Murine and Chinese cobra venom-derived nerve growth factor stimulate chondrogenic differentiation of BMSCs *in vitro*: A comparative study

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Abstract. Mesenchymal stem cell (MSC)-based therapy has been commonly used in cartilage reconstruction, due to its self-renewing ability and multi-differentiation potential. Nerve growth factor (NGF) from cobra venom has been reported to regulate chondrogenesis of bone-derived MSCs (BMSCs) and chondrocyte metabolism. Therefore, the present study aimed to determine whether other sources of NGF behave in the same manner as NGF from natural venom. The present study compared the effects of NGF from two sources, the commercially purchased recombinant murine β-NGF (mNGF) and cobra venom-derived NGF (cvNGF), on chondrogenesis of BMSCs by performing hematoxylin and eosin and fluorescein diacetate/propidium iodide staining, DNA and glycosaminoglycan quantization and reverse transcription-quantitative polymerase chain reaction to investigate cell morphology, viability, proliferation, glycosaminoglycan synthesis and cartilage-specific gene expression. The results demonstrated that cvNGF significantly accelerated cell proliferation and upregulated the expression of cartilage-specific genes, including aggrecan, SRY-box 9 and collagen type II α1 chain. Conversely, cvNGF reduced the expression levels of collagen type I α1 chain (a fibrocartilage marker), runt-related transcription factor 2 and enolase 2 compared with in the mNGF and control groups. In addition, Chinese cobra venom, which is the main resource of cvNGF, is abundant and inexpensive, thus greatly decreasing the cost. In conclusion, the present study demonstrated that cvNGF may be considered a potential growth factor for inducing chondrogenic differentiation of BMSCs.

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Introduction

Articular cartilage has limited regenerative capabilities following injury, due to the lack of vascularization, reduced progenitor cell supply and the presence of few cells with low mitotic activity (1,2). Cell-based therapies are considered useful approaches for cartilage restoration (3,4). Mesenchymal stem cells (MSCs) can be isolated from multitudinous tissues, including bone marrow, adipose tissue and the placenta, and are frequently used as a source of cartilage restoration due to high yields, easy accessibility and the capability to differentiate along the chondrogenic lineage (5). Bone-derived MSCs (BMSCs) appear to be the most promising choice for cartilage regeneration, due to the ease with which they can be acquired, and their high proliferative capacity and chondrogenic differentiation ability (6). Growth factors, such as transforming growth factor-β1, serve critical roles in the regulation the chondrogenic differentiation of MSCs. Although conditioned medium that regulates chondrogenesis of MSCs has been well established (7), the high cost, short half-life, versatility and functional heterogeneity of traditional growth factors remain an obstacle for stem cell-based therapy.

In our previous study, it was revealed that a novel growth factor, nerve growth factor (NGF) extracted from Chinese

cobra venom, specifically induces the chondrogenic differentiation of MSCs and further promotes cartilage repair (8). The chondral specificity of NGF is better than traditionally used growth factors, which may lead to osteophyte formation instead of chondrogenesis during cartilage regeneration (9-11). Other peptide neurotrophins, such as Nel-like molecule-1, which is abundantly secreted in neural tissue, are also reported to promote the proliferation of chondrocyte and maintain its phenotype *in vitro* (12). Grassel (13) also demonstrated the roles of sensory and sympathetic neurotransmitters on limb formation during the process of embryonic skeletal development. Notably, venom-derived NGF rarely induces BMSCs to differentiate into a neuronal phenotype (14).

There are numerous sources of NGF, which has been isolated from venom and body fluids, and formed by recombination. Lipps (15,16) studied the bioactivity of NGF from bee, scorpion and spider venoms, and NGF from body fluids, including serum, saliva and urine, and compared these NGFs with cobra venom-derived NGF (cvNGF). The findings of these previous studies indicated that the biological activities of NGFs obtained from human body fluids on PC12 cells were minor in comparison to cvNGF, and the biological activity of bee NGF on PC12 cells was 1/10 that of cvNGF. It remains to be determined as to whether NGF from other sources behaves the same as NGF extracted from natural venom.

The present study compared the effects of commercially purchased recombinant murine β-NGF (mNGF) and cvNGF on the chondrogenic differentiation of MSCs by detecting cell proliferation, glycosaminoglycan (GAG) synthesis and cartilage-specific gene expression. The findings of this study may aid in the clinical application of NGF for cartilage repair.

Materials and methods

Cell culture. BMSCs were isolated from bone marrow that was extracted from two male Sprague-Dawley rats (weight, 8-10 g; age, 3 days), which were purchased from the Animal Experimental Center of Guangxi Medical University (Nanning, China). The rats were housed at a constant temperature (26˚C) and relative humidity (60%) under a reversed 12-h dark/light cycle (lights on until 8:00 p.m.), with free access to a standard diet and water. All experiments were conducted in accordance with the standard guidelines approved by the Animal Care and Experiment Committee of Guangxi Medical University (Guangxi, China; protocol number: 2014-12-3), and the present study was approved by the ethics committee of Guangxi Medical University. Briefly, the rats were sacrificed with an injected overdose of pentobarbital, and bone marrow was collected from the bilateral femur by flushing with α-modified Eagle's medium (α-MEM; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 1% penicillin/streptomycin (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China). Following density gradient centrifugation (1,100 x g at 25˚C for 30 min), the isolated BMSCs were cultured in α-MEM supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin/streptomycin. The culture medium was changed every 2 days. Characterization of isolated BMSCs was performed as described previously (5).

