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Wnt/β-catenin signaling regulates neuronal differentiation of mesenchymal stem cells

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ABSTRACT

Mesenchymal stem cells (MSCs) have been demonstrated to be able to differentiate into neuron-like cells, but the precise mechanisms controlling this process are unclear. Using neuron-specific enolase (NSE) and nestin as neuronal markers, we examined the role of Wnt/β-catenin signaling in MSC neuronal differentiation in present study. The results indicated that the expression of β-catenin increased markedly during the neuronal differentiation of MSCs. Blocking Wnt signaling by treating MSCs with β-catenin siRNA could decrease the differentiation of MSCs into neuron-like cells and up-regulation of Wnt signaling by treating MSCs with Wnt-3a could promote neuronal differentiation of MSCs. Above results suggest that Wnt/β-catenin signaling may play a pivotal role in neuronal differentiation of MSCs. Our data broaden the knowledge of molecular mechanisms involved in the neuronal differentiation of MSCs and provide a potential target for directing differentiation of MSCs for clinical application.

1. Introduction

Mesenchymal stem cells (MSCs) are an adherent, fibroblast-like population with the potential for extensive self-renewal and multilineage differentiation, presenting exciting possibilities for cellular therapy [1]. In addition to their differentiation into osteoblasts, chondroblasts, adipocytes and myoblasts, recent studies have demonstrated that both human and rodent MSCs are able to differentiate into neuron-like cells [2,3]. As a potential alternative source of neurons, MSCs have been applied to replace damaged cells in the nervous system using animal models of neurological disorders or traumatic brain injury [4,5]. Though MSCs have been shown to be able to differentiate into neurons, the precise mechanisms controlling this process are still poorly understood.

Wnt signaling was known to be crucial in regulating embryonic development, cell proliferation and cell fate determination. In canonical Wnt signaling pathway, β-catenin plays a central role as a transcriptional coactivator. When extracellular secreted Wnts interact with the receptor, Frizzled and low density lipoprotein receptor related protein (LRP), β-catenin is hypophosphorylated, stabilized and translocated into the nucleus where it induces transcription of target gene expression. Recently, Wnt signaling has been implicated in the control of MSC differentiation, including osteogenic, chondrogenic, adipogenic, and myogenic differentiation [6]. In addition, a growing body of evidence suggests that Wnt signaling plays pivotal roles in cell fate specification in the nervous systems [7]. Wnt has also been shown to promote neuronal differentiation from embryonic, somatic, and neural stem cells [8,9]. On the basis of these previous studies, we hypothesized that Wnt signaling was involved in the neuronal differentiation of MSCs. In present study, we therefore examined the effects of Wnt/β-catenin signaling on rat MSC neuronal differentiation in vitro.

2. Materials and methods

2.1. Generation and culture of MSCs

Male Sprague–Dawley (SD) rats (weighing about 100 g) were purchased from Zhejiang University Animal Center. All animal investigations were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by NIH and approved by the Institutional Animal Care Committee of Zhejiang University. MSCs were obtained from rat femoral and tibial bone marrow as previously described [10]. Briefly, muscles and the entire connective tissue were detached, and the epiphyses were removed. Marrow was harvested by inserting an 18-gauge syringe needle into one end of the bone shaft and flushing the contents into a 60-mm culture dish containing proliferation culture medium, consisting of Dulbecco's Modified Eagle's Medium-F12

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(DMEM-F12) (Invitrogen) supplemented with 10% (vol/vol) fetal bovine serum (FBS) (Invitrogen). Cells were then centrifuged, and nucleated cells were counted and seeded at a density of 5×10^5 - cells/cm^2 in culture medium at 37 °C in 5% CO_2. After 24 h, all non-adherent cells were removed by medium exchange; medium was subsequently replaced every 3 days. The monolayer of adherent cells was trypsinized (0.25% trypsin-EDTA; Invitrogen) at 80% confluence, resuspended in culture medium, and seeded at a density of 10,000 cells/cm^2. Passage 3–6 was used in this study.

2.2. Neuronal induction

Neuronal induction of MSCs was performed according to the protocol of Woodbury et al. [2]. Briefly, MSCs were incubated in the preinduction medium containing of DMEM-F12/20% FBS/ 1 mM β-mercaptoethanol (BME) for 24 h. After removal of the preinduction medium, the cells were transferred into the neuronal induction medium consisting of DMEM-F12/5 mM BME. MSCs were collected before preinduction, at 6 h, 12 h, 24 h of preinduction and at 1 h, 3 h, 5 h of post-induction. Both protein and mRNA levels of β-catenin and the specific marking proteins of neurons were detected.

