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Isolation, Culture and Biological Characteristics of Tibetan Mastiff (Canis lupus Familiaris) Muscle Satellite Cells

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With the ability of self-renewal and multipotenntial differentiation, muscle satellite cells (MSCs) are recognized as a population of tissue-specific progenitors which play an important role in the growth, repair and regeneration of muscles. Satellite cells have been isolated for a very long time, but the techniques of isolation are still to be improved. In this study, MSCs were obtained from aborted Tibetan mastiff fetuses (about 60 days) under sterile conditions. Primary MSCs were sub-cultured to passage 25 *in vitro*. The gene of C-met, MyoD1, Desmin and Pax7 were detected by RT-polymerase chain reaction and immunofluorescence assays. The result showed that they were positive in MSCs of Tibetan mastiff. The growth of different passages cells typically appeared in S curve. Furthermore, myogenic, osteogenic and adipogenic differenciation of MSCs were successfully induced. The results denoted that the MSCs obtained from Tibetan Mastiffs exhibited the characteristics of multipotent stem cells. Therefore, this work provided a theoretical and technical basis for Tibetan mastiff, even its genetic resources preservation and laid a solid foundation for studying the mechanism of skeletal muscle growth and development in future.

Keywords: Tibetan Mastiff, Muscle Satellite Cells, Identification, Pluripotent, Differentiation.

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1. INTRODUCTION

Skeletal muscle is the key component of supporting body weight and its growth is the major factor affecting body growth for animals. The skeletal muscle is composed of a polynucleated muscle fiber. Muscle satellite cells (MSCs), located between the basal lamina and basement membrane of the myofiber, are inactive mononucleated myogenic cells. MSCs play a critical role in the growth of skeletal muscle, and control muscle growth and development in the posthatch. At first, MSCs were identified in frog muscle by Mauro in 1961.[1] And MSCs were isolated in different species, e.g., human,[2] rat,[3] bovine,[4] chick[5] and porcine[6] by a wide variety of methods. MSCs in adult are quiescent under normal physiology position, but they can rapidly back into the cell cycle following injuries or growth signals.[7] The MSCs that entered into cell cycle express muscle regulatory factors (MRFs) such as Myf-5 and MyoD, and get into the myogenic pathway, eventually repair muscle structure and function.[8–10] However, many pathological conditions weaken their regenerative potential, such as muscle wasting or muscular dystrophies.[11,12]

In this study, we have modified the methods for isolation, culture and identification of Tibetan Mastiff MSCs. Meanwhile, these cells were induced to differentiate into osteocytes, adipocytes and myoblasts following treatment with different inducers, respectively. Through this work, skeletal satellite cells from Tibetan Mastiff could be reserved as genetic resources to study the mechanism of muscle development and laid a solid foundation for studying the dog in future.

2. MATERIALS AND METHODS

2.1. Experimental Animals and Reagents

Aborted Tibetan mastiff fetuses (about 60 days) were provided by Institute of Animal Science and Veterinary, Chinese Academy of Agricultural Sciences, Beijing.

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DMEM/F12 (Gibco, USA), fetal bovine serum (FBS, Gibco), Trypsin (Amresco), non-essential amino acids (Gibco); rabbit anti-chicken Pax7, Myod1, Desmin and myogenin polyclonal primary antibody (Bioss, China), Goat serum (Bioss, China), bovine serum albumin (BSA), EDTA, fluorescein isothiocyanate (FITC) conjugated goat-anti-rabbit secondary antibody IgG (Zhongshan Golden Bridge, China), basic fibroblast growth factor (bFGF, Peprotech), horse serum (Sigma), indometacin (Sigma), hepatocyte growth factor (Sigma), insulin transferrin (Sigma), fibroblast growth factor-4 (Sigma), β-glycerophosphate (Sigma), vitamin C (Sigma), alizarin red (Boster, China), isobutyl methylxanthine (IBMX), Oil red O (Sigma), dexamethasone (Sigma), 4′,6-diamidino-2-phenylindole (DAPI), collagenase I (Sigma), penicillin and streptomycin (Gibco), Trizol reagent (Invitrogen, USA) and cDNA reverse transcription system (Takara, China).

2.2. Isolation and Culture of MSCs

Muscular tissue was obtained from thigh muscle of aborted Tibetan mastiff fetuses (about 60 days) under sterile conditions. The tissue was washed several times with phosphate-buffered saline (PBS) supplemented with 100 IU/ml penicillin and 100 μg/ml streptomycin to remove any other tissues including connective tissue and small vessels. The samples were finely minced into small fragments with ophthalmic scissors. The small pieces were digested with 0.1% (m/v) collagenase I at 37 °C for 40 minutes, and then incubated with 0.25% trypsin for 1 h at 37 °C to further digest the tissues. The enzymatic activity was then neutralized with DMEM/F12 containing 15% (v/v) fetal bovine serum. The suspension was filtered with a 74-mm-mesh sieve and centrifuged at 1500 rpm for 10 min at room temperature. After centrifugation of the sample, haemocytes and epithelial cells will float and can be discarded with the supernatant. The remaining pellet was resuspended in complete medium containing DMEM/F-12, 10% (v/v) FBS, 2 mM L-glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin and 10 ng/mL basic fibroblast growth factor. After counting, cells were plated into flasks at 1×10^5 cells/ml, and cultured at 37 °C, 5% CO_2. After 24 h, the cells were washed with PBS to remove nonadherent cells.

