

Original Article

Nerve growth factor induces cord formation of mesenchymal stem cell by promoting proliferation and activating the PI3K/Akt signaling pathway

Wen-xia WANG¹, Xin-yang HU^{1,2}, Xiao-jie XIE^{1,2}, Xian-bao LIU^{1,2}, Rong-rong WU¹, Ya-ping WANG¹, Feng GAO¹, Jian-an WANG^{1,2,*}

¹Cardiovascular Key Lab of Zhejiang Province, ²Department of Cardiology, Second Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou 310009, China

Aim: To investigate whether nerve growth factor (NGF) induced angiogenesis of bone marrow mesenchymal stem cells (MSCs) and the underlying mechanisms.

Methods: Bone marrow MSCs were isolated from femors or tibias of Sprague-Dawley rat, and cultured. The cells were purified after 3 to 5 passages, seeded on Matrigel-coated 24-well plates and treated with NGF. Tube formation was observed 24 h later. Tropomyosin-related kinase A (TrkA) and p75NTR gene expression was examined using PCR analysis and flow cytometry. Growth curves were determined via cell counting. Expression of VEGF and pAkt/Akt were analyzed with Western blot.

Results: NGF (25, 50, 100 and 200 µg/L) promoted tube formation of MSCs. The tubular length reached the maximum of a 2.24-fold increase, when the cells were treated with NGF (50 µg/L). NGF (50 µg/L) significantly enhanced Akt phosphorylation. Pretreatment with the specific PI3K inhibitor LY294002 (10 µmol/L) blocked NGF-stimulated Akt phosphorylation, tube formation and angiogenesis. NGF (25–200 µg/L) did not affect the expression of TrkA and vascular endothelial growth factor (VEGF), but significantly suppressed the expression of p75NTR. NGF (50 µg/L) markedly increased the proliferation of MSCs.

Conclusion: NGF promoted proliferation of MSCs and activated the PI3K/Akt signaling pathway, which may be responsible for NGF induction of MSC angiogenesis.

Keywords: nerve growth factor; mesenchymal stem cells; angiogenesis; PI3K/Akt signaling pathway; TrkA; p75 neurotrophin receptor

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Introduction

Stem cell transplantation is one of newest therapeutic methods proposed to improve the outcome of patients with heart failure or infarctions^[1, 2]. Mesenchymal stem cells (MSCs) are a group of clonogenic cells capable of multilineage differentiation and self-reproduction. They can differentiate into mesoderm-type cells, such as osteoblasts, adipocytes and chondrocytes. MSCs are present in adult tissues, most notably in bone marrow stroma^[3, 4]. MSC transplantation could improve the outcomes of patients with myocardial infarction. The cause of improvement from this method is still unknown, but autocrine and paracrine growth mechanisms and angiogenesis are likely candidates^[5].

In the peripheral and central nervous systems, NGF plays a crucial role in regulating growth, differentiation and survival of neurocytes^[6, 7]. These physiological effects are mediated by two types of membrane receptors: tyrosine kinase receptor A (*TrkA*), which shows a high affinity for NGF, and *p75* neurotrophin receptor (*p75NTR*), which exhibits a low affinity for NGF^[8]. The cellular effects of NGF are mediated mainly by the high affinity receptor, *TrkA*^[9].

The physiological effect of NGF on the survival and differentiation of neurons has been well described, and there is now growing evidence that NGF displays potential for inducing angiogenesis in physiological and pathological conditions^[10–13]. However, whether NGF could enhance angiogenesis in MSCs remains unclear.

In this study, we investigated the effect of NGF on MSC angiogenesis. Our findings indicated that NGF could promote MSC angiogenesis *in vitro* by promoting proliferation and activating the PI3K/Akt signaling pathway.

* To whom correspondence should be addressed.

E-mail wang_jian_an@tom.com

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Materials and methods

MSC isolation and expansion

MSCs were isolated and harvested as previously described^[14]. Briefly, male Sprague-Dawley rats (weight: 80 g, 2–3 weeks) were sacrificed, and we collected bone marrow samples by flushing their femoral and tibial cavities with low glucose Dulbecco's modified Eagle's medium (DMEM-LG). Samples were transferred to sterile centrifuge tubes. Tubes were centrifuged at 900×g for 5–8 min. The precipitate was resuspended in DMEM-LG containing 10% fetal bovine serum (FBS) (Sijiqing, Zhejiang, China), penicillin (100 U/mL) and streptomycin (100 g/L), then seeded into 25-cm² flasks (Falcon, Oxnard, CA, USA) and cultured at 37°C in humidified air with 5% CO₂. After 24 h, the media was replaced and nonadherent hematopoietic cells were removed. Spindle-shaped adherent MSCs were expanded and purified with 3 to 5 passages after initial plating.

Flow cytometry analysis

Flow cytometry analysis was performed to identify MSC surface markers and to examine *p75* expression. MSCs were lifted with 0.25% trypsin and rinsed with phosphate buffered saline (PBS). Cells were centrifuged for 5 min at 900×g, resuspended in 0.5 mL PBS, and primary antibody was added (CD34, CD45: Santa Cruz, CA. CD29-PE: eBioscience, USA. CD31-PE, CD90-FITC: BD, USA. *P75*: Abcam, Cambridge, UK). Samples were incubated at room temperature for 1 h. After the cells were washed with PBS, they were incubated with secondary antibody for 1 h and then analyzed by flow cytometry.