Cell seeding. Two sources of NGF were added to the cell cultures. mNGF was purchased from Peprotech, Inc. (Rocky Hill, NJ, USA), and cvNGF was extracted and purified from the venom of Chinese cobra (*Naja naja atra*) as described in our previous study (8). Briefly, BMSCs were cultured in chondrogenic medium including 50 μg/ml ascorbic acid (A7506; Sigma-Aldrich; USA), 100 nM dexamethasone (D1756; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany), 1% insulin-transferrin-selenium solution (41400045; Gibco; Thermo Fisher Scientific, Inc.) and 0.06 μg/ml mNGF or 6 μg/ml vNGF. For each experiment, cells at passage 2 were used and the seeding density was $2x10^4$ cells/cm^2. Experiments were performed at 7, 14 and 21 days.

Cytotoxicity assay. NGF toxicity on BMSCs was detected using the MTT assay (Gibco; Thermo Fisher Scientific, Inc.). BMSCs were seeded onto 96-well plates and treated with various concentrations of mNGF (0, 0.0075, 0.015, 0.03, 0.06, 0.12, 0.24, 0.48 and 0.96 μg/ml) and cvNGF (0, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24 and 32) and incubated at 37˚C. After 3 days, 20 μl MTT solution (5 mg/ml) was added and incubated at 37˚C for 4 h in the dark. After removing the medium, the formazan crystals in each well were dissolved with 200 μl dimethyl sulfoxide (Gibco; Thermo Fisher Scientific, Inc.). The absorbance was measured at 570 nm using a microplate reader (Thermo Fisher Scientific, Inc.).

Cell viability assay. Cell viability was determined by fluorescein diacetate (FDA; GenWay Biotech, Inc., San Diego, CA, USA) and propidium iodide (PI; Sigma-Aldrich; Merck KGaA) staining on days 7, 14 and 21. Briefly, after rinsing with PBS for three times, 0.5 μM FDA and 2 μM PI in 1 ml PBS was added to the cells and incubated in the dark for 5 min. Images were captured under a laser scanning confocal microscope (Olympus Corporation, Tokyo, Japan).

Cell morphological examination. The BMSCs were seeded onto 24-well-plates and were cultured with mNGF (0.06 μg/ml) or cvNGF (6 μg/ml) for 7, 14 and 21 days. The cells were then washed three times with PBS and fixed with 95% alcohol for 30 min at room temperature. Subsequently, the cells were washed with PBS and stained with 10% hematoxylin and 2% eosin (H&E; Nanjing Jiancheng Bioengineering Institute, Nanjing, China) at room temperature for 5 min and 30 sec, respectively. Finally, cells were observed and images were captured under an inverted phase contrast microscope (Olympus Corporation).

Cell proliferation analysis and biochemical assay. After being cultured for 7, 14 or 21 days, cells were washed with PBS and then digested with 0.25% trypsin/ETDA. Cells were centrifuged at 200 x g for 5 min at room temperature, and digested with proteinase K (20 mg/ml; Sigma-Aldrich; Merck KGaA) for 16 h at 56˚C. After staining with 10 μg/ml Hoechst 33258 dye at room temperature for 10 min (Sigma-Aldrich; Merck KGaA), the DNA content was determined using a fluorescence microplate reader (BioTek Instruments, Inc., Winooski, VT, USA) at 460 nm; calf thymus DNA (Sigma-Aldrich; Merck KGaA) was used as a standard and absorption of Hoechst 33258 dye was considered the baseline. The total production

Table I. Primers for reverse transcription-quantitative polymerase chain reaction.

Gene	Forward primer	Reverse primer
Acan	5'-GACAAGGACGAGTTCCCTGG-3'	5'-CTCCGGGGATGTGGCATAAA-3'
Sox9	5'-TCCAGCAAGAACAAGCCACA-3'	5'-TCCAGCAAGAACAAGCCACA-3'
Col2a1	5'-AGATGGCTGGAGGATTTGACG-3'	5'-TTTCCGGGCTTTCCAGCTT-3'
Col1a1	5'-GATCCTGCCGATGTCGCTAT-3'	5'-GGGACTTCTTGAGGTTGCCA-3'
RUNX2	5'-GTGGCCAGGTTCAACGATCT-3'	5'-TGAGGAATGCGCCCTAAATCA-3'
ENO2	5'-TCAAGTCTCCTGCTGACCCT-3'	5'-AACGTGTCCTCGGTTTCTCC-3'
β-actin	5'-CCCATCTATGAGGGTTACGC-3'	5'-TTTAATGTCACGCACGATTTC-3'

Acan, aggrecan; *Sox9*, SRY-box 9; *Col2a1*, collagen type II; *Col1a1*, collagen type I; *RUNX2*, runt-related transcription factor 2; *ENO2*, enolase 2.

of GAG was quantified by detecting absorbance at 525 nm, following treatment with 16 mg/l 1, 9-dimeth-ylmethylene blue (DMMB; Sigma-Aldrich; Merck KGaA) at room temperature for 10 min; chondroitin sulfate (Sigma-Aldrich; Merck KGaA) was used as a standard. The GAG content in each cell was normalized to the total DNA content of all cells, which represented its biosynthetic activity under various culture conditions.

