics and phenotype of adipose-derived mesenchymal stem cells**

Dense fusiform cell colonies were visible after primary cells were cultured for 12 h. On day 4 of culture, cell colonies were significantly enlarged and cells were fused and required their first passage. The interval between subsequent generations was about 3 days. The third-generation of ADSCs appeared



small and spindle shaped. They had abundant cytoplasm, large nuclei, and prominent nucleoli and were arranged in a vortex shape after cell fusion; and resembled fibroblasts when visualized under a microscope [Figure 1a]. Flow cytometry analysis of ADSC surface antigens showed that CD90, CD73, and CD29 expressions were positive, whereas CD34, CD45, and CD11b/c expressions were negative [Figure 1b].

### Verification of differentiation of adipose-derived mesenchymal stem cells

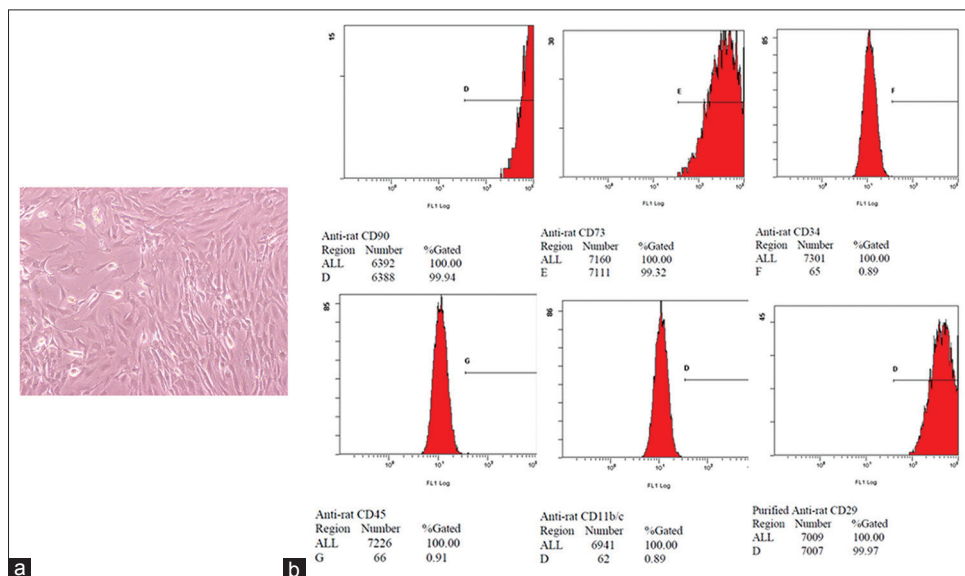
Digested third-generation ADSCs were induced to differentiate into adipocytes, chondrocytes, and osteoblasts by the addition of adipogenic, chondrocytic, and osteogenic medium, respectively. As shown in Figure 2, with oil red O staining, cells differentiated into adipocytes contained circular orange lipid droplets. In cells that were induced to differentiate into chondrocytes, the extracellular matrix stained with alcian blue. With alizarin red staining, cells that were differentiated into osteoblasts contained red calcium depositions.

### Transfection efficiency

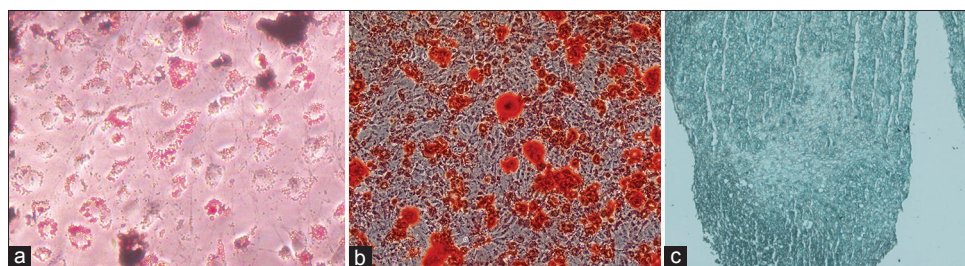
Lentiviral infection with different MOI gradients revealed that the optimal ADSC transfection efficiency was achieved using the LV5-CMV-GFP-EF1a-Puro-ChABC lentiviral vector when the MOI was 100. Use of higher MOI gradients resulted in the death of some ADSCs, with the proportion of dead cells positively correlated with the MOI value. This effect is presumably due to the toxicity of the lentiviral vector and the reagents used. In summary, the study determined that the optimal MOI value for lentiviral vector transfection of ADSCs was 100. To further improve the rate of ChABC-ADSCs formation, unsuccessfully infected ADSCs were screened with puromycin (2 µg/ml) [Figure 3].