Differentiation

Adipogenic differentiation

Passage p5 MSCs were seeded at 5×10⁴ cells on culture plates in DMEM-LG. The following day, the medium was replaced with adipogenic induction medium composed of high glucose DMEM (DMEM-HG) with 1 μmol/L dexamethasone (Sigma-Aldrich, St Louis, MO, USA), 0.2 mmol/L indomethacin (Sigma), and 1×liquid media supplement (ITS) (Sigma). Media were changed every three days. After induction for 10 d, Oil Red O staining (Sigma) was used to examine adipogenic differentiation. Cells were observed and photographed with an inverted microscope.

Chondrocyte differentiation

Chondrocyte differentiation was performed using cell adherent culture methods. MSCs were cultured with DMEM-HG with 10 μg/L transforming growth factor β 3 (Santa Cruz), 100 μmol/L dexamethasone and 50 μmol/L ascorbic acid (Sigma). Media were changed every three days. After 14 d, toluidine blue staining was used to examine chondrocyte differentiation.

Matrigel assay

Cord formation was induced using Matrigel (Sigma)^[15]. For the MSC cord formation assay, 200 μL of Matrigel was paved on a well of a 24-well plate and incubated for 1 h at 37°C.

MSCs were then trypsinized, resuspended and seeded onto the Matrigel (1×10⁵ cells/well). MSCs were treated with NGF (R&D Systems, Minneapolis, MN) at different concentrations (0, 25, 50, 100, and 200 μg/L) with or without the PI3K specific inhibitor, LY294002 (10 μmol/L) (Sigma). The ability of MSC cord formation was observed and photographed using an inverted phase contrast microscope after 24 h. Pictures were taken from triplicate experiments and the tubular lengths of the MSCs were measured using Image-Pro Plus.

Immunocytochemistry

MSCs were cultured on Matrigel on 24-well plates, gently pulled off media and washed twice with PBS. Cells were fixed in 10% paraformaldehyde (dissolved in PBS) for 30 min at 37°C, and permeabilized by immersion in 0.5% TritonX-100 for 15 min. After washing with PBS, the cells were blocked with 5% FBS/PBS for 1 h, and then labeled with appropriate primary antibodies (vWF: Santa Cruz, GAP43: Abcam). After overnight incubation at 4°C, the cells were rinsed and incubated with TRITC-conjugated secondary antibodies for 1 h at RT, then washed and incubated with Hoechst 33258 (Invitrogen, CA, USA). Cells were analyzed using a fluorescent microscope (Olympus, Tokyo, Japan).

Reverse transcription-polymerase chain reaction (RT-PCR) and real-time PCR analysis

Total RNA was extracted using Trizol reagent (Invitrogen, USA) to analyze gene expression in the MSCs. RNA was reverse-transcribed to obtain cDNA. The sequences of PCR primers for *p75* were: 5' TTGCTTGCTGTTGGAATGAG 3' (forward), 5' AGCTCCTGGGGAGGAAAATA 3' (reverse) (Sangon, Shanghai, China). The sequences of *TrkA* primers were 5' GTCTGGTGGGTCAGGGACTA 3' (forward), 5' GGGTTGCTTTCCATAGGTGA 3' (reverse) (Sangon). The sequences of GAPDH primers were 5' AGACAGCCGCATCTTCTTGT 3' (forward), 5' CTTGCCGTGGGTAGAGTCAT 3' (reverse) (Sangon). Real-time PCR analysis of *p75* gene expression was performed with the ABI PRISM 7000 Sequence Detection System using the SYBR Green I Real Time PCR Kit (Bioer, Hangzhou, China). Relative quantification of gene expression was performed using the 2^{-ΔΔCt} method. Analysis of *TrkA* gene expression was performed by RT-PCR. Agarose gel electrophoresis (1.7%) was performed to analyze the products.

Western blot analysis

MSCs were collected in an Eppendorf tube, then lysed with RIPA lysis buffer (Beyotime, Shanghai, China) for 30 min. Cell and nuclear lysates were centrifuged at 13000×g for 25 min at 4°C. Samples containing equal amounts (60 μg) of protein were run on SDS-PAGE gels and transferred onto PVDF membranes. Membranes were blocked with 0.1% Tween-20 TBS (TBS-T) containing 1% BSA for 1 h. Then the membrane was incubated in diluted primary antibodies (VEGF and β-actin: Santa Cruz, USA. Akt, pAkt: Cell Signaling Technology, Beverly, MA, USA). After washing in 0.1% TBS-T, the membranes were incubated for 2 h with HRP-conjugated secondary anti-

bodies. Bands were visualized by ECL. Quantity One software was used to semi-quantify protein levels in every lane.

Cell growth curve

Equal numbers (5×10^3) of MSCs with or without NGF were plated on 24-well plates. Cell number was determined using a cell counting chamber at 0, 1, 2, 3, 4, and 5 d after cell plating. Triplicate wells were used for each time point.

Statistical analysis

Data were expressed as the mean \pm SD and analyzed with SPSS 16.0 statistical software (SPSS Inc, Chicago, IL). Statistical analysis was performed by one-way ANOVA, and a P value <0.05 was considered statistically significant.