Safranin O staining. Safranin O staining was performed to assess the synthesis of GAG. After being fixed with 95% alcohol for 30 min, at room temperature the cells were stained with 0.1% safranin O (Sigma-Aldrich; Merck KGaA) for 10 min. Subsequently, the cells were washed with water and sealed with neutral gum. Eventually, cells were observed and images were captured under an inverted phase contrast microscope (Olympus Corporation).

Immunohistochemical staining. Immunohistochemistry was performed to analyze the expression levels of collagen type I α1 chain (COL1A1) and collagen type II α1 chain (COL2A1). After 7, 14 and 21 days, cells were washed three times with PBS, fixed with 95% alcohol at room temperature for 30 min and treated with Triton X-100 (Sigma-Aldrich; Merck KGaA) at room temperature for 10 min. To eliminate endogenous peroxidase activity, cells were incubated with 3% H_2O_2 at room temperature for 15 min and blocked with goat serum (Beijing Solarbio Science & Technology Co., Ltd.) for 15 min at room temperature. After incubating with COL1A1 (1:200; Wuhan Boster Biological Technology, Ltd., Wuhan, China) and COL2A1 (BA0533; 1:200; Wuhan Boster Biological Technology Ltd.) primary antibodies overnight at 4˚C, cells were incubated with the secondary antibody (G1080, 1:200; Beijing Solarbio Science & Technology Co., Ltd.) and followed by biotin-labeled horseradish peroxidase (Zhongshanjinqiao Biotechnology Inc., Wuhan, China) at room temperature for 15 min. A DAB kit (Wuhan Boster Biological Technology, Ltd.) was used to visualize antibody binding. Finally, images of the cells were captured under an inverted phase contrast microscope (Olympus Corporation).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis. RT-qPCR was conducted to analyze the expression levels of aggrecan (*Acan*), SRY-box 9 (*Sox9*), *Col2a1, Col1a1*, runt-related transcription factor 2 (*RUNX2*) and enolase 2 (*ENO2*). The sequences of primers used for RT-qPCR are presented in Table I. Total RNA was extracted using a RNA isolation kit (Wuhan Megentec Biological Technology, Ltd., Wuhan, China), according to the manufacturer's protocol. RT of RNA was performed using a RT kit (Fermentas; Thermo Fisher Scientific, Inc.) and was carried out at 25˚C for 5 min, 42˚C for 60 min and 72˚C for 5 min. RT-qPCR was performed on a qPCR Detection system with Fast Start Universal SYBR Green Master Mix (Roche Diagnostics, Basel, Switzerland) under the following conditions: 10 min at 95˚C for initial denaturation, 15 sec at 95˚C and 1 min at 60˚C for final extension (40 cycles). The melting curve data were collected to verify PCR specificity; each gene was analyzed in triplicate. The relative mRNA expression levels were calculated using the $2^{-\Delta\Delta Cq}$ (17) method with β-actin as a reliable internal control.

Statistical analysis. Data are presented as the means ± standard deviation from 4 repeated experiments. Data were analyzed using one-way analysis of variance followed by Dunnett's post hoc test (SPSS version 21; IBM Corp., Armonk, NY, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Cytotoxicity of NGF. An MTT assay was performed to detect the cytotoxicity of NGF on BMSCs. Cells were treated with increasing concentrations of mNGF (0-0.96 μg/ml) or cvNGF (0-32 μg/ml). As shown in Fig. 1, mNGF at concentrations ranging between 0.03 and 0.12 μg/ml and cvNGF at 1.5-16 μg/ml significantly accelerated cell growth ($P<0.05$). On the basis of these results, 0.06 μg/ml mNGF (Fig. 1A) and 6 μg/ml cvNGF (Fig. 1B), which exhibited the most obvious effect on BMSC proliferation, were chosen for further investigation.

Cell viability. To evaluate the effects of NGF on cell viability, a FDA/PI staining kit (Fig. 2) was used. The majority of the cells in all groups were stained green, which indicated a greater amount of viable cells. More viable cells and fewer apoptotic cells (stained red) were detected in the cvNGF group compared with the other groups.

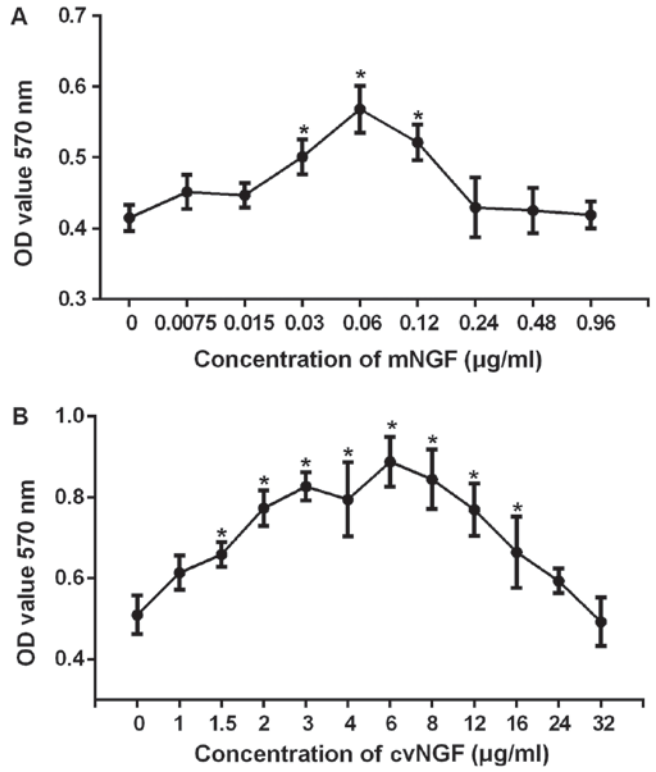

Figure 1. Cytotoxicity of (A) mNGF and (B) cvNGF on bone-derived mesenchymal stem cells following treatment for 3 days. Data are presented as the means ± standard deviation, n=6. *P<0.05 vs. 0 μg/l. cvNGF, cobra-venom-derived NGF; mNGF, murine β-NGF; NGF, nerve growth factor; OD, optical density.