### Proliferation curves of adipose-derived mesenchymal stem cells and adipose-derived mesenchymal stem cells that stably express chondroitinase ABC

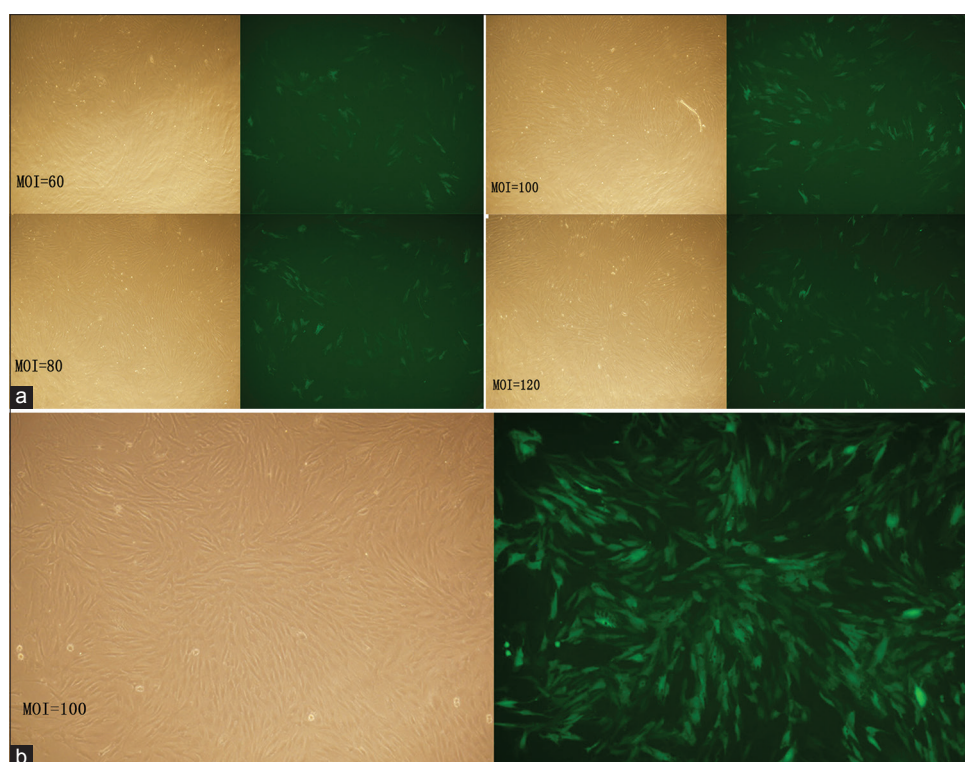
Proliferation curves of ADSCs and ChABC-ADSCs were determined using the CCK-8 method. Comparisons between these groups using paired *t*-tests showed ADSCs



**Figure 1:** Representative cell culture images and flow cytometry identification of ADSCs. (a) ADSCs showed a flattened fibroblast-like and fusiform shape morphology (original magnification, ×100). (b) Flow cytometry analysis of CD marker expression in third-passaged ADSCs. ADSCs: Adipose-derived mesenchymal stem cells; ChABC-ADSCs: ADSCs that stably express chondroitinase ABC.