2.3. siRNA assay

MSCs were planted into a 6-well plate at a density of 1×10^5 - per well and cultured in proliferation medium containing DMEM-F12 and 20% FBS (antibiotics free). The cells reached 50–60% confluence, followed by serum starvation for 12 h. For siRNA inhibition studies, MSCs were transfected with validated β-catenin siRNA or negative control siRNA using the Lipofectamine protocol (Invitrogen). GFP expression was established by fluorescence microscopy and transfection efficiency was tested. After siRNA transfection for 72 h, the cells were prepared for neuronal induction. The treated cells were collected for mRNA and protein analysis.

2.4. Wnt-3a treatment

MSCs were planted into a 6-well plate at a density of 1×10^5 - per well. On reaching 70%, MSCs were incubated in the neuronal induction media in presence or absence of 100 ng/ml Wnt-3a (Peprotech). The treated cells were collected for mRNA and protein detection.

2.5. RT-PCR assay

Total cellular RNA was extracted using Trizol reagent (Invitrogen) according to the manufacturer's protocol. Total RNA (1 μg) was used for cDNA synthesis using PrimeScript™ RT-PCR Kit (Takara). The expression level of each gene was determined by semi-qRT-PCR method. The PCR was carried out in 25 μl reaction volume using the Taq™ PCR Kit (Takara). Each cycle consisted of a denaturation step at 94 °C for 30 s, an annealing step at appropriate annealing temperature for 30 s and extension step at 72 °C for 1 min. The final extension step was followed by a 7 min extension reaction at 72 °C. Primer sequences were listed as follows: β-catenin: F: GACACCTCCCAAGTCCTTTATG R: GTACAACGGGCTGTTTCTACG; NSE: F: CCCTCTATCGCCACATTGCTC R: AAGGGTCAGCGG GAGACTTGA; nestin: F: GGAGCCATTGTGGTCTACTGA R: GATGCAACTCTGCCTTATCC; Ngn1: F: CACTCGGCCCACATTCAAGC R: TCGTCGGTGAGGAAACTGGA. The amplified fragments were separated by 1% agarose gel electrophoresis. Images of the RT-PCR stained with ethidium bromide were analyzed with Gel-PRO Analyzer (Bio-Rad). The band intensity of the genes of interest was normalized to β-actin.

2.6. Western blotting

The protein contents of the cell lysates were determined using a Micro BCA Kit (Thermo). Protein from the cell lysates was mixed with 4× loading buffer containing 10 mM dithiothreitol and boiled for 10 min, before electrophoresis on 10% sodium dodecyl sulfate–polyacrylamide gels. Following transfer onto polyvinylidene fluoride membranes and blocking, membranes were incubated overnight at 4 °C with β-catenin (1:1000, Cell Signal Technology), neuron-specific enolase (NSE) (1:500, ENZO Life Science), nestin (1:500, R&D), Ngn1 (1:500, Cell Signal Technology) or β-actin mAb (1:2000, Sigma–Aldrich). Following several washes in TBST, membranes were subsequently incubated for 1 h with horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse IgG antibody (Sigma–Aldrich) diluted 1:2000 in blocking buffer. The membrane was washed 3 times with TBST. The signals were detected by enhanced chemiluminescence reagents (Biological Industries) and exposure to X-ray film. The density of the bands was quantified using ImageJ software (National Institutes of Health). Expression of protein was normalized to β-actin expression in the same lane.

2.7. Immunofluorescence assay

For immunofluorescence analysis, cells were first fixed with 4% paraformaldehyde for 20 min and washed with PBS. Next, the cells were incubated with 0.2% Triton-100 for 5 min. Nonspecific binding was blocked by incubation with PBS containing 10% normal goat serum at room temperature for 1 h. After removing excess serum, the slides were incubated overnight at 4 °C with the mouse monoclonal antibody against β-catenin (1:1000, Cell Signal Technology) or nestin (1:500, R&D). After washes with PBS, cells were incubated with FITC or Cy3-conjugated goat anti-rabbit secondary antibodies in the dark for 30 min. Nuclei were stained with DAPI (Boster). After washing the slides, immunofluorescence was detected using a fluorescent microscope (Nikon, Japan).