Cell morphology. Morphological alterations in BMSCs were detected following treatment with NGF for 7, 14 and 21 days using H&E staining. As shown in Fig. 3, more cells displayed chondrocyte-like morphology in the cvNGF group at the same culture period compared with the control and mNGF groups. In addition, more cell colonies were observed in the cv-NGF group.

Cell proliferation. The proliferation of NGF-treated BMSCs was analyzed according to DNA content. The DNA content in all groups was increased in a time-dependent manner (Fig. 4A). The growth rate of BMSCs was 126 and 59% higher in the cvNGF and mNGF groups at day 21, respectively, compared with in the control group, as evidenced by the significantly higher DNA content. In particular, cvNGF promoted cell growth compared with the other two groups; these findings were consistent with the results of the cell viability assay (Fig. 2) and H&E staining (Fig. 3).

GAG secretion. GAG deposition in BMSCs treated with NGFs for 7, 14 and 21 days was measured by DMMB assay and Safranin O staining. As shown in Fig. 4B, GAG production was gradually increased in a time-dependent manner. GAG synthesis in NGF-treated BMSCs was significantly higher compared with in the control group at the same timepoint. Compared with the control group, cvNGF promoted GAG synthesis most effectively among all groups with an increase of 60% at day 21. Safranin O staining also detected intense staining in the NGF groups compared with in the control group (Fig. 5). In addition, more abundant and homogeneously distributed GAG was detected in BMSCs in the cvNGF group.

Production of collagen type I and type II. Immunohistochemical staining was used to detect the expression of type I and type II collagen in BMSCs following treatment with NGFs *in vitro* (Fig. 6). Strong type II collagen-positive staining (Fig. 6B) and weaker type I collagen staining (Fig. 6A) were detected in the NGF groups after 7, 14 and 21 days of culturing, particularly in the cvNGF group.

Gene expression. The mRNA expression levels of *Acan, Sox9*, *Col2a1*, *Col1a1*, *RUNX2* and *ENO2* were detected by RT-qPCR. As shown in Fig. 7, cartilage-specific genes, *Acan*, *Sox9* and *Col2a1*, were significantly upregulated by cvNGF compared with the control and mNGF groups. Conversely, *Col1a1* was significantly downregulated in the cvNGF group. The expression levels of *RUNX2*, a specific gene associated with hypertrophy and osteogenic differentiation, were similar to those of *Col1a1*. Furthermore, *ENO2*, a specific gene marker for neural differentiation, was not induced by NGF, as evidenced by reduced expression compared with in the control group.

Discussion

Articular cartilage repair remains a huge challenge for researchers and clinicians. MSC-based therapies have been employed to tackle these obstacles, due to their high proliferation rate, easy availability and capacity to differentiate into numerous cell types (18). Growth factors are involved in MSC-based therapy. In our previous study, NGF was extracted from Chinese cobra venom using an improved three-step chromatography method, and its specific chondrogenesis-promoting effects on BMSCs were verified (8). Furthermore, cvNGF has been reported to exhibit higher bioactivity compared with other natural sources of NGF (15,16).

The present study focused on comparing the chondrogenic effects of NGF from two sources, commercially purchased recombinant mNGF and extracted cvNGF, on BMSCs. The results of this study verified that cvNGF greatly accelerated the survival and proliferation of BMSCs compared with the control and mNGF groups, as evidenced by the results of cell viability, morphological and proliferation analyses.

The results of an RT-qPCR analysis demonstrated that the expression levels of *Acan*, *Sox9* and *Col2a1*, which are cartilage specific markers (19,20), were significantly upregulated in the cvNGF group compared with in the other two groups. *SOX9* has been reported to act as a chondrogenic transcription factor and is a marker of early cartilage formation that has an important role in the process of chondrogenisis though promoting the production of *Acan* and activating co-expression with collagen type II (21,22). Consistent with the upregulation of cartilage-specific genes, the deposition of GAG, which serves a pivotal role in maintaining cartilage load-bearing capacity (23), was markedly enhanced by cvNGF, as indicated in the results of a biochemical assay and safranin O staining. In addition, more collagen type II-positive staining was detected in the cvNGF group compared with in the other two groups. These results suggested that cvNGF may