Results

Characterization of MSCs

To demonstrate that the cells isolated from rat bone marrow were indeed MSCs, we examined their differentiation capacity and phenotypic markers. MSCs appeared spindle-shaped and adhered to the bottom of the culture-flask (Figure 1A). After adipogenic induction for 10 d, MSCs contained abundant amounts of vacuoles, and Oil Red O staining demonstrated that these vacuoles contained neutral lipids (Figure 1B). After chondrogenic induction, MSCs showed metachromasia following toluidine blue treatment (Figure 1C). These results indicate that the MSCs differentiated into adipocytes and chondrocytes. Flow cytometry demonstrated that the MSCs were negative for the hematopoietic markers CD31, CD34, and CD45 but were positive for the MSC markers CD29 and CD90 (Figure 1D).

NGF promoted cord formation of MSCs in Matrigel

3-D Matrigel basement models closely mimic the structure, composition, physical properties and functional characteristics of the neovascularization microenvironment. To assess the effect of NGF on angiogenesis in MSCs, Matrigel assays were performed. After incubation for 24 h, MSCs formed tubes and networks on the Matrigel basement models *in vitro* (Figure 2A). After culturing with NGF for 24 h, the ability of the MSCs to form tubes was enhanced. As shown in Figure 2B–2E, the number of tubes, and the tubular lengths, were promoted in groups of NGF-treated MSCs compared to controls. NGF 50 and 100 $\mu\text{g/L}$ enhanced cord formation in MSCs ($P < 0.05$) (Figure 2F). The tubular length of 50 $\mu\text{g/L}$ NGF-treated MSCs was 2.24-fold greater than controls. As such, the following experiments were performed using 50 $\mu\text{g/L}$ NGF.

MSCs cultured in Matrigel may differentiate into endothelial cells

Matrigel has been widely used in cell-culture applications, and the Matrigel assay is commonly recognized as an angiogenesis model *in vitro*. Von Willebrand Factor (vWF) is a surface marker used to identify endothelial cells. The MSCs that formed tubes in Matrigel expressed vWF, which was used as a specific marker of endothelial cells (Figure 3B). However, the MSCs that did not form tubes were negative for vWF. This result indicated that tubes formed in Matrigel may differentiate into endothelial cells. Because the Matrigel can also support neuronal differentiation of neural precursor cells^[16], we examined whether the MSCs differentiated into neurocytes. MSCs were positively expressing early neuronal markers and some mature neuronal markers. Immunofluorescence analyses showed that the MSCs were negative for GAP43

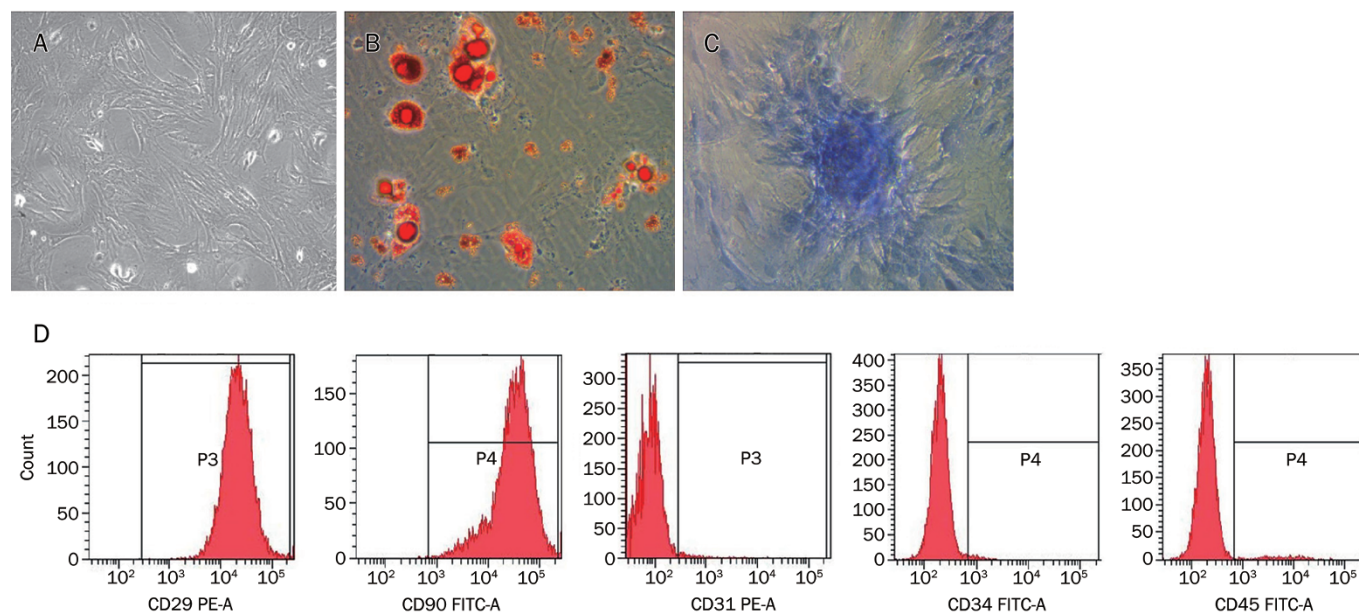


Figure 1. Characterization of mesenchymal stem cells (MSCs). (A) Representative morphological image of MSCs. Magnification $\times 100$. (B) Oil Red O staining of the adipogenic induction of MSCs. Magnification $\times 200$. (C) Toluidine blue staining of the chondrogenic induction medium-induced MSCs. Magnification $\times 200$. (D) Expression of cell surface markers on cultured MSCs. Flow cytometry analysis showed that the positivity of selected markers were as follows: CD29 99.6%, CD90 99.3%, CD31 2.4%, CD34 1.5%, CD45 3.2%.