2.8. Statistical analysis

All data are presented as mean ± SD. Differences between group means were assessed by analysis of variance for multiple comparisons using SPSS 16.0. A P-value of <0.05 was considered significant.

3. Results

3.1. MSCs differentiated into neuron-like cells after induction in vitro

After three passages, rat bone marrow derived MSCs were shown a homogeneous fibroblast-like and spindle-shaped morphology (Fig. 1A). To exclude the possibility of haematopoietic contamination, we examine the phenotype of MSCs. FACS analysis demonstrated that MSCs expressed CD44, CD90 and CD29, but not CD34 and CD45 (data not shown). In addition, cell adipogenic, osteogenic, and chondrogenic differentiation capacity was assessed as our previous study demonstrated [10].

Neuronal differentiation of MSCs was induced as previous studies [2]. To induce the neuronal phenotype, MSCs were maintained in preinduction medium containing 20% serum and 1 mM BME for 24 h. After preinduction, the changes in morphology of the MSCs were not apparent (Fig. 1B). To induce efficient neuronal differentiation, the cells were transferred to serum-free medium containing 5 mM BME. Within 60 min of exposure to serum-free induction medium, cytoplasm in the flat MSCs retracted towards the nucleus, forming a contracted multipolar cell body. Over the

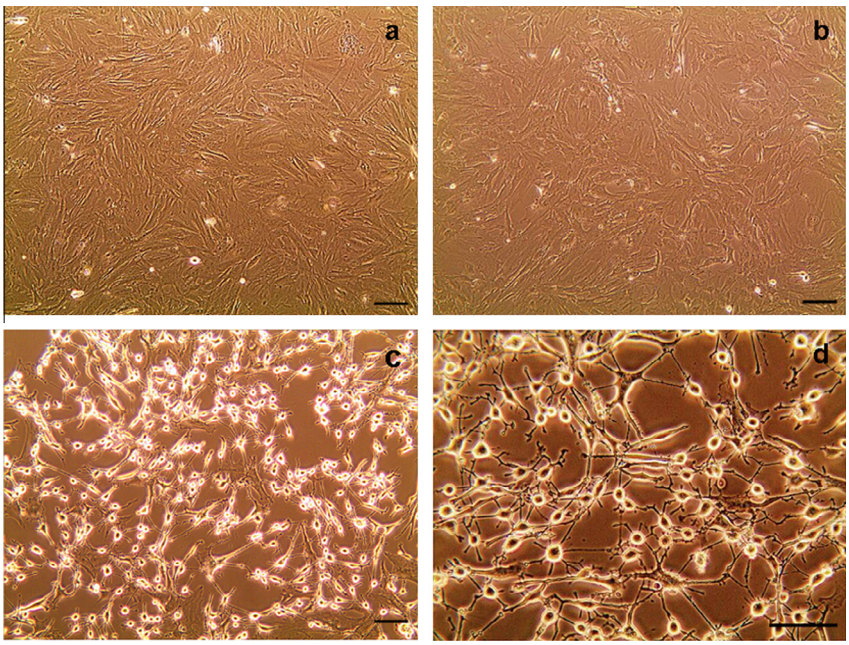

Fig. 1. Neuronal differentiation of rat MSCs. (A) Morphology of undifferentiated MSCs (100×). (B) Morphology of MSCs treated with pre-induction medium for 24 h (100×). (C) Morphology of neuron-like cells differentiated from MSCs (100×). (D) Morphology of neuron-like cells differentiated from MSCs (200×).