The cells could be called P0 generation when the confluence of cells hit 70%–80%. The cells were washed with PBS after the old medium was removed. Then 0.125% (m/v) trypsin was added to dissociate the cells from the plates, when the vast majority of cells gleamed and retracted into smoother, medium containing required nutrients was added and mixed softly. Then trypsinization was neutralized with fresh complete medium. The cells were subcultured at the ratio of 1:2. This passage was entitled as P1 generation. With the time going of subculture, the cells were purified after four to five passages. At that point, the cells can be harvested for expansion and freezing or directly induced to differentiate.

2.3. Growth Kinetics

Cells from P5, P10 and P15 were harvested and seeded in 24-well plates with a density of 1.0×10^4/well, cultured for 5–7 days, and numbers of colony-forming units were counted to calculate colony-forming rate, which is calculated by colony forming unit numbers/starting cell number per 24-well plates $\times 100\%$.

P5, P10 and P15 were collected and plated in 24-well plates with a density of 1.0×10^4/ml, respectively. Viability and counting are performed 3 wells every day and successively for 8 days. Cell counting per well is repeated for 3 times to calculate the mean. The population doubling time (PDT) is calculated based on the formula $PDT = (t - t0)\lg 2/(\lg Nt - \lg N0)$, $t0$: starting time of culture; t: termination time of culture; $N0$: initial cell number of culture; Nt: ultimate cell number of culture.

2.4. RT-PCR Analyses of Cell Surface Markers

Total RNA was obtained from MSCs of P5 using Trizol extraction and subjected to reverse transcription procedure. Primers were designed according to the sequences of C-met, Desmin, Pax7, MyoD1, myogenin and GAPDH (internal control) from GeneBank. The template cDNA was amplified by PCR using the gene specific primers listed in Table I. The PCR products were visualized by 2% agarose gel electrophoresis, and bands were visualized with a UV transilluminator.

2.5. Immunofluorescent Detection of Cell Surface Markers

MSCs of P5 were fixed in 4% (m/v) paraformaldehyde for 15 min, then washed twice with PBS. The cells were permeabilized using 0.2% (v/v) Triton X-100 for 20 min and washed twice with PBS. The samples were blocked by 10% (v/v) goat serum for 30 min, then incubated in 1% BSA containing the following polyclonal antibodies: (1) rabbit anti-dog Desmin (1:100); (2) rabbit anti-dog Pax7 (1:100); (3) rabbit anti-dog MyoD1 (1:100) and (4) rabbit anti-dog c-met (1:100) for 1 h at room temperature. PBS was used to replace primary antibodies for the negative control. Then, the samples were washed two times with PBS, and incubated in PBS containing FITC-conjugated goat anti-rabbit secondary antibody for 1 h at room temperature. After incubation, the cells were washed two times with PBS. The samples finally were counterstained with DAPI. Photomicropraphs were taken using Nikon TE-2000-E confocal microscope with an attached Nikon EZ-C1 3.70 digital camera system.

2.6. Flow Cytometry

The expression of cell-associated surface markers can be measured by flow cytometric analysis. Briefly, MSCs of P5 were dissociated with 0.125% trypsin, centrifuged, separated into 1 mL aliquots, and labeled with custom-made. The cells were fixed and permeabilized in 70% (v/v)

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Table I. Primer sequences used in RT-PCR assay.

Gene	Primer sequence	Tm (°C)	Cycle	Fragment size (bp)
C-met	F 5′ CTCGGACTTTTCCTGTGGCT 3′ R 5′ TGCACAATCAGGCTACTGGG 3′	50	30	392
Pax7	F 5′ CCCCTTTGGGCAGAAATCCA 3′ R 5′ CGGGTATGAACCAGGACACC 3′	55	30	230
MyoD1	F 5′ TCAGACGCCCCCGTACTTTA 3′ R 5′ TCCAGGTCCTCGAAGAAACG 3′	55	30	245
Desmin	F 5′ AATTGACTTGGAGCGCAGGA 3′ R 5′ TTCTTAGCCGCGATGGTCTC 3′	55	30	198
Myogenin	F 5′ AGCTCTATGAGACGTCCCCC 3′ R 5′ CAGTCACTGGGCACCTTGG 3′	59	30	364
GAPDH	F 5′ CCCACTCTTCCACCTTCGAC 3′ R 5′ GTGGTCCAGGAGGCTCTTAC 3′	60	30	156
PPAR-γ	F 5′ CATTGACCCAGAAAGCGACT 3′ R 5′ AAGCCCTTGCATCCTTCACA 3′	48	30	459
LPL	F 5′ CGGTGCAACTCAAAGGAAGC 3′ R 5′ TCAGCCACAGTGCCATACAG 3′	49	30	248
ALP	F 5′ CTGAAATACGCCCTCAGGCT 3′ R 5′ CTTGGAGAGGGCCACGTAAG 3′	49	30	189
Osteopontin	F 5′ CTGAGGAAAAGCAGAATGCTGTG 3′ R 5′ GTCTTTTGCATGGCTGTCCC 3′	51	30	464
MHC	F 5′ CCAGGGCTCTGATGTGTCTC 3′ R 5′ AGGTTCTCAGGGCTTCCATTTAT 3′	58	30	445

ice ethanol for 12 h. Then other steps were same as Immunofluorescent detection. Finally, cells were detected by using the flow cytometer. Flow cytometric data were analyzed using CXP software version 2.0 (Beckman Coulter).