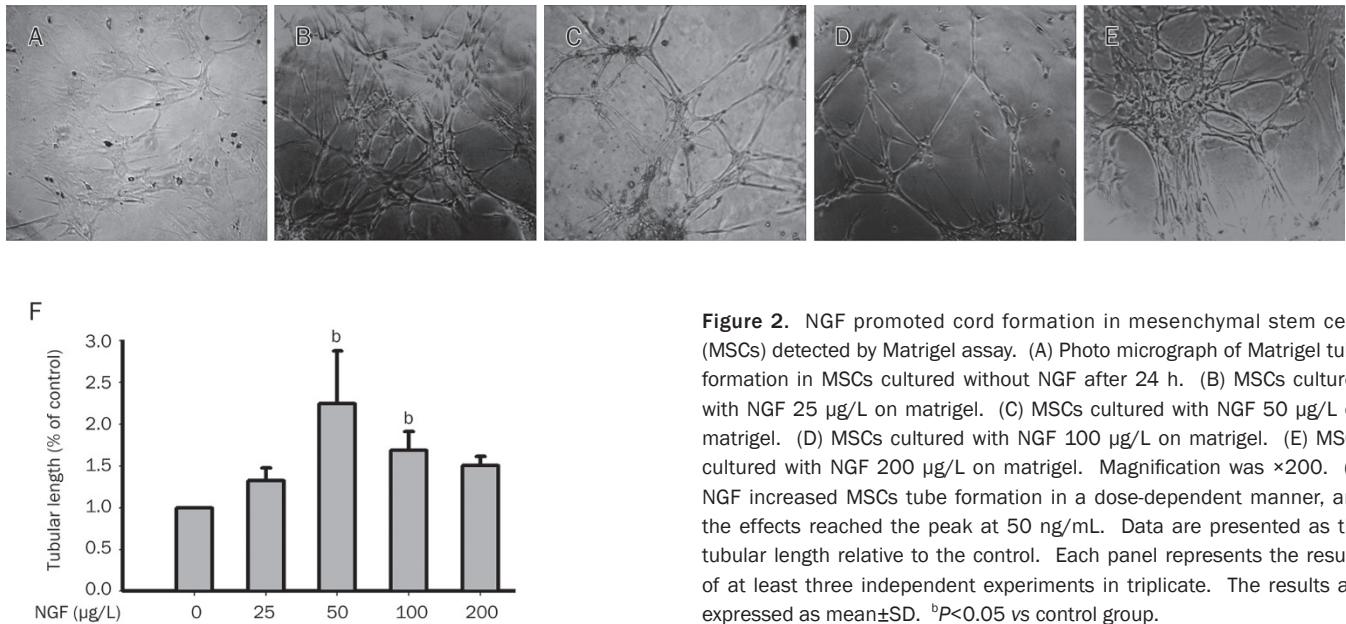


Figure 2. NGF promoted cord formation in mesenchymal stem cells (MSCs) detected by Matrigel assay. (A) Photo micrograph of Matrigel tube formation in MSCs cultured without NGF after 24 h. (B) MSCs cultured with NGF 25 μg/L on matrigel. (C) MSCs cultured with NGF 50 μg/L on matrigel. (D) MSCs cultured with NGF 100 μg/L on matrigel. (E) MSCs cultured with NGF 200 μg/L on matrigel. Magnification was ×200. (F) NGF increased MSCs tube formation in a dose-dependent manner, and the effects reached the peak at 50 ng/mL. Data are presented as the tubular length relative to the control. Each panel represents the results of at least three independent experiments in triplicate. The results are expressed as mean±SD. ^b*P*<0.05 vs control group.