**Figure 2:** Staining results of differentiation of ADSCs. (a) Adipogenesis was demonstrated by oil red O staining which indicated lipid droplets in the cytoplasm. (b) Osteogenesis was demonstrated by alizarin red S staining which verified the formation of calcium nodes. (c) Chondrogenesis was demonstrated by alcian blue staining which showed the acid mucopolysaccharide in chondrocyte balls. Original magnification, ×40. ADSCs: Adipose-derived mesenchymal stem cells.



**Figure 3:** The GFP expression of ChABC-ADSCs in different MOI and final outcome of infection. (a) Three days after lentivirus-infected adipose-ADSCs, fluorescent microscope to observe the expression of GFP in different MOI values. (b) Lentivirus-infected adipose-ADSCs were treated with 2  $\mu\text{g/ml}$  puromycin for 24 h (original magnification,  $\times 40$ ). GFP: Green fluorescent protein; MOI: Multiplicity of infection; ADSCs: Adipose-derived mesenchymal stem cells; ChABC-ADSCs: ADSCs that stably express chondroitinase ABC.

proliferative capacity was decreased in 48 h ( $P < 0.05$ ) meanwhile ChABC-ADSCs still in a period of quick proliferation [Figure 4].

### Determination of chondroitinase ABC synthesis with Western blotting

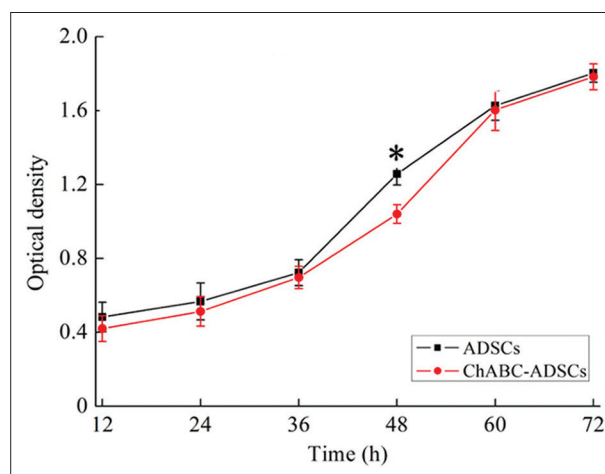
Western blotting was used to determine the relative level of ChABC expression in ChABC-ADSCs in comparison with an internal reference protein. Using gray scale values, the signal intensity of ChABC was compared with that of the reference protein  $\beta$ -actin. Results revealed that the level of ChABC expression in the ChABC-ADSC group was significantly higher than that of the ADSC group [ $P < 0.05$ , Figure 5a].

### Effect of chondroitinase ABC on adipose-derived mesenchymal stem cell migration in transwell assay

After 24 h, the number of cells penetrating into the matrigel (mean of 5 views with an equal area) from the ChABC-ADSC group was significantly higher than that of the ADSC group [ $P < 0.05$ , Figure 5b].

## DISCUSSION

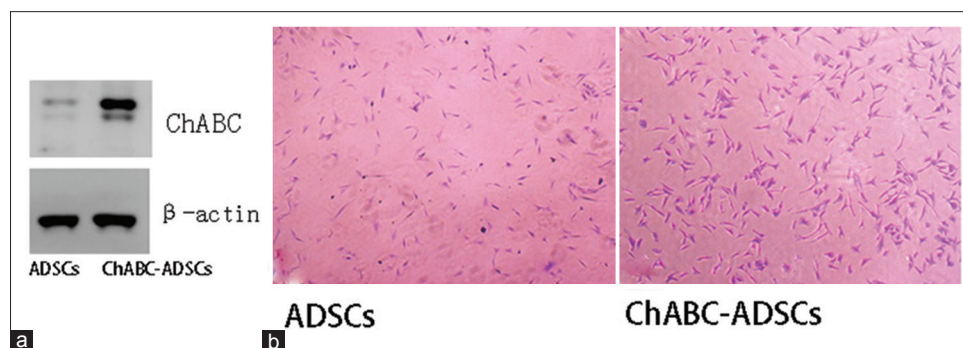
Traumatic SCI seriously affects the health and quality of life of patients. Although there are many methods to treat SCI, there are still many challenges to the regeneration and repair of nerves following SCI.<sup>[15]</sup> Stem cell transplantation has brought with it a new possibility of rehabilitation of patients suffering from SCI. ADSCs have the advantages that they are easy to obtain, have strong proliferation, can