2.7. Adipogenic Differentiation

MSCs were separated into the control group and the induced group. When the cells grew to 60–70% confluence, the induced group were incubated in adipogenic medium consisted of DMEM/F12 containing 10% FBS, 200 μM indomethacin, 1 mM dexamethasone, 10 μM insulin transferrin and 0.5 mM IBMX. Cells in the control group were cultured in complete medium without factors of differentiation. After 1 week, the two groups were tested for accumulation of intracellular lipid using oil red O staining and expression of adipogenic cells specific genes by RT-PCR.

2.8. Osteogenic Differentiation

The cells were separated into the control group and the induced group. When cultures hit 60%–70% confluence, cells in the induced group were cultured in osteogenic medium that containing DMEM/F12 supplemented with 10% FBS, 0.1 mM dexamethasone, 10 mM β-glycerophosphate and 50 μg/ml ascorbate. Meanwhile, cells in the control group were cultured in primary medium. The medium was replaced by fresh medium every 2 days. Three weeks later, the ability of the cells forming calcium node was tested by alizarin red staining, and osteoblast specific genes were detected by RT-PCR.

2.9. Myogenic Differentiation

The cells were divided into two groups as described above. When cultures reached 60%–70% confluence, the induced group was cultured in myogenic medium that containing DMEM/F12 supplemented with 2% horse serum. Meanwhile, cells in the control group were maintained in primary medium. The medium was renewed every 2 days. Ten days later, the morphology of MSCs was observed by inverted microscope, and specific genes of myogenic differentiation were detected by RT-PCR and Immunofluorescence.

3. RESULTS

3.1. Culture and Morphological Observation of MSCs

The primary MSCs obtained from muscular tissue under sterile conditions were seeded into 60 mm Petri plate, after about 48 h, various shapes of cells began to stretch, a small number of polygonous cells could be observed under the inverted microscope, and tiny minority of cells showed irregular shape (Fig. 1(A)). The cells expanded rapidly, approximately 5 days later, the cells confluence degree could reach 70%–80% confluence (Fig. 1(B)). The cells of P1 were inhomogenous (Fig. 1(C)). From P5 the MSCs were fully purified, and the shape was also homogenous. MSCs exhibited fusiform pattern (Fig. 1(D)). There was no apparently difference in morphology among different passages, and the biological properties were steady after several generation (Figs. 1(D–F)). The cells were cultured to passage 24 and passage 25 and exhibited typical senescent appearance, such as vacuole, tabular shape, and karyopyknosis in most cells (Figs. 1(G, H)). As time goes by, the cells would depart from the plates after all.

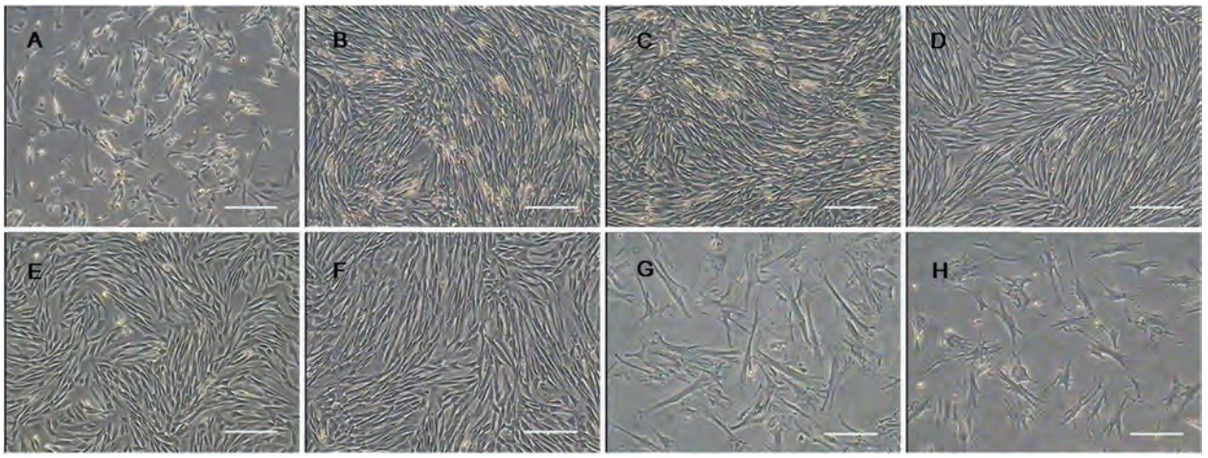

Fig. 1. Morphology of primary and subcultured MSCs. (A) Primary cells were cultured after 48 h. Most of cells began to adhere and stretch. (B) MSCs were cultured about 5 days, the cells grew to 70%–80% confluence. (C) The cells of P1 were inhomogenous. (D) After P5, MSCs were purified, and there was no apparently difference in morphology among successive passages. MSCs were spindle-shaped or fusoid. (D–F) Morphology of P5, P10 and P15 MSCs. (G, H) The MSCs of P24 and P25 displayed a typical senescent. Scale bar = 100 μm.