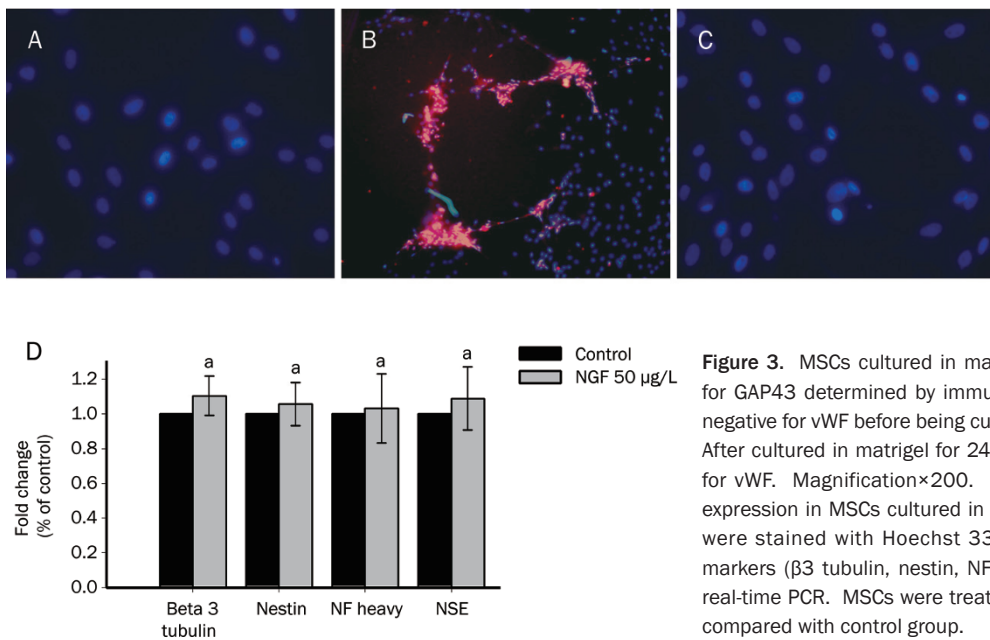


Figure 3. MSCs cultured in matrigel were positive for vWF and negative for GAP43 determined by immunofluorescence analysis. (A) MSCs were negative for vWF before being cultured in matrigel. Magnification×400. (B) After cultured in matrigel for 24 h, MSCs formed tubes and were positive for vWF. Magnification×200. (C) Immunofluorescence image of GAP43 expression in MSCs cultured in matrigel. Magnification×400. Nucleuses were stained with Hoechst 33258. (D) The expressions of neuronal markers (β3 tubulin, nestin, NF heavy, NSE) in MSCs were examined by real-time PCR. MSCs were treated with or without NGF for 24 h. ^a*P*>0.05 compared with control group.

both before and after culture in Matrigel (Figure 3C). RT-PCR analyses demonstrated that pretreatment with only NGF had no influence on the expression of β3 tubulin, nestin, neurofilament (NF) or neuron-specific enolase (NSE) (Figure 3D).

Influence on TrkA and p75 receptor expression

TrkA gene expression was examined using RT-PCR. Total RNA was extracted after MSCs were either untreated or treated with NGF for 24 h. As shown in Figure 4A, the *TrkA* mRNA expression was barely detectable and was not influenced by NGF at any concentration. However, real-time PCR demonstrated that NGF-treatment decreased the expression

of *p75* mRNA (Figure 4B). It induced a 0.27- to 0.55-fold decrease in gene expression in the NGF-treated MSC group when compared with controls, and NGF concentrations of 50 μg/L showed the strongest inhibiting effect. Moreover, flow cytometry further confirmed inhibition of *p75* gene expression by NGF (Figure 4C).

Activation of the PI3K/Akt signaling pathway in MSCs

Western blot experiments were used to identify the downstream signaling pathway involved in NGF-induced cord formation in MSCs. The results revealed that the phosphorylated form of Akt, a downstream target of PI3K, was elevated