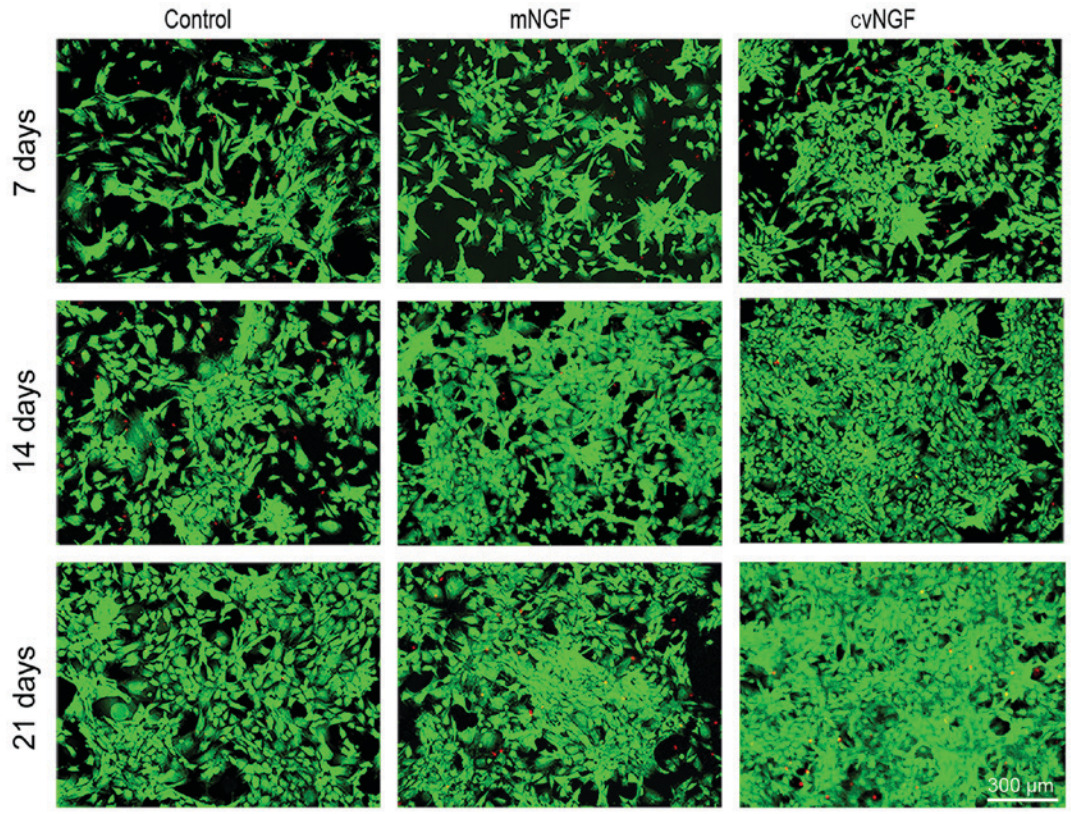

Figure 2. Fluorescein diacetate/propidium iodide staining was used to detect the viability of bone-derived mesenchymal stem cells cultured alone (control) or with NGFs (mNGF, 0.06 μg/ml; cvNGF, 6 μg/ml) for 7, 14 and 21 days. Cell seeding density, 2x10^4/ml (original magnification, x100; scale bar, 300 μm). cvNGF, cobra-venom-derived NGF; mNGF, murine β-NGF; NGF, nerve growth factor.

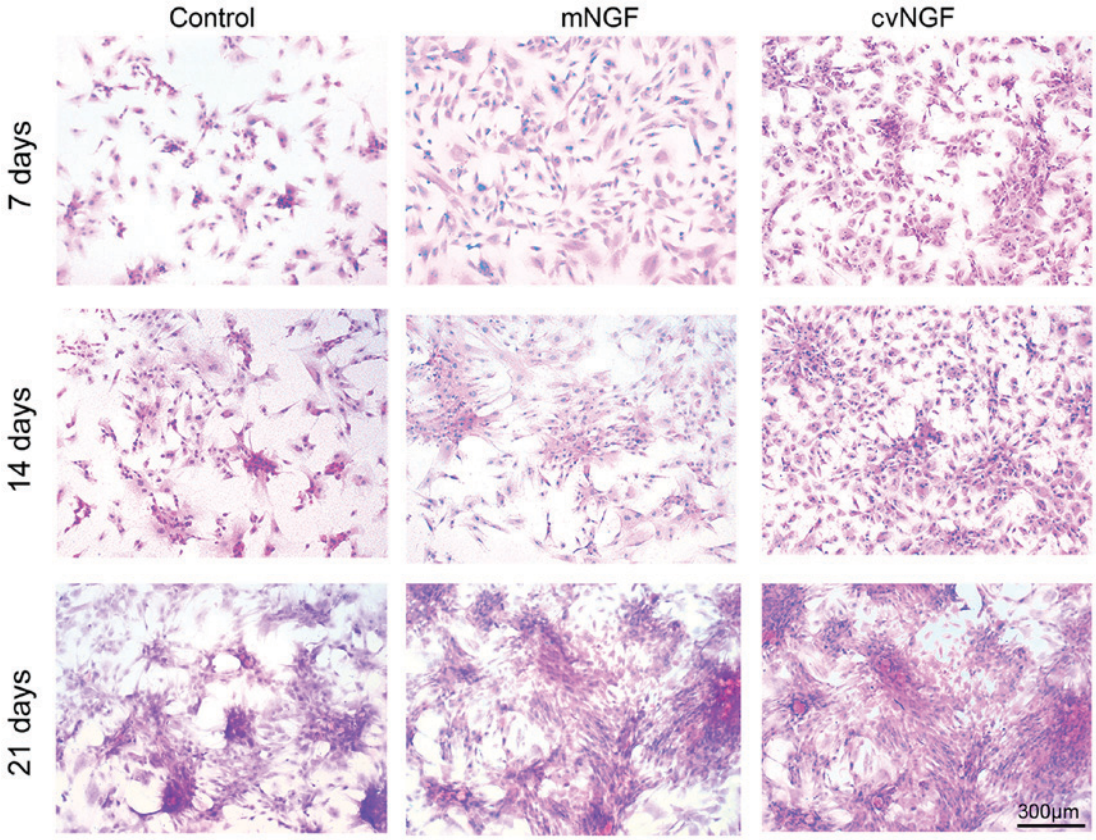

Figure 3. Hematoxylin and eosin staining was used to detect the morphology of bone-derived mesenchymal stem cells cultured alone (control) or with NGFs (mNGF, 0.06 μg/ml; cvNGF, 6 μg/ml) for 7, 14 and 21 days. Cell seeding density, 2x10^4/ml (original magnification, x100; scale bar, 300 μm). cvNGF, cobra-venom-derived NGF; mNGF, murine β-NGF; NGF, nerve growth factor.