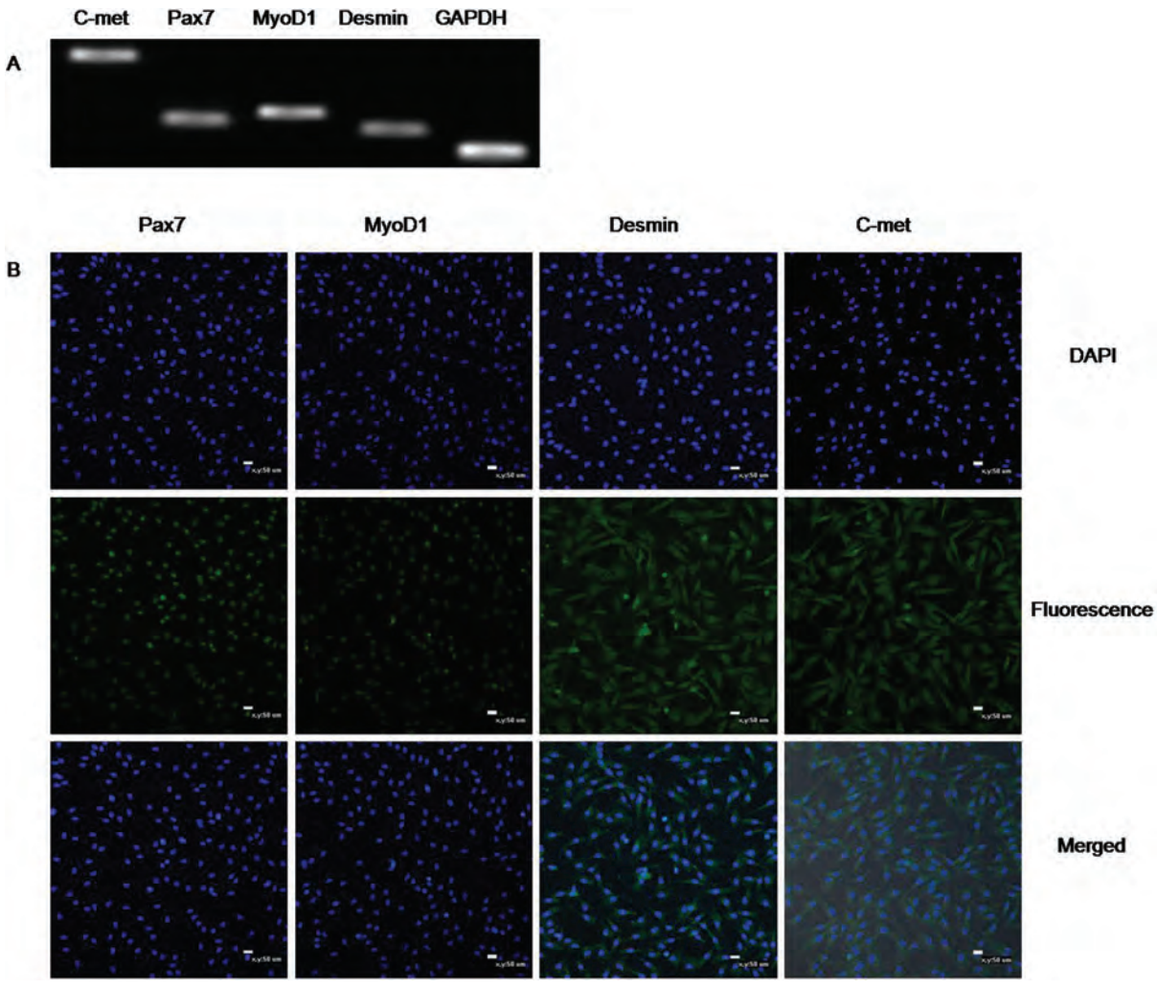

Fig. 2. Surface markers of the MSCs. Several surface markers of MSCs were detected by RT-PCR and immunofluorescence. (A) RT-PCR analysis exhibited that the MSCs expressed C-met, Desmin, Pax7 and MyoD1. And GAPDH was used as an internal control. (B) Immunofluorescence exhibited that the expression of Desmin, Pax7 and MyoD1 were positive. Scale bar = 50 μm.

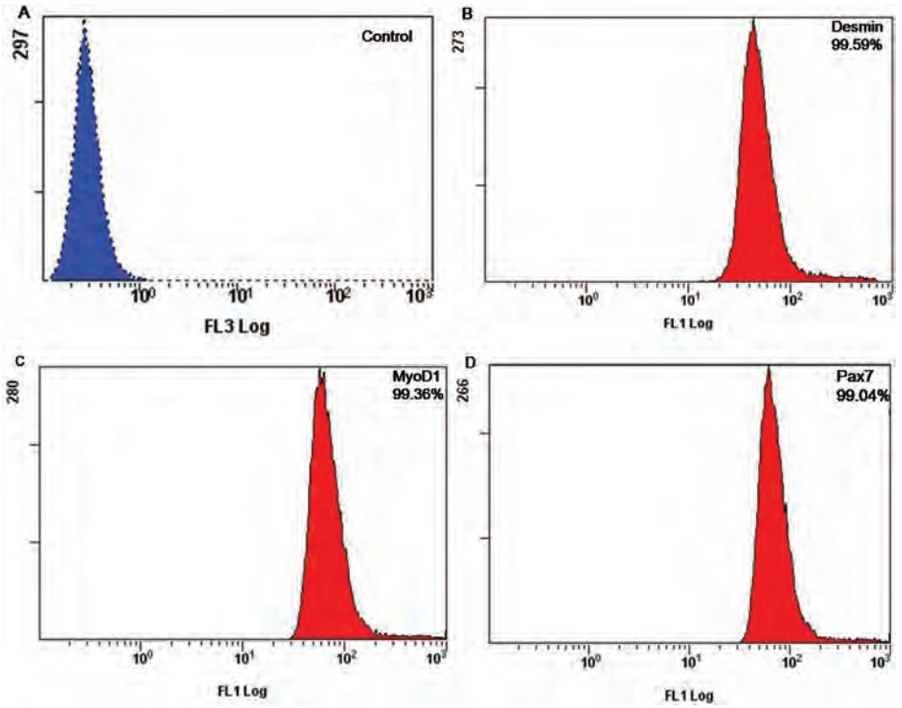

Fig. 3. Flow cytometric analysis of MSCs. The expression of Desmin, MyoD1 and Pax7 were detected by flow cytometry. The result has shown that P5 of MSCs were high expression for Desmin, MyoD1 and Pax7.