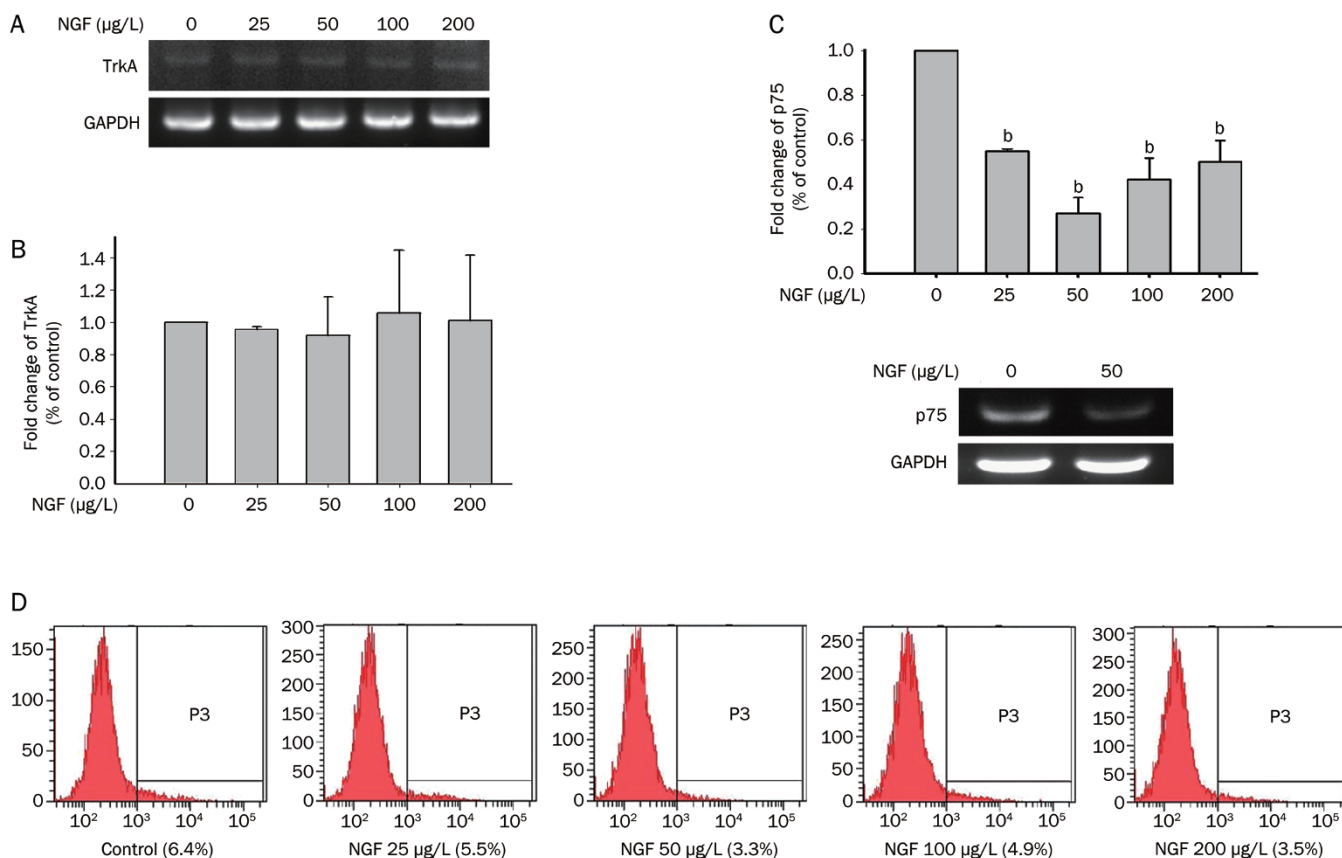


Figure 4. PCR and FACS analysis of TrkA and p75 expression. (A) Total RNA was isolated from MSCs treated or untreated with NGF. TrkA and a housekeeping gene (GAPDH) were analyzed by RT-PCR. (B) Data were expressed as the fold change of TrkA relative to the control. (C) P75NTR and GAPDH gene expressions of MSCs at different concentrations were examined by real-time PCR. Data were represented with mean±SD of three replicates. ^b*P*<0.05 vs control group. (D) The effect of different concentrations of NGF on P75NTR protein level of MSCs was analyzed by FACS. The percentage of positive cells was listed in parentheses.

in NGF-treated MSCs within 1 h of treatment (Figure 5A). LY294002 was used to inhibit the activation of PI3K, which inhibited the expression of pAkt activated by NGF (Figure 5B). The addition of LY294002 (10 µmol/L) to cultures containing 50 µg/L NGF almost entirely attenuated NGF-induced capillary-like structure formation (Figure 5C–5G).

Enhancement of MSCs proliferation

The increased angiogenesis potential of MSCs after NGF pretreatment prompted us to investigate whether it was associated with increased proliferation of MSCs. Figure 6 shows the growth curves of untreated MSCs and 50 µg/L NGF-treated MSCs. Following NGF treatment for 1 d, the cell growth was markedly enhanced, and the growth trend increased over time.

NGF had no influence on VEGF expression

To determine whether NGF-promoted MSC cord formation was related to VEGF expression, we determined the level of VEGF expression in treated and untreated MSCs. Figure 7 shows that NGF treatment had no significant effect on the expression level of VEGF in MSCs.

Discussion

The major findings of the present study were as follows: (1) NGF can induce MSC cord formation on Matrigel *in vitro*. (2) NGF had no influence on the expression of TrkA mRNA, but could inhibit p75 expression in MSCs. (3) The effect of NGF-promoted MSC angiogenesis may be mediated by the PI3K/Akt signaling pathway. It also may be associated with the promotion of MSC proliferation, and has no correlation to VEGF expression in MSCs.

MSC transplantation was reported to improve myocardial function following myocardial infarction, but the underlying mechanisms remain to be elucidated^[4]. Recent evidence has indicated that intramyocardial implantation of bone marrow MSCs induced revascularization, and these MSCs may differentiate into endothelial cells after myocardial infarction^[5,17]. Enhancing the angiogenesis ability of MSCs may be beneficial to improving myocardial function, and many studies have focused on accomplishing this aim. For instance, Annabi *et al* found hypoxic culture conditions could rapidly induce MSC migration and three-dimensional capillary-like structure formation on Matrigel through paracrine and autocrine regulatory mechanisms^[18]. Wu *et al* found that calcium was a