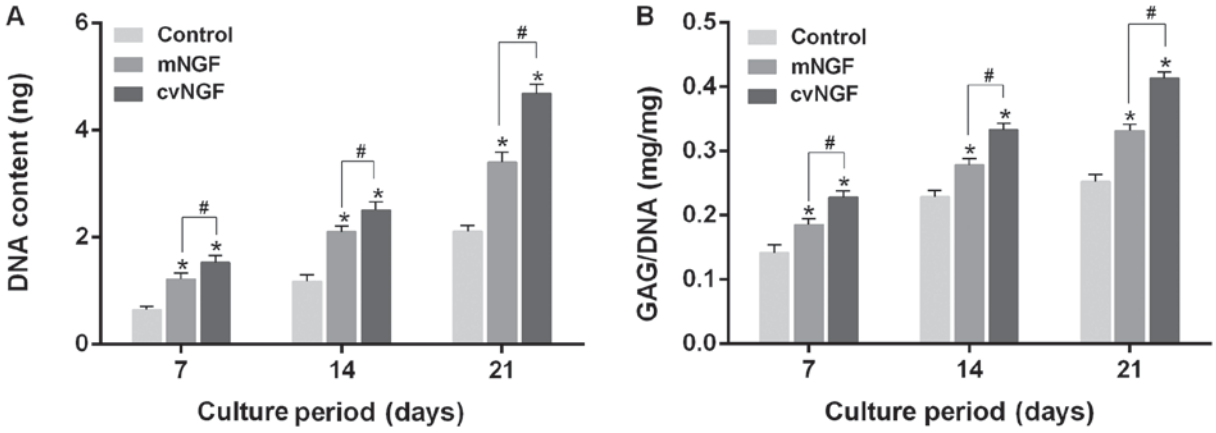

Figure 4. Quantification of cell proliferation, as detected by (A) DNA content and (B) matrix production by GAG. (A) Proliferation of bone-derived mesenchymal stem cells cultured alone (control) or with NGFs (mNGF, 0.06 μg/ml; cvNGF, 6 μg/ml) for 7, 14 and 21 days. (B) GAG (mg) normalized to DNA (mg). Data from four independent experiments were evaluated, and are presented as the means ± standard deviation. *P<0.05 vs. the control group; #P<0.05, as indicated. cvNGF, cobra-venom-derived NGF; GAG, glycosaminoglycan; mNGF, murine β-NGF; NGF, nerve growth factor.