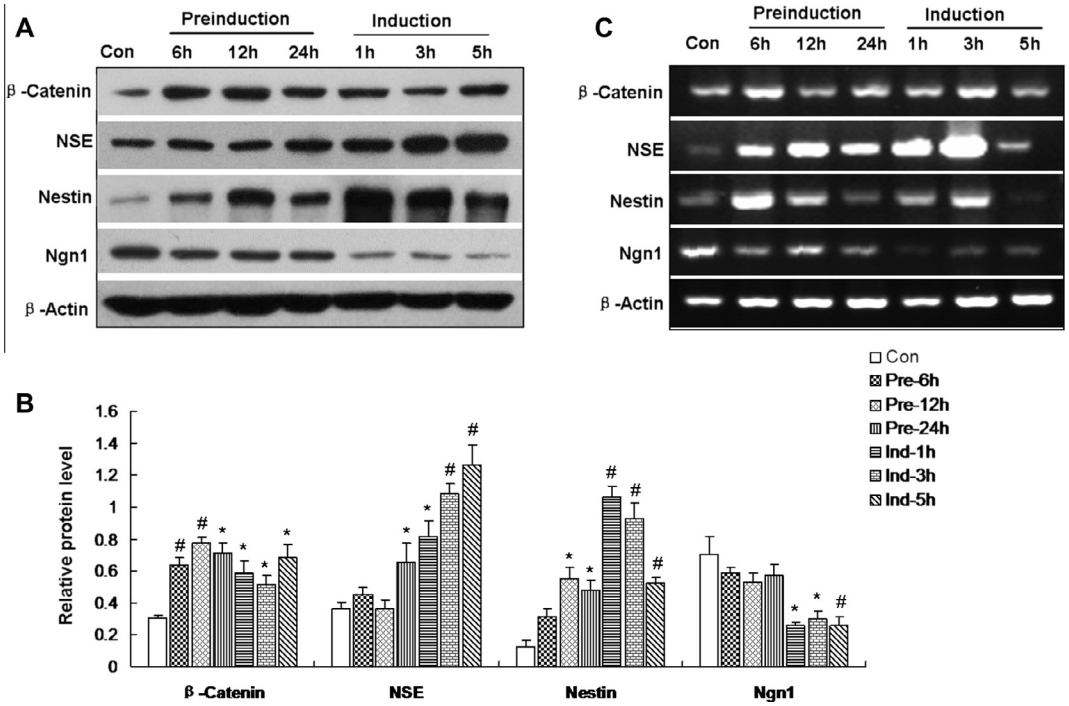

Fig. 2. β-Catenin increased during neuronal differentiation of MSCs. (A) Protein level of β-catenin, NSE, nesin and Ngn1 during neuronal induction of MSCs. β-Actin was used as an inner control. (B) Quantification of β-catenin, NSE, nesin and Ngn1 protein expression normalized to β-actin. $^{*}P < 0.05$ vs. control. $^{\#}P < 0.01$ vs. control. (C) mRNA levels of β-catenin, NSE, nesin and Ngn1 during neuronal induction of MSCs.

subsequent 2 h, cell bodies became increasingly spherical and refractile, exhibiting a typical neuronal appearance (Fig. 1C and D).

To characterize neuronal differentiation further, we detected the expression of neuronal marker neuron-specific enolase (NSE) and nestin. The results showed that uninduced MSCs expressed low levels of NSE and nestin both at protein and mRNA levels (Lane 1 in Fig. 2A and Lane 1 in Fig. 2B). Induction of the neuronal phenotype resulted in a dramatic increase in NSE and nestin expression (last three lanes in Fig. 2A and B). These results confirmed that rat MSCs can differentiate into neuron-like cells after induction in vitro.

3.2. β-Catenin increased during neuronal differentiation of MSCs

To investigate the role of Wnt/β-catenin signaling in the neuronal differentiation of MSCs, we detected the expression kinetics of β-catenin, the key member of Wnt signaling. As shown in Fig. 2A, β-catenin was expressed at a low level at the undifferentiated

state, whereas the protein levels of β-catenin increased significantly from 6 h of preinduction, reach a peak at 12 h, and maintained at a steady level until 5 h of post-induction. Consistent with the results of protein detection, the mRNA level of β-catenin exhibited the similar trend (Fig. 2C). Correspondently, the protein and mRNA levels of both neuronal markers NSE and nestin were increased in a time dependent manner during the induction process (Fig. 2). In addition, we observed the change in the expression of neuron-specific transcription factors, Neurogenin1 (Ngn1). The undifferentiated MSCs expressed a high level of Ngn1, indicating that MSCs possess the potential of neuronal differentiation. During the pre-induction process, the expression of Ngn1 decreased slightly. However, both protein and mRNA levels of Ngn1 were decreased obviously while neuronal induction (Fig. 2).