3.2. Identification of the MSCs

The specific surface markers of MSCs were detected by RT-PCR and immunofluorescence. RT-PCR experimental results emerged that the MSCs were positive for C-met, Desmin, Pax7 and MyoD1. And GAPDH served as internal control in the picture (Fig. 2(A)). The results of immunofluorescence also depicted that Desmin, Pax7, MyoD1 and c-met were positively expressed under the confocal microscopy (Fig. 2(B)). Flow cytometry has shown that P5 of MSCs were highly expressed for Desmin, MyoD1 and Pax7, the positive rates of Desmin, MyoD1 and Pax7 were 99.59%, 99.36% and 99.04%, respectively (Fig. 3).

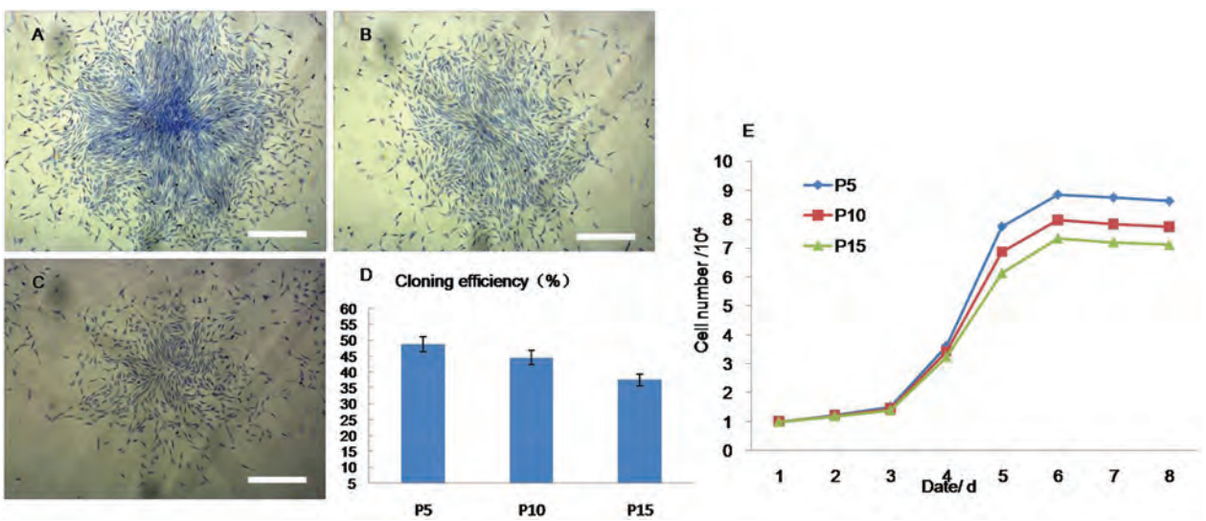

Fig. 4. Colony-forming cell assay. (A–C) were colony-forming units of P5, P10, and P15 respectively. (D) was the bar chart of colony-forming rates for different passages of MSCs. Scale bar = 100 μm. (E) Growth curves of the MSCs. The growth curves of P5, P10, and P15 MSCs were all typical S curve, with cell number reflected by the vertical axis. There are a latent phase, a logarithmic phase, and a plateau phase in a growth curve.

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3.3. Growth Kinetics

Colony formation was observed after culturing for 7 days by microscopy. The CFU were $48.68 \pm 1.63\%$, $44.52 \pm 1.72\%$, and $37.46 \pm 1.69\%$ for passage 5, passage 10 and passage 15 respectively (Figs. 4(A–C)), showing the ability of obtained MSCs for self-renewal.

The proliferation regularity of MSCs at P5, P10 and P15 was detected by cell hemocytometer, and the growth curve was drawn following the counting data (Fig. 4(E)). Through analyzing the growth curve, we could see that there was a latency phase of about 24 h; then the cells proliferated rapidly and entered logarithmic phase. Along with the density of MSCs increase, proliferation was inhibited and the cells entered the plateau phase at approximately 6–7 days and began to degenerate. The growth kinetics was all typically sigmoidal. The PDT was 28.32 h, 29.27 h and 30.01 h for passage 5, passage 10, and passage 15, respectively.

3.4. Adipogenic Differentiation of the MSCs

Adipogenic differentiation of the MSCs could be demonstrated by positive Oil Red O staining.[13, 14] After cultured in adipogenic medium for 7 days, the MSCs changed from fibroblast-like to oblate and produced a large number of lipid droplets in cells (Fig. 5(A)). With prolonged induction, the number of lipid droplets increased gradually and assembled into the larger droplets. The differentiation result was determined by the way of oil red O staining; the induced cells were positive for the staining result (Fig. 5(B)). Cells cultured in complete medium were not stained by Oil Red O (Fig. 5(C)).