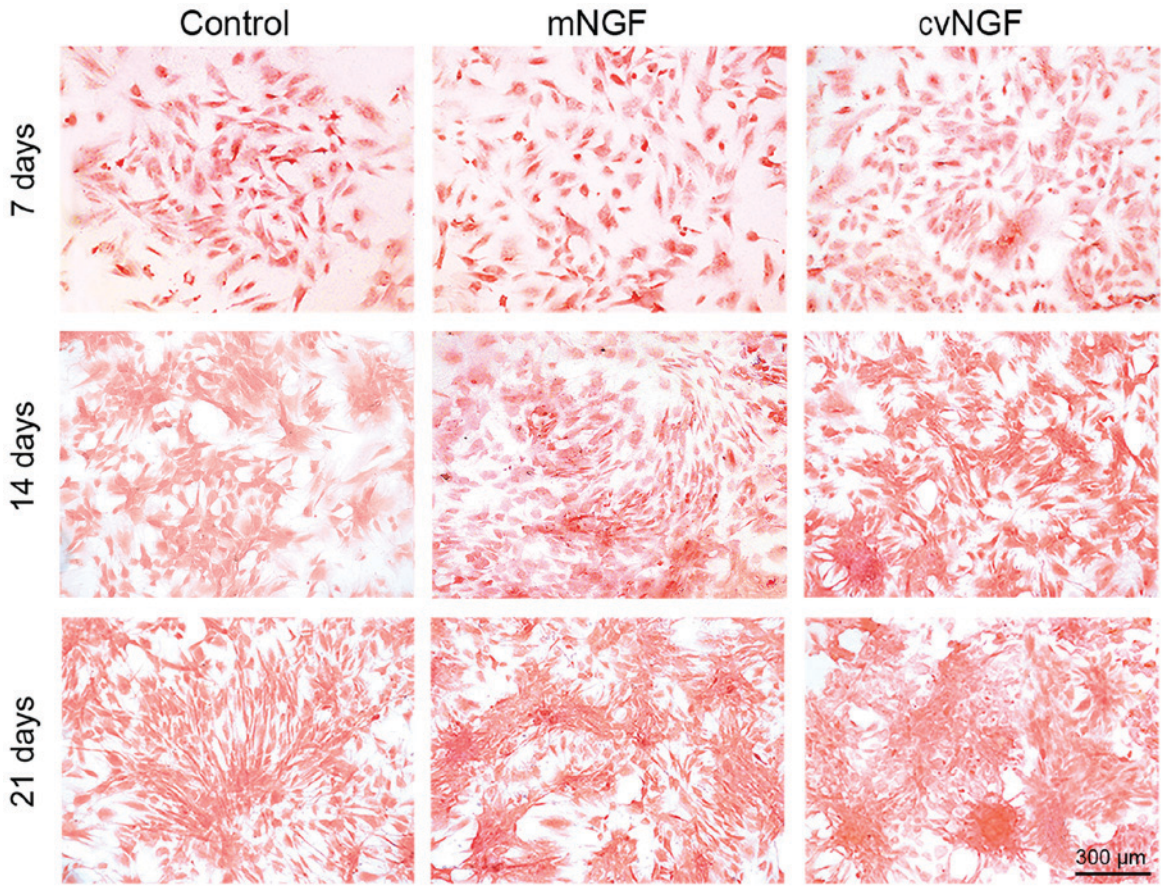

Figure 5. Safranin O staining demonstrated the extracellular matrix synthesis of bone-derived mesenchymal stem cells cultured alone (control) or with NGFs (mNGF, 0.06 μg/ml; cvNGF, 6 μg/ml) for 7, 14 and 21 days. Cell seeding density, 2x10^4/ml (original magnification, x100; scale bar, 300 μm). cvNGF, cobra-venom-derived NGF; mNGF, murine β-NGF; NGF, nerve growth factor.

accelerate chondrocyte proliferation and stimulate cartilage matrix secretion via regulating the key activators of the chondrocyte-specific enhancer, including *Acan*, *Sox9* and *Col2a1*.

Collagen type I is a marker of fibrocartilage (24), which was downregulated in response to treatment with cvNGF, as evidenced by the results of PCR and immunohistochemistry. When type II collagen and cartilage-specific proteoglycan are lost and replaced by a complex collagen phenotype, which is predominately associated with type I collagen and a low level of proteoglycan synthesis, dedifferentiation of chondrocytes ensues. The present study indicated that cvNGF could maintain a phenotype of induced chondrocytes, which was in accord with our previous study that revealed cvNGF protected chondrocytes from differentiation *in vitro*. Furthermore,