**Figure 4:** ChABC-ADSCs proliferation affected by lentivirus. ADSCs proliferative capacity was decreased at 48 h. \*Compared with ChABC-ADSCs ( $P < 0.05$ ,  $n = 3$ ). ADSCs: Adipose-derived mesenchymal stem cells; ChABC-ADSCs: ADSCs that stably express chondroitinase ABC.

be stably passaged,<sup>[4]</sup> and can be induced to differentiate into neural tissue. They also do not express human tissue histocompatibility antigens,<sup>[16]</sup> can inhibit proliferation and secretion of T lymphocytes *in vitro*, and are not associated with graft versus host diseases *in vivo*.<sup>[17]</sup> Kingham *et al.*<sup>[18]</sup> differentiated ADSCs into Schwann cell precursors to treat SCI. In this study, ADSCs were selected as seed cells for the treatment of SCI.





**Figure 5:** Western blotting results of ChABC expression and transwell analysis of cell migration. (a) Western blotting results revealed that the level of ChABC in the ChABC-ADSCs group was higher compared with the ADSCs group ( $P < 0.05$ ,  $n = 3$ ). (b) Representative images of transwell assay results of ADSCs and ChABC-ADSCs. Cells were stained with crystal violet. Cell migration in ChABC-ADSCs group was increased compared with the ADSCs group ( $P < 0.05$ ,  $n = 3$ ) (original magnification,  $\times 40$ ). ADSCs: Adipose-derived mesenchymal stem cells; ChABC-ADSCs: ADSCs that stably express chondroitinase ABC.

Zuk *et al.*<sup>[4]</sup> first proposed a trypsin digestion method for isolation and culture of ADSCs, together with a direct culture method.<sup>[19]</sup> However, they found that it took more than 7 days to obtain primary ADSCs and only a small number of cell colonies could be obtained. After repeated attempts, the present study successfully developed a modified secondary digestion method to obtain ADSCs. Inguinal fat pads of SD rats were cut into pieces, predigested with 0.1% collagenase for 10 min and centrifuged to discard the precipitate. The uppermost layer of white adipose tissue was again digested with an equal volume of 0.1% collagenase for 50 min, and then the ADSCs were precipitated, resuspended, and cultured. After 12 h, the adherent growth of cell colonies was visible. These results show that this modified method can effectively improve the number of primary colonies of ADSCs that can be obtained.

Cell surface antigens were verified using flow cytometry, with following results: CD90 (+), CD73 (+), CD29 (+), CD34 (-), CD45 (-), and CD11b/c (-). These results are consistent with ADSC surface antigen expression profiles obtained in previous studies.<sup>[20]</sup> In addition, the present study has successfully differentiated ADSCs into adipocytes, osteoblasts, and chondrocytes verifying their differentiation capacity. These results suggest that using the modified secondary digestion method, the obtained ADSCs retain their differentiation capacity. These cells express low levels of hematopoietic-stem-cell-specific surface antigens and high levels of stem-cell-specific surface antigens.

The modified secondary digestion method removed most of the fibrous connective tissue, red blood cells, and other impurities from the adipose pieces. It reduced the amount of fibrous material that adhered to the primary cells and increased the number of adherent cells in primary culture. Together, these features effectively improved the isolation efficiency of ADSCs from the adipose tissue. Using this modified ADSC extraction method, the first generation passage can be achieved at around 4 days, significantly shortening the required proliferation time of the primary cells.