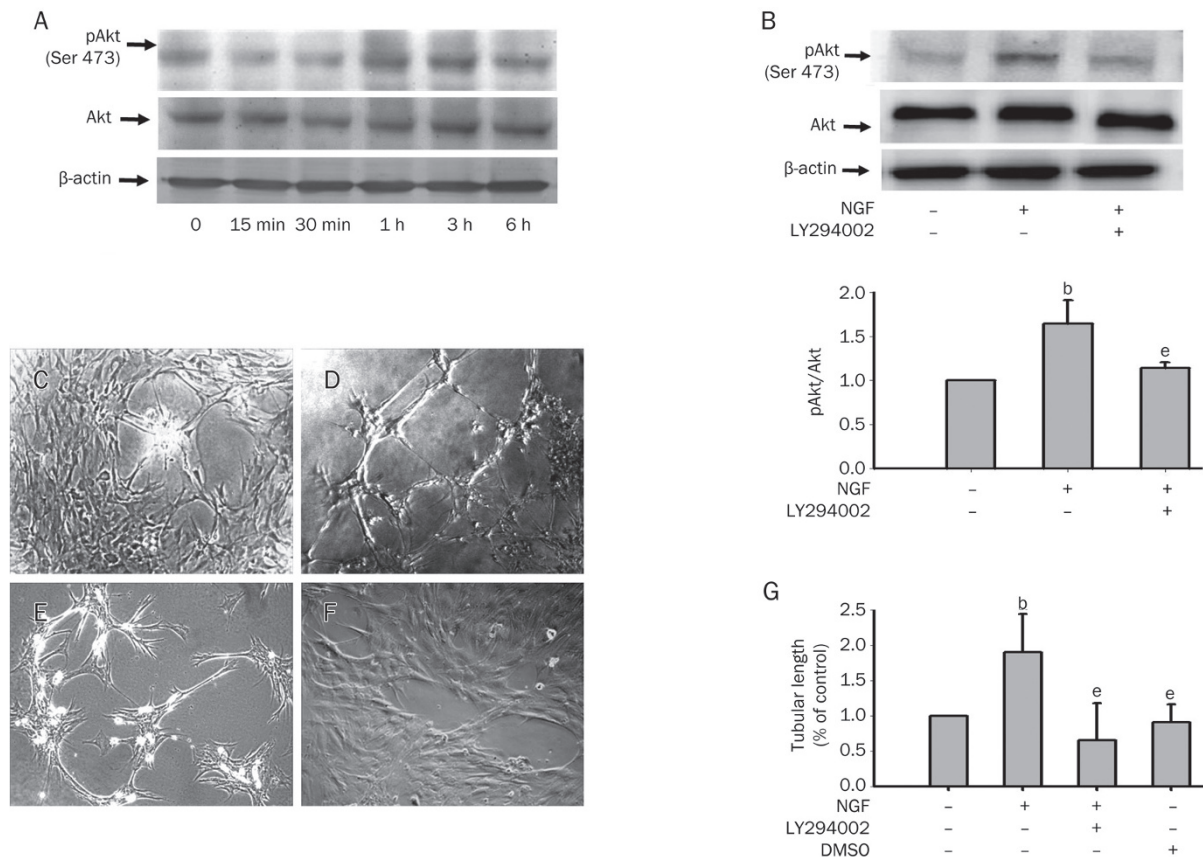


Figure 5. Effect of PI3K/Akt signaling pathway involved in the promotion of cord formation. (A) Western blot analysis of pAkt and Akt at the indicated time points after treated with NGF (50 $\mu\text{g/L}$). (B) Western blot analysis of pAkt and Akt of NGF treated MSCs. Cells were pretreated with or without LY294002 (10 $\mu\text{mol/L}$) for 1 h, and then stimulated with or without NGF (50 $\mu\text{g/L}$) for 1 h. ^b $P < 0.05$ vs control group. ^e $P < 0.05$ vs NGF treated MSCs. (C) Photomicrograph shows the matrigel tube formation of MSCs cultured without NGF. (D) MSCs cultured with NGF (50 $\mu\text{g/L}$) on matrigel for 24 h. (E) MSCs pretreated with LY294002 (10 $\mu\text{mol/L}$) for 1 h and cultured with NGF (50 $\mu\text{g/L}$) for 24 h on matrigel. Magnification $\times 200$. (F) MSCs cultured with DMSO (1 $\mu\text{mol/L}$) for 24 h on matrigel. (Because LY294002 was reconstituted in DMSO, so we evaluated the effect of DMSO on MSCs cord formation.) (G) LY294002 attenuated the angiogenic effect of NGF. Data were representative of three independent experiments. The results are expressed as mean \pm SD. ^b $P < 0.05$ vs control group. ^e $P < 0.05$ vs NGF treated MSCs.

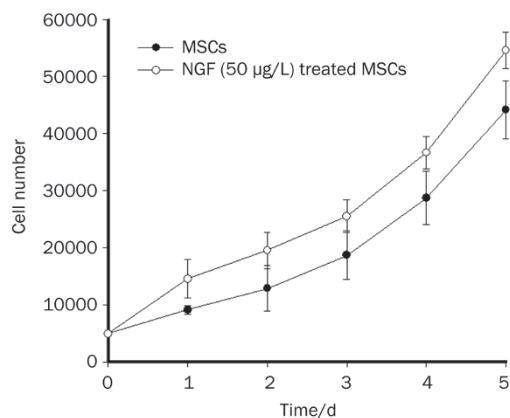


Figure 6. Growth curves of MSCs with or without NGF. Cell counting method was applied to examine the proliferation ability of MSCs. Data were representative of three independent experiments.

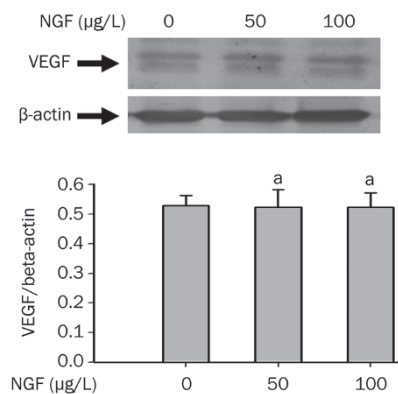


Figure 7. NGF treatment had no influence on the expression level of VEGF in MSCs. Western blot was performed to evaluate the expression of VEGF in MSCs after treatment with or without NGF (50 and 100 $\mu\text{g/L}$). Western blot image and quantitation of VEGF was normalized by β -actin. ^a $P > 0.05$ vs control group.

positive regulator of CXCR4 expression that enhanced bone marrow stem cell pro-angiogenesis therapy^[19]. In the present study, we focused on NGF regulation of MSC tube formation and investigated the possible mechanism behind it.

NGF has been shown to trigger angiogenesis both directly and indirectly. NGF may induce expression of specific proteins, including VEGF and MMP-2, and may contribute to the maintenance, survival, and function of endothelial cells^[10-13, 20, 21]. We found that NGF promoted MSC cord formation when grown on Matrigel, as evidenced by a 2.24-fold increase in tubular length when grown with a concentration of 50 µg/L NGF.