To determine differentiation of the MSCs, the expression of adipogenic markers was tested by RT-PCR in the both groups. Adipocyte specific genes PPAR-γ and LPL were positive (Fig. 5(D)), which did not occur in the control group.

3.5. Osteogenic Differentiation of the MSCs

The capacity of MSCs to differentiate into osteogenic cells was verified, and the morphological and phenotypic marker analysis was carried on for the induced cells. The cell shape changed at the seventh days after induction; the cells became confluent and formed mineralized nodules which were bigger for further induction. After cultured in osteogenic medium for 21 days, morphological changes of the MSCs was obvious (Fig. 6(A)). Further, the nodules were Alizarin Red staining positive (Fig. 6(B)). Besides, with prolonged effects of induction, the nodules increased

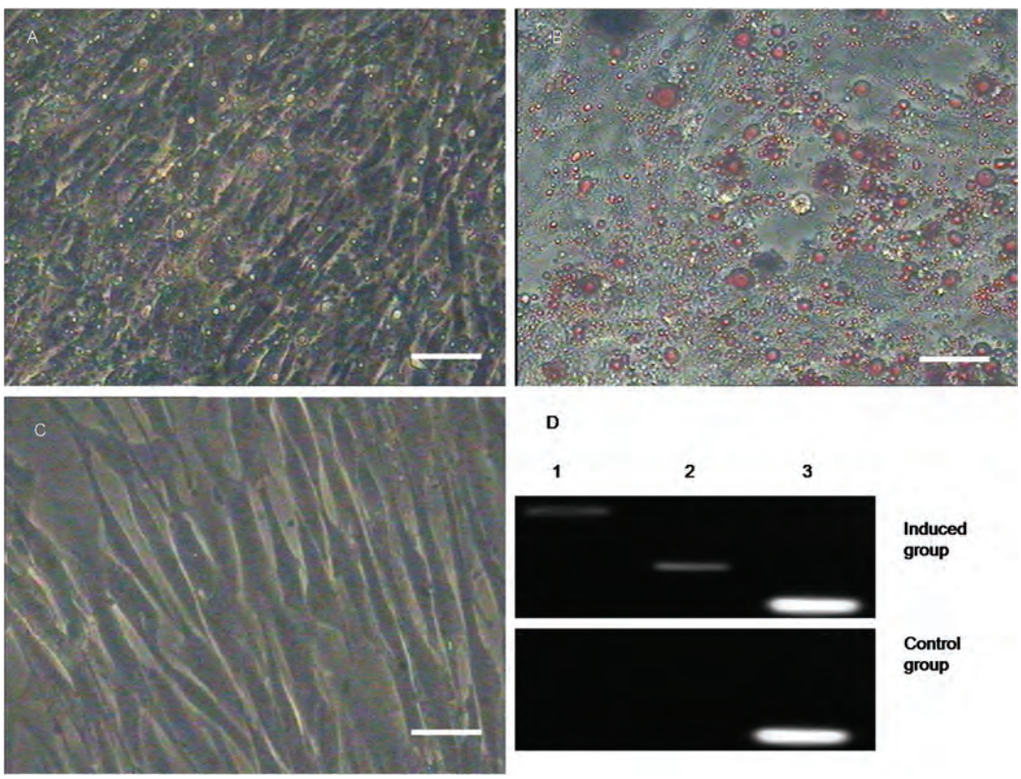

Fig. 5. Adipogenic differentiation of the MSCs. (A) MSCs were inducted after 1 weeks, they began to changed from shuttle to oblate, and there were many lipid droplets in the cells. Droplets increased and assembled to form larger ones along with the extension of induction time. (B) They were positive for oil red O staining. (C) The cells cultured in primary medium had no difference in the morphology and phenotype, and they were also negative for oil red O staining. Scale bar = 50 μm. (D) RT-PCR detection of the adipogenic markers LPL and PPAR-γ expression in induced group and control group. Induced cells were positive for LPL and PPAR-γ, but the control cells were not. 1: PPAR-γ; 2: LPL; 3: GAPDH.