3.3. β-Catenin knockdown inhibited neuronal differentiation of MSCs

To further confirm the roles of Wnt/β-catenin pathway in the neuronal differentiation, we performed the knockdown experiments by transfecting MSCs with β-catenin siRNA. As shown in Fig. 3A, siRNA transfection leads to a 70% down expression of β-catenin in neuronal induction cells. Accordingly, the expression of neuronal markers NSE and nestin decreased dramatically on the differentiated MSCs with β-catenin siRNA transfection. In addition, β-catenin inhibition resulted in an increase of Ngn1 in neuronal induced MSCs. Consistent with the results of Western blotting, the results of immunofluorescence assay also indicated that β-catenin knockdown inhibited neuronal differentiation of MSCs (Fig. 4). In summary, blockade of the β-catenin by siRNA inhibited the neuronal differentiation of MSCs.

3.4. Wnt-3a promoted neuronal differentiation of MSCs

To up-regulate Wnt signaling pathway, recombinant Wnt-3a (100 ng/mL) was added in the neuronal induction medium. Total β-catenin expression of differentiated MSCs was increased by Wnt-3a (Fig. 3C and D). Accordingly, incubation of MSCs in neuronal induction medium supplemented with Wnt-3a resulted in a significant upregulation of NSE and nestin. The immunofluorescence results showed that the neuron marker nestin was increased dramatically in presence of Wnt-3a accompanied by the enhancing of β-catenin (Fig. 4). Taken together, stimulation of the Wnt signaling pathway by Wnt-3a promoted the neuronal differentiation of MSCs.

4. Discussion

MSCs from bone marrow have been broadly studied for use in the treatment of neurological disorders [11–13]. Many studies have demonstrated that MSCs can give rise to neuronal cells, both in vitro and in vivo [2,3,14]. Transdifferentiation of bone-marrow-derived MSCs into neuronal cells can be obtained by manipulation of culture conditions and biochemical supplements to which the cells were exposed and in which they were maintained. In this study, we used BME, the chemical compound widely used, for neuronal induction in vitro. Consistent with previous studies, our results showed that MSCs acquired neuronal-like morphology and neuronal marker expression after induction. Nestin, a class VI intermediate filament protein, is expressed exclusively on neural progenitor cells in normal brain [15]. Our data showed that most of the induced neuron-like cells expressed nestin strongly, indicating that the differentiated cells were under a progenitor cell state. Using this differentiation model, we explored the mechanisms of neuronal differentiation of MSCs.

Considerable evidence suggested a crucial role of Wnt/β-catenin signaling in cell fate decision of MSCs and neurogenesis. Etheridge et al. [16] demonstrated that MSCs express a wide range of components of Wnt signaling including LRP-5, secreted Frizzled-related peptide (sFRP)-2, sFRP3, sFRP4, GSK-3β, β-catenin, T-cell factor (TCF)-1 and TCF-4. Wnt signaling controls the self-renewal and multipotent differentiation of MSCs (review in [6]). In addition, Wnt signaling participates in the regulation of almost every aspect