Activation of astrocytes and oligodendrocytes precursors appears 24 h after SCI, with glial scars forming through

the secretion of CSPGs.<sup>[21,22]</sup> CSPGs are potent inhibitors of neurite outgrowth and may play a key role in preventing regeneration following the central nervous system injury. This process may occur through the formation of physical barriers and chemical suppression,<sup>[23-25]</sup> as well as by strongly inhibiting the formation of the nervous lateral bud.<sup>[26]</sup> Because CSPG-mediated inhibition of nerve regeneration is mainly through the GAG side chains, it is generally believed that removing the GAG side chains or interfering with their synthesis could promote axonal regeneration.<sup>[27]</sup> ChABC derived from actinomycetes specifically degrades the GAG side chains of most CSPGs and has been widely used in the treatment of SCI. Tom *et al.*<sup>[28]</sup> achieved positive results after intrathecally injecting SCI rats with ChABC, which could increase regeneration of related proteins in neurons and consequently increase neuronal regeneration and promote the recovery of motor and sensory functions. Sekiya *et al.*<sup>[29]</sup> found that ChABC on the surface of glial scars improved cell transplantation outcomes in the treatment of nerve injury. ChABC is a nontumorigenic gene<sup>[30]</sup> and its long-term expression *in vivo* is unlikely to increase tumorigenic risk. This property makes it possible to construct ADSC lines that stably express ChABC by using lentiviral vectors.

In this study, an LV5-CMV-GFP-EF1a-Puro-ChABC lentiviral vector was used to construct stable ADSC lines expressing ChABC. When the optimal MOI (transduction unit per cell, found to be 100) for ADSC transfection was used, GFP expression was more than 50%. This transfection efficiency was increased to more than 90% after puromycin screening, providing a reliable safeguard for subsequent experiments. Western blotting confirmed that passage of ChABC-ADSC lines was unlikely to affect ChABC expression efficiency after two passages. Cell proliferation assays using the CCK-8 method demonstrated that ADSCs proliferative capacity was decreased at 48 h after seeding, meanwhile ChABC-ADSCs still in a period of quick proliferation. However, the concrete mechanism of the decrease needs further study. Combined, the results in this study demonstrate that ChABC-ADSC lines can express ChABC *in vitro* stably and efficiently over a long period.

Cattin *et al.*<sup>[31]</sup> found that nerve regeneration requires migration and accumulation of Schwann cells to form a “bridge” to conduct the severed axons. In the peripheral nervous system, axons can grow but cannot penetrate the glial scar.<sup>[12]</sup> This study further explored the impact of ChABC intervention on the migration of ADSCs using a transwell assay. Results confirmed that ChABC-ADSCs can uniformly penetrate the matrix membrane and ChABC expression enhances their migration. Reginensi *et al.*<sup>[32]</sup> found that by degrading CSPGs, ChABC could weaken the glial scar barrier, makes it much easier to traverse. Ikegami *et al.*<sup>[28]</sup> found that CSPGs inhibited the migration of neural stem/progenitor cells and ChABC attenuated this inhibitory effect *in vitro*. ChABC-ADSCs synthesize biologically active ChABC that both degrades the glial scar and provides favorable conditions for ADSCs to migrate through the matrix. A previous study has shown that when donor cells are placed on the surface of a ChABC-treated gliotic nerve, cells migrate into the nerve autonomously.<sup>[29]</sup> Furthermore, the channel generated by the migration of ADSCs can open up a pilot hole for the later growth of nerve cells and axons, creating conditions for axon regeneration and penetration of the glial scar. This process thereby promotes recovery of sensory and motor functions after SCI.

In summary, the secondary collagenase digestion method is effective for isolating ADSCs. ChABC-ADSCs constructed using lentiviral vectors stably express ChABC and this expression can significantly enhance the migration of ADSCs in matrigel.

### Financial support and sponsorship

This work was supported by a grant from the National Natural Science Foundation of China (No. 81472073).

### Conflicts of interest

There are no conflicts of interest.

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