The Matrigel assay is commonly considered to be an experimental method that measures the ability of cells to promote angiogenesis *in vitro*^[15] and is also used to support the differentiation of neural precursor cells into neuronal cells^[16]. NGF is a common neurotrophic factor, so we needed to exclude the possibility that MSCs normally have the ability to differentiate into neuronal cells. Immunofluorescence analyses showed MSC-formed tubes may have differentiated into endothelial cells. MSCs were negative for GAP43 before and after culture in Matrigel. Because of the positive expression of early neuronal markers and a few mature markers, it is difficult to use immunofluorescence to judge whether the cells have a tendency to differentiate into neurocytes. Real-time PCR demonstrated that pretreatment with NGF had no influence on the expression of neuronal markers. Therefore, we believe that the cells were not differentiating into neurons.

Results indicated that tubular lengths responded to increasing concentrations of NGF in a pattern similar to a Gaussian distribution. It is unknown whether this distribution was caused by regulation of the NGF receptors. *TrkA* is a specific receptor of NGF and is involved in angiogenesis^[22]. Myung-Jin Park *et al* demonstrated that *TrkA* activation induces endothelial cell invasion and cord formation^[20]. However, our results showed *TrkA* mRNA were hardly detectable in MSCs, and NGF treatment had no significant effect on *TrkA* expression levels. Western blot analyses further confirmed that MSCs had low levels of *TrkA* protein expression (data not shown). *P75NTR* is a low affinity NGF receptor. To our surprise, real-time PCR and flow cytometry analyses both demonstrated that NGF could inhibit *p75* receptor expression in MSCs, and *p75*-positive cells only comprised 6.4% of the population. Previous studies show that *p75NTR* over-expression can promote apoptosis. We found that *TrkA* and *p75* were scarcely expressed in bone marrow MSCs, so we postulated that the inhibition of *p75* may be related to the effect of NGF in promoting MSC proliferation, and its association with MSC angiogenesis. Because our experiments show low levels of *TrkA* and *p75NTR* expression, there may be other mechanisms involved that require further study.

The PI3K/Akt signaling pathway is reported to be involved in angiogenesis in endothelial cells, and it plays an important role in the physiological effect of NGF, especially in the angiogenesis process^[22]. Park *et al* demonstrated that NGF stimulates endothelial cell invasion and cord formation, and that the

PI3K/Akt signaling pathway may be responsible for triggering angiogenesis^[20].

Many reports have shown that the PI3K/Akt signaling pathway is critical for MSC proliferation, anti-apoptosis and migration, and promoted angiogenesis through specific stimulation^[23, 24]. Activating PI3K could induce the assembly of receptor-PI3K complexes, followed by activation of Akt by a second messenger. Through phosphorylation, activated Akt mediated the activation and inhibition of several targets, resulting in cellular growth, survival and proliferation through various mechanisms^[25].

Our Western blot analysis results showed that NGF stimulated Akt phosphorylation in MSCs. LY294002 inhibition of the catalytic activity of the p110 subunit of PI3K have been widely used *in vitro* for many years^[25]. The Matrigel assay showed that NGF-induced MSC tube formation was completely blocked by LY294002. Using the specific kinase inhibitor, we showed that the PI3K/Akt signaling pathway was critical for NGF-induced MSC tube formation on Matrigel *in vitro*.

Cell proliferation plays an important role in angiogenesis. We used a cell growth curve method to evaluate whether the increased angiogenic potential of NGF-treated MSCs was associated with increased proliferation. We found that NGF could markedly promote MSC proliferation. During angiogenesis, NGF may promote MSC sprouting and proliferation during tube formation.

It has been reported that NGF up-regulated VEGF expression both *in vitro* and *in vivo*. NGF may be involved in promoting endothelial cell growth, which is associated with increased expression of VEGF. The result of this increased expression may be capillary sprouting^[26]. VEGF is a crucial mediator of vascular hyperpermeability, angiogenesis, and inflammation. These processes are intimately involved in tissue repair and regeneration^[27]. NGF played a functional role in reparative neovascularization through a VEGF-mediated mechanism^[10]. Park *et al* showed that NGF may induce reparative angiogenesis during thymic regeneration in adults through the up-regulation of VEGF expression^[13]. Wu *et al* showed that bone marrow MSCs stimulated endothelial cell proliferation, migration, and organization into tubules through the expression of high levels of VEGF^[28]. Our data showed that MSCs could synthesize VEGF. However, the expression of VEGF was not different in both the NGF-treated and control groups. Our findings were different from the studies described above, which may be due to our use of different cells. NGF can promote VEGF expression in vascular endothelial cell and thymic epithelial cells, but it has no effect on VEGF expression in MSCs. Accordingly, we suggested that NGF-induced angiogenesis in MSCs are not related to VEGF expression.

Angiogenesis is very complex and consists of several processes, including proliferation, migration and invasion of endothelial cells, and is necessary for their survival. We investigated the effects of NGF on MSC migration through scratch tests and trans-well assays, but we observed that NGF did not promote migration (data not shown). We presume that

the enhancement of MSC angiogenesis by NGF contributes to increased cell proliferation and cord formation, though there may be other mechanisms involved.

In summary, this study demonstrates that NGF enhances proliferation in MSCs, and activates the PI3K/Akt signaling pathway. These effects may play an important role in angiogenesis, and these results may have value in the treatment of myocardial infarction using MSC transplantation.

Acknowledgements

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