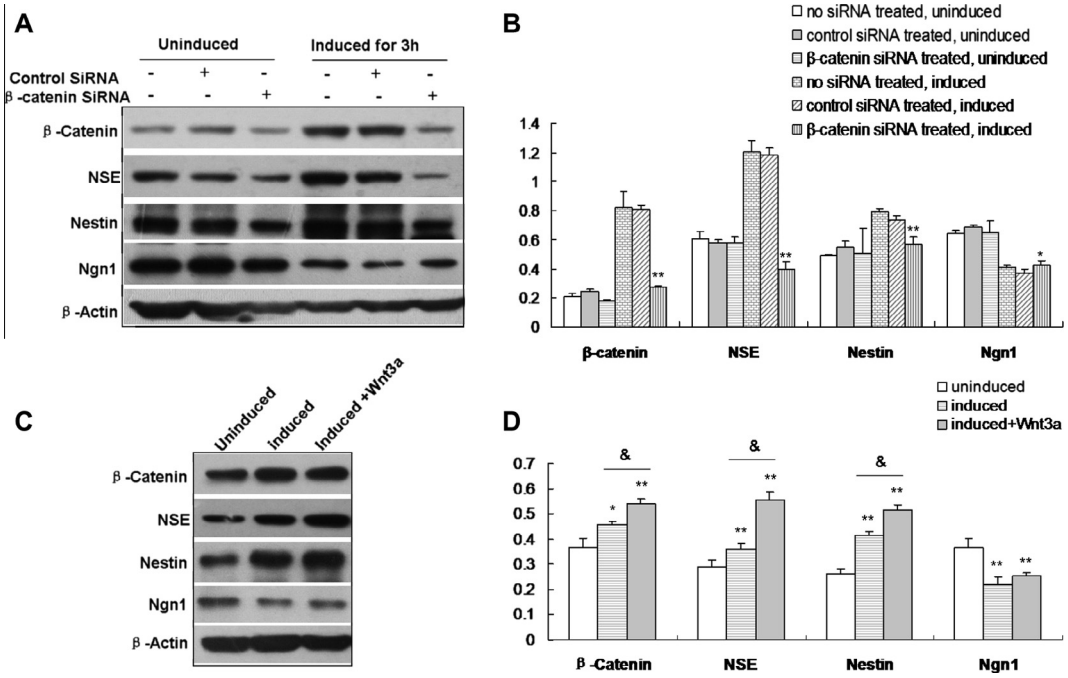

Fig. 3. β-Catenin knockdown inhibited neuronal differentiation and Wnt-3a promoted neuronal differentiation of MSCs. (A) Protein levels of β-catenin, NSE, nesin and Ngn1. MSCs were transfected with control siRNA or β-catenin siRNA. After siRNA transfection for 72 h, the cells were prepared for neuronal induction. Expression of β-catenin and neuronal markers in the treated cells were identified by Western-blotting. (B) Quantification of β-catenin, NSE, nesin and Ngn1 protein expression. $^{*}P < 0.05$ vs. control of induction for 3 h group. $^{**}P < 0.01$ vs. control of indiction for 3 h group. (C) Expression of β-catenin, NSE, nesin and Ngn1 in MSCs treated with Wnt-3a. (D) Quantification of β-catenin, NSE, nesin and Ngn1 protein expression normalized to β-actin. $^{*}P < 0.05$ vs. uninduced group. $^{**}P < 0.01$ vs. uninduced group.

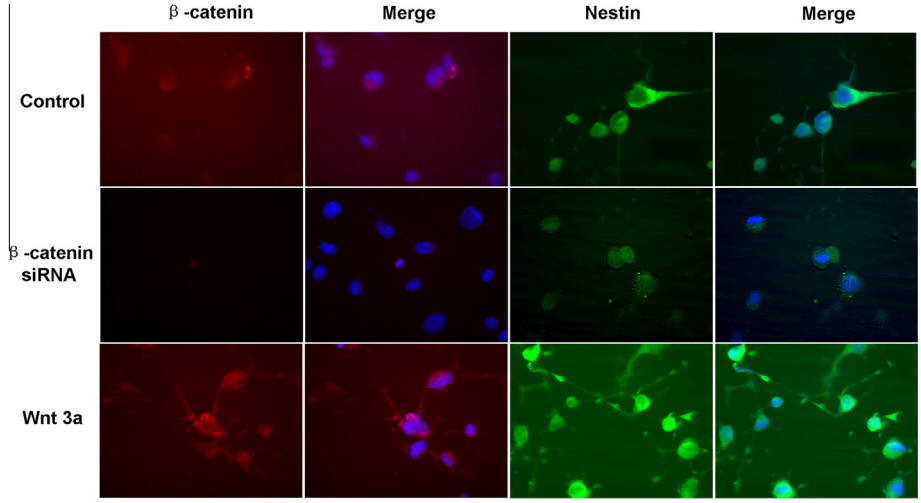

Fig. 4. Immunofluorescent analysis of β-catenin and nestin expression in neuronal differentiated MSCs including control, siRNA group and Wnt-3a group. β-Catenin was stained with secondary Cy3-labeled antibodies. Nestin was stained with secondary FITC-labeled antibodies.

of neural development including patterning of the neural tube, neural stem cell maintenance, proliferation, fate determination, axon guidance, dendrite development, and synapse formation [8]. Previous studies indicated that dynamic changes in Wnt signaling are required for neuronal differentiation of mouse embryonic stem cells [7]. Munji et al. [17] reported that Wnt/β-catenin pathway regulates neuronal cortical intermediate progenitor cells differentiation into neurons. Kondo et al. [18] identified that Tlx3 is a novel target for the canonical Wnt signaling that promotes sensory neuronal differentiation from bone marrow-derived somatic stem cells. However, the role of Wnt signaling on neuronal differentiation of MSCs was not fully investigated. In this study, our data indicated that β-catenin was up-regulated during MSCs differentiation into neuron-like cells, which was basically consistent with previous observations. We also demonstrated that activation of the canonical Wnt pathway promoted, and inhibition of this pathway blocked, neuronal differentiation of MSCs in vitro. Our results suggested that Wnt signaling played a pivotal role in the neuronal differentiation of MSCs.