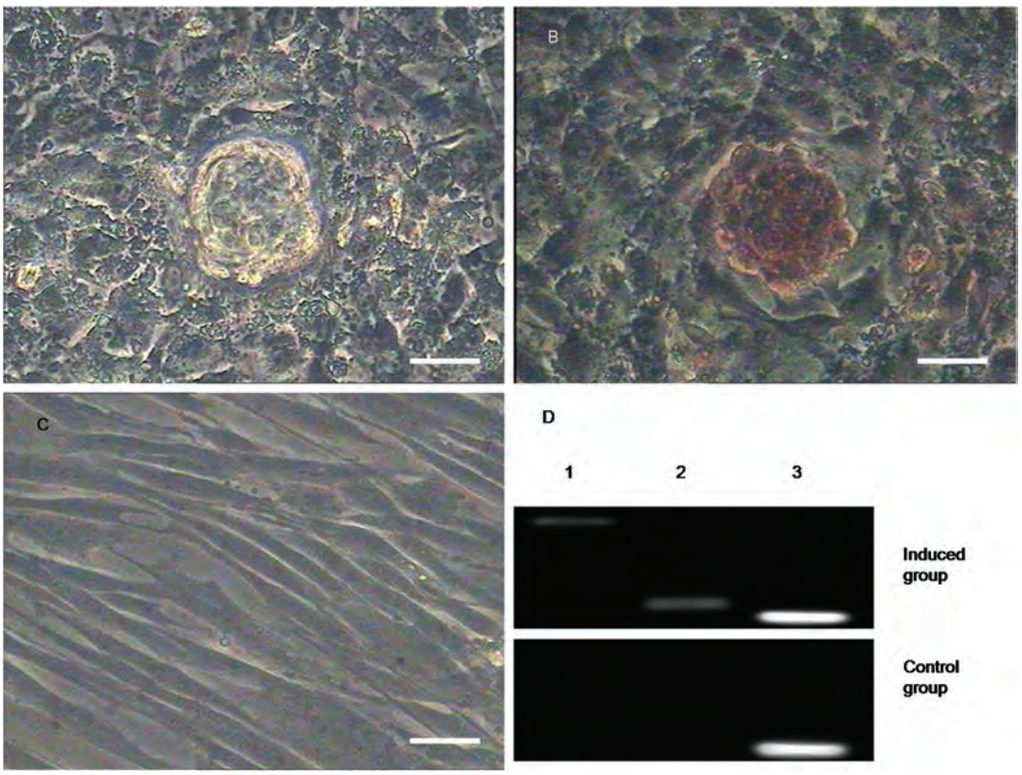

Fig. 6. Osteogenic differentiation of the MSCs. (A) The quantity and size of nodules increased as induction progressed. (B) After culture in osteogenic medium for 21 days, the cell shape was changed, and Alizarin Red staining was positive. (C) Cells cultured in primary medium were not changed in morphology or stained by Alizarin Red. Scale bar = 50 μm. (D) RT-PCR detection of the osteogenic markers ALP and osteopontin expression in the both groups. Induced cells were positive for osteopontin and ALP, but the control cells were not. 1: Osteopontin; 2: ALP; 3: GAPDH.

and were bigger. The cells cultured in primary medium were not changed in morphology or stained by Alizarin Red (Fig. 6(C)).

To determine that differentiation had happened, the expression of osteogenic markers was tested by RT-PCR in the both groups. Osteogenic-specific genes osteopontin and ALP were positive in the induced group, but not in the control group (Fig. 6(D)).

3.6. Myogenic Differentiation

The shape of the cells cultured in myogenic medium was changed and proliferated slowly. Anastomosis among cells could be observed apparently after 10 days. And the MSCs initiated terminal differentiation to form multinucleated myotubes (Fig. 7(A)). Immunofluorescence result showed that the multinucleated myotubes were observed with DAPI (Fig. 7(B)). But Cells cultured in complete medium were not changed in morphology after 10 d (Fig. 7(C)). MHC and myogenin and were expressed in the differentiated cells (Fig. 7(D)).

4. DISCUSSION

MSCs are useful source to investigate the mechanisms of muscle growth and development. Skeletal muscle satellite cells are a kind of mononuclear stem cell in adult. And they are located between the basal lamina and sarcolemma of the muscle fibers, so it is difficult to obtain the cells. At present, the classical and efficient method to obtain muscle satellite cells, is to break down the connective tissue network and myofibers in order to release the MSCs based on the mincing and enzymatic digestion of the muscle mass.[15] MSCs were isolated in different species including frog, human, rat, bovine, porcine and chick. There is no corresponding research on Tibetan Mastiff.

In this work, skeletal muscle satellite cells of Tibetan mastiff were isolated from thigh muscle using combined enzymatic digestion of 0.1% collagenase I and 0.25% trypsin under sterile conditions. Muscle fibers can be separated by collagenase I and trypsin which part MSCs out from the basal lamina and sarcolemma of the muscle fibers. Tibetan mastiffs MSCs were purified by the differential adherence technique, because fibroblasts adhere to the culture plate more rapidly than MSCs.[16]

Our research showed that Tibetan mastiff MSCs could be cultured at least 25 passages *in vitro*. The markers of inactive MSCs include C-met,[17] Pax7,[18,19] and M-cadherin,[19] while the markers of proliferating MSCs include Myf5, MyoD and Desmin.[2,20] In this work, the gene of C-met, Desmin, Pax7 and MyoD1 were detected