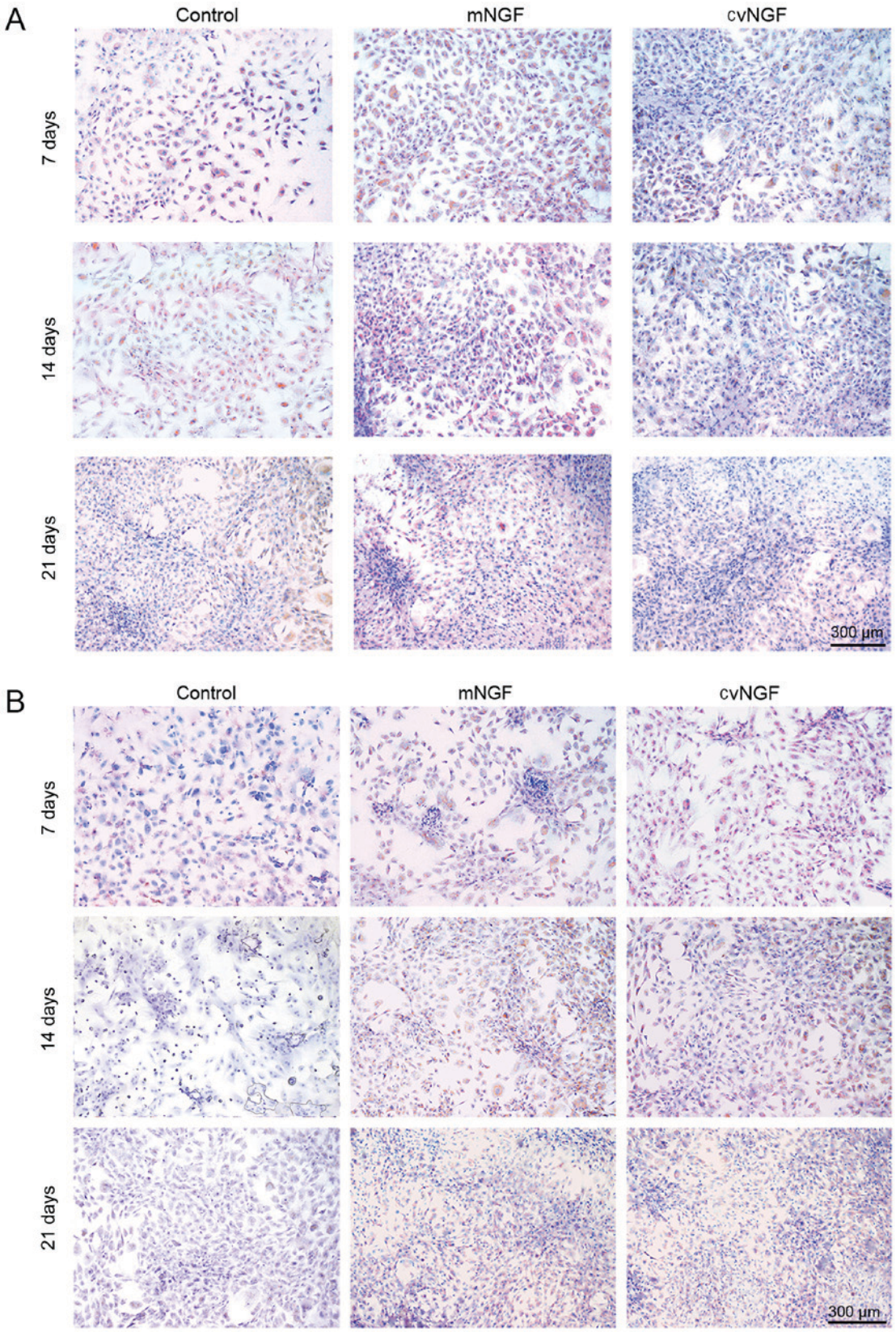

Figure 6. Immunohistochemical staining was used to detect the secretion of (A) type I and (B) type II collagens in bone-derived mesenchymal stem cells cultured alone (control) or with NGFs (mNGF, 0.06 μg/ml; cvNGF, 6 μg/ml) for 7, 14 and 21 days. Cell seeding density, 2x10^4/ml (original magnification, x100; scale bar, 300 μm). cvNGF, cobra-venom-derived NGF; mNGF, murine β-NGF; NGF, nerve growth factor.

RUNX2, a transcription factor for chondrocyte terminal differentiation (25), was also downregulated in the cvNGF group. *ENO2*, which has a role in neural differentiation, was also suppressed by cvNGF (26) compared with in the mNGF and control groups, thus indicating that cvNGF did not induce BMSCs neural differentiation.

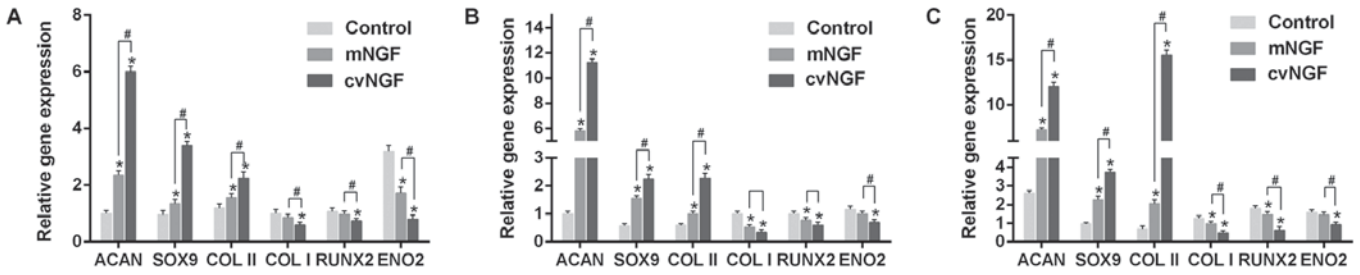

Figure 7. Quantitative comparison of *Acan*, *Sox9*, Col2a1, Col1a1, *RUNX2* and *ENO2* mRNA expression, as detected by reverse transcription-quantitative polymerase chain reaction. Bone-derived mesenchymal stem cells cultured alone (control) or with NGFs (mNGF, 0.06 μg/ml; cvNGF, 6 μg/ml) for (A) 7, (B) 14 and (C) 21 days. Data are presented as the means ± standard deviation from 3 repeated experiments. The mRNA expression levels were analyzed using the $2^{-\Delta\Delta Cq}$ method, with β-actin as the internal control. *P<0.05 vs. the control group; #P<0.05, as indicated. *Acan*, aggrecan; *Col2a1*, collagen type II; *Col1a1*, collagen type I; cvNGF, cobra-venom-derived NGF; *ENO2*, enolase 2; mNGF, murine β-NGF; NGF, nerve growth factor; *RUNX2*, runt-related transcription factor 2; *Sox9*, SRY-box 9.

The present results indicated that 6 μg/ml cvNGF and 0.06 μg/ml mNGF resulted in increased cell proliferation. Although the effective concentration of mNGF was lower than that of cvNGF, the chondrogenic effects of cvNGF on BMSCs were superior to mNGF. Furthermore, the high cost and sophisticated manufacturing process of mNGF limits its clinical application. Conversely, cvNGF is separated from natural venom, which is easily accessed and low cost, and is therefore conducive to clinical use. The present results suggested that cvNGF may be a promising growth factor for cartilage reconstruction.

In conclusion, the present study compared the chondrogenic effects of NGF from two sources, the commercially purchased mNGF and the extracted cvNGF, on BMSCs *in vitro*. The results suggested that cvNGF was more effective at inducing chondrogenic differentiation of BMSCs compared with mNGF. The easy acquirement and low cost of cvNGF indicated that it may be considered a favorable growth factor for cartilage reconstruction and beneficial for clinical applications. However, the present study conducted only *in vitro* experiments; therefore, these findings need to be further confirmed *in vivo*.

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Not applicable

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

LZ and JZ designed the study and directed its structure. ZM and ZL performed the experiments and contributed to the writing and revision of the manuscript. SL contributed to the revision of the manuscript, performed the PCR and processed the data. DL, YH and HW were responsible for the statistical analysis.

Ethics approval and consent to participate

All experiments were conducted in accordance with the standard guidelines approved by the Animal Care and Experiment Committee of Guangxi Medical University (Guangxi, China; protocol number: 2014-12-3), and the present study was approved by the ethics committee of Guangxi Medical University.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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