The Wnt family of secreted cell signaling proteins consists of at least 19 members in mammals, such as Wnt-1, Wnt-2, Wnt-3 and Wnt-3a, some of which are expressed and play an important role in the central nervous system developing. The absence of Wnt-1 and Wnt-3a resulted in a deficiency in neural crest derivatives, which originate from the dorsal neural tube [19]. Lee et al. indicated that Wnt-3a signaling is crucial for the normal growth of the hippocampus [20]. Specifically, Wnt-3a/canonical β-catenin signaling, through the downregulation of Axin, plays an essential role in the neuronal differentiation of P19 cells [21]. Yu et al. reported that Wnt-3a and Wnt-5a increased neuronal differentiation of neural progenitor cells [22]. Our results showed that Wnt3a up-regulated the β-catenin expression and promoted the neuronal differentiation of MSCs.

Neuronal differentiation involves the proneural basic helixloop-helix (bHLH) transcription factors. For example, the bHLH proteins Neurogenin (Ngn)1 and Ngn2 are essential for neurogenesis in the neocortex [23]. Ngn1 is a proneural bHLH transcription factor expressed in newly committed neuronal progenitors and immature neurons, and plays an essential role in neurogenesis and regional specification in the neocortex, together with Ngn2. Previous study proved that a β-catenin/TCF complex directly regulates the Ngn1 promoter [24]. Our results indicated that Ngn1 expressed at a high level in the early stage and decreased in the late stage of neuronal differentiation of MSCs. In addition, we found that β-catenin inhibition resulted in an increase of Ngn1 in neuronal differentiated MSCs. These data suggested that Wnt signaling might regulate the neuronal differentiation of MSCs through Ngn1.

Our data broaden the knowledge of molecular mechanisms involved in the neuronal differentiation of MSCs and provide a potential target for directing differentiation of MSCs for clinical application.

Disclosure

The authors declare that no competing financial interests exist.

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References

[1] M.F. Pittenger, A.M. Mackay, S.C. Beck, R.K. Jaiswal, R. Douglas, J.D. Mosca, M.A. Moorman, D.W. Simonetti, S. Craig, D.R. Marshak, Multilineage potential of adult human mesenchymal stem cells, Science 284 (1999) 143–147.
[2] D. Woodbury, E.J. Schwarz, D.J. Prockop, I.B. Black, Adult rat and human bone marrow stromal cells differentiate into neurons, J. Neurosci. Res. 61 (2000) 364–370.
[3] J. Sanchez-Ramos, S. Song, F. Cardozo-Pelaez, C. Hazzi, T. Stedeford, A. Willing, T.B. Freeman, S. Saporta, W. Janssen, N. Patel, D.R. Cooper, P.R. Sanberg, Adult bone marrow stromal cells differentiate into neural cells in vitro, Exp. Neurol. 164 (2000) 247–256.
[4] J.S. Bae, H.S. Han, D.H. Youn, J.E. Carter, M. Modo, E.H. Schuchman, H.K. Jin, Bone marrow-derived mesenchymal stem cells promote neuronal networks with functional synaptic transmission after transplantation into mice with neurodegeneration, Stem Cells 25 (2007) 1307–1316.
[5] M. Dezawa, H. Kanno, M. Hoshino, H. Cho, N. Matsumoto, Y. Itokazu, N. Tajima, H. Yamada, H. Sawada, H. Ishikawa, T. Mimura, M. Kitada, Y. Suzuki, C. Ide, Specific induction of neuronal cells from bone marrow stromal cells and application for autologous transplantation, J. Clin. Invest. 113 (2004) 1701–1710.
[6] L. Ling, V. Nurcombe, S.M. Cool, Wnt signaling controls the fate of mesenchymal stem cells, Gene 433 (2009) 1–7.
[7] N.A. Slawny, K.S. O'Shea, Dynamic changes in Wnt signaling are required for neuronal differentiation of mouse embryonic stem cells, Mol. Cell. Neurosci. 48 (2011) 205–216.
[8] T.M. Michaelidis, D.C. Lie, Wnt signaling and neural stem cells: caught in the Wnt web, Cell Tissue Res. 331 (2008) 193–210.
[9] R. Hubner, A.C. Schmole, A. Liedmann, M.J. Frech, A. Rolfs, J. Luo, Differentiation of human neural progenitor cells regulated by Wnt-3a, Biochem. Biophys. Res. Commun. 400 (2010) 358–362.
[10] L. Liu, Q. Yu, J. Lin, X. Lai, W. Cao, K. Du, Y. Wang, K. Wu, Y. Hu, L. Zhang, H. Xiao, Y. Duan, H. Huang, Hypoxia-inducible factor-1alpha is essential for hypoxia-