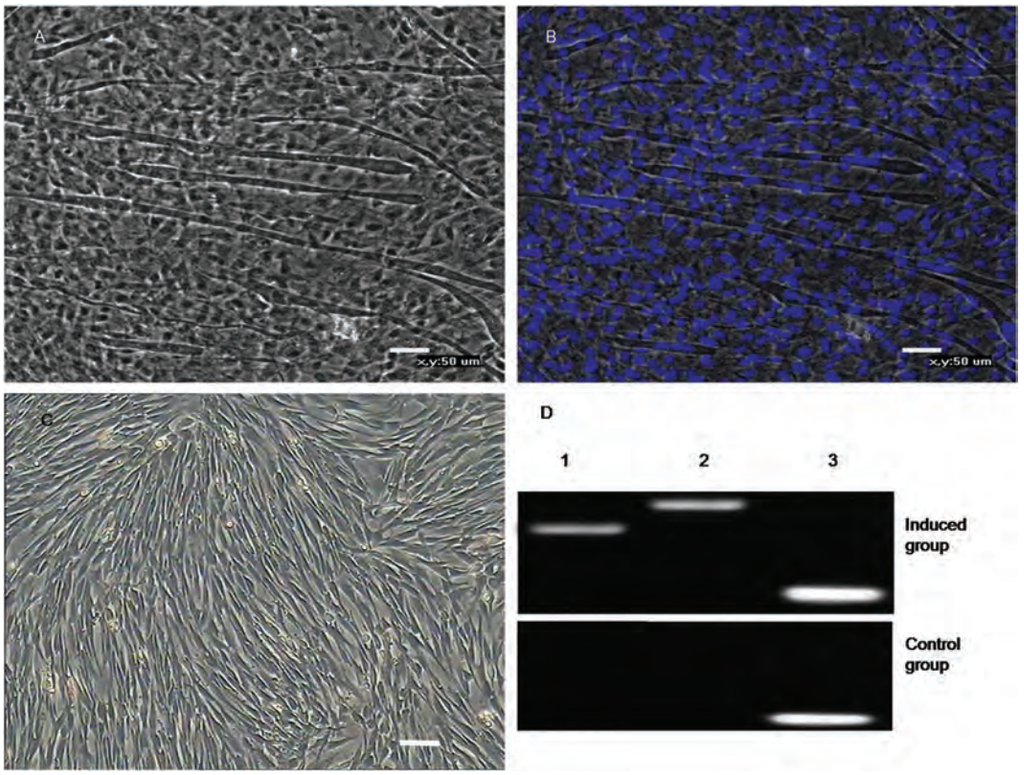

Fig. 7. Myogenic differentiation of the MSCs. (A) After culture in myogenic medium for 10 days. (B) On the tenth day, the cell shape was changed, and multinucleated myotubes were observed with DAPI. (C) Cells cultured in complete medium were not changed in morphology after 10 d. Scale bar = 50 μm. (D) RT-PCR analysis of the myogenic differentiation markers myogenin and MHC expression in induced group and control group. Induced cells were positive for myogenin and MHC, but the control cells were not. 1: Myogenin; 2: MHC; 3: GAPDH.

by RT-polymerase chain reaction and immunofluorescence assays. The result showed that they were positive in Tibetan mastiffs MSCs.

Pax7 is specific marker of skeletal MSCs, it probably acts through the recruitment of a histone methyltransferase complexin, promoting expansion of the activated pool,[21–22] but repressing premature differentiation of satellite cells.[23] All MSCs in adult express Pax7, which acts as a survival factor.[24] C-Met, also called hepatocyte growth factor receptor, it involved in cell information regulation, proliferation and differentiation.[25] MyoD1 is also a very important protein expressed in MSCs; nearly all satellite cells express MyoD1, even at a very early stage of the MSCs. When MSCs are activated, all the cells express MyoD1 before entering into a MRF-negative, quiescent state when muscles are repaired.[26, 27] This gene encodes a nuclear protein that belongs to the basic helix-loop-helix family of transcription factors and the myogenic factors subfamily. It regulates muscle cell differentiation by inducing cell cycle arrest, a prerequisite for myogenic initiation. The protein is also involved in muscle regeneration. It activates its own transcription which may stabilize commitment to myogenesis.[28] The function of Desmin has been deduced through studies in knockout mice. Desmin is one of the earliest protein markers for muscle tissue in embryogenesis as it is detected in the somites.[29] Although it is present early in the development of muscle cells, it is only expressed at low levels, and increases as the cell nears terminal differentiation.

The traditional view is that the skeletal muscle satellite cells are myogenic cells but not the pluripotent cells. However, this view is being challenged. It was reported that myogenic cells or clonal myogenic cells isolated from adult skeletal muscles display osteogenic[30] or adipogenic[30, 31] differentiation capability. In addition, blocking the Wnt signaling pathway also induces adipogenic differentiation of C2C12 cells.[32]

The growth of different passages cells typically appeared in S type curve and had a natural population doubling time. Furthermore, MSCs were successfully induced to differentiate into osteoblasts, adipocytes, and myoblasts. All the results showed that the biological properties of the MSCs obtained from Tibetan mastiff were stable. Our *in vitro* differentiation studies support the findings already reported in mammals. Therefore, MSCs may be a good option for cellular transplantation therapy and regenerative medical projects.

The multipotency of stem cells is the most notable characteristic for autologous cell therapy. *In vitro*, under the action of some inducing factors, the expressing of

some key genes in the signaling pathway related to stem cell differentiation can change.[33] Consequently, differentiation in specific directions was achieved. In our research, we induced Tibetan mastiff MSCs to differentiate into osteoblasts, adipocytes, and myoblasts and tested corresponding to genes of the cell types above. The autologous features of these stem cells, in combination with their distinct multipotentiality and easy acquirement, make MSCs an attractive chioce for future tissue-engineering and cell-based therapies. The results above suggested that Tibetan mastiffs MSCs had strong self-renewal ability and the potential to differentiate towards mesodermal and endodermal cells. Although the multilineage differentiation of MSCs was successful *in vitro*, there are many technical challenges on utilizing MSCs in clinical application for therapeutic purposes, such as high rate of reduction. These aspects need to be taken into account in future research and clinical applications.

5. CONCLUSION

In conclusion, in this work, MSCs were successfully obtained from muscular tissue of aborted Tibetan mastiff fetuses, and the ability of expansion and differential potential were tested *in vitro*. This investigation offers an important sense on the research of Tibetan Mastiff and the potential application of MSCs as a stem cell material for its genetic resources preservation and lays a solid foundation for studying the mechanism of skeletal muscle growth and development in future.

Conflicts of Interest

The authors declare that they have no competing interests.

Ethics Statement

The study was approved by the Chinese Academy of Agriculture Sciences Institutional Animal Care and Use Committee. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Chinese Academy of Agricultural Sciences. All procedures were treated in accordance with the regional ethical committee for the use and care of laboratory animals.

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