induced mesenchymal stem cell mobilization into the peripheral blood, Stem Cells Dev. 20 (2011) 1961–1971.
[11] A.M. Parr, C.H. Tator, A. Keating, Bone marrow-derived mesenchymal stromal cells for the repair of central nervous system injury, Bone Marrow Transplant. 40 (2007) 609–619.
[12] A. Uccelli, A. Laroni, M.S. Freedman, Mesenchymal stem cells for the treatment of multiple sclerosis and other neurological diseases, Lancet Neurol. 10 (2011) 649–656.
[13] K.T. Wright, W. El Masri, A. Osman, J. Chowdhury, W.E. Johnson, Concise review: bone marrow for the treatment of spinal cord injury: mechanisms and clinical applications, Stem Cells 29 (2011) 169–178.
[14] M. Dezawa, Insights into autotransplantation: the unexpected discovery of specific induction systems in bone marrow stromal cells, Cell. Mol. Life Sci. 63 (2006) 2764–2772.
[15] M.L. Hendrickson, A.J. Rao, O.N. Demerdash, R.E. Kalil, Expression of nestin by neural cells in the adult rat and human brain, PLoS One 6 (2011) e18535.
[16] S.L. Etheridge, G.J. Spencer, D.J. Heath, P.G. Genever, Expression profiling and functional analysis of Wnt signaling mechanisms in mesenchymal stem cells, Stem Cells 22 (2004) 849–860.
[17] R.N. Munji, Y. Choe, G. Li, J.A. Siegenthaler, S.J. Pleasure, Wnt signaling regulates neuronal differentiation of cortical intermediate progenitors, J. Neurosci. 31 (2011) 1676–1687.
[18] T. Kondo, A.J. Matsuoka, A. Shimomura, K.R. Koehler, R.J. Chan, J.M. Miller, E.F. Srour, E. Hashino, Wnt signaling promotes neuronal differentiation from mesenchymal stem cells through activation of Tlx3, Stem Cells 29 (2011) 836–846.
[19] M. Ikeya, S.M. Lee, J.E. Johnson, A.P. McMahon, S. Takada, Wnt signalling required for expansion of neural crest and CNS progenitors, Nature 389 (1997) 966–970.
[20] S.M. Lee, S. Tole, E. Grove, A.P. McMahon, A local Wnt-3a signal is required for development of the mammalian hippocampus, Development 127 (2000) 457–467.
[21] J. Lyu, F. Costantini, E.H. Jho, C.K. Joo, Ectopic expression of Axin blocks neuronal differentiation of embryonic carcinoma P19 cells, J. Biol. Chem. 278 (2003) 13487–13495.
[22] J.M. Yu, J.H. Kim, G.S. Song, J.S. Jung, Increase in proliferation and differentiation of neural progenitor cells isolated from postnatal and adult mice brain by Wnt-3a and Wnt-5a, Mol. Cell. Biochem. 288 (2006) 17–28.
[23] C. Schuurmans, F. Guillemot, Molecular mechanisms underlying cell fate specification in the developing telencephalon, Curr. Opin. Neurobiol. 12 (2002) 26–34.
[24] Y. Hirabayashi, Y. Itoh, H. Tabata, K. Nakajima, T. Akiyama, N. Masuyama, Y. Gotoh, The Wnt/beta-catenin pathway directs neuronal differentiation of cortical neural precursor cells, Development 131 (2004) 2791–2801.