



***Oct4* and *Sox2* overexpression improves the proliferation and differentiation of bone mesenchymal stem cells in Xiaomeishan porcine**

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ABSTRACT. Mesenchymal stem cells derived from bone marrow (BMSCs) are a population of self-renewing multipotent cells that are capable of differentiating into various cellular lineages, and are widely employed in tissue engineering and cell therapy. Recently, clinical research involving BMSCs has become increasingly popular. In order to conduct appropriate research, it is first necessary to amplify large amounts of functional BMSCs *in vitro*. However, after several passages of expanding *in vitro*, the proliferation and differentiation potential of BMSCs gradually decline. To determine whether overexpression of *Oct4* or *Sox2* might prevent this decline, we transfected *Oct4* or *Sox2*, which are essential for the pluripotency and self-renewal of embryonic stem cells, into BMSCs of Xiaomeishan porcine by a lentivirus. The results showed that overexpression of *Sox2* or *Oct4* BMSCs in culture media containing a basic fibroblast growth factor resulted in higher proliferation

and differentiation compared to controls, suggesting that genetic modification of stemness-related genes is an efficient way to maintain the proliferation and differentiation potential of BMSCs.

Key words: *Oct4*; *Sox2*; Bone mesenchymal stem cells; Overexpression; Xiaomeishan porcine

INTRODUCTION

Bone mesenchymal stem cells (BMSCs) are a population of self-renewing multipotent cells. These stem cells are capable of differentiating into various cellular lineages, including the osteogenic, chondrogenic, adipogenic, and myogenic lineages (Pittenger et al., 1999; Jiang et al., 2002; Imabayashi et al., 2003; Bosch et al., 2006), and are widely used in clinical investigations (Friedenstein et al., 1966; Horwitz et al., 2002; Kadivar et al., 2006; Yang et al., 2008). Before human clinical trials can be approved, scaled-up cell production and delivery into an animal model with cell doses (number of cells) comparable to those anticipated for human trials are often required in order to satisfy regulatory safety concerns. Porcines display significant morphological and functional similarities to humans. Therefore, isolation and thorough characterization of porcine BMSCs offer great potential and would represent an advantageous experimental tool for tissue engineering and cell therapy. The main line of local Taihu pig in China, Xiaomeishan, is famous for its outstanding fecundity and meat quality, and is recognized worldwide for its outstanding genetic resources. Consequently, this breed is not only valuable for its resource conservation, but also for use in transgenic breeding to establish a culture of Xiaomeishan BMSCs. However, these cells tend to lose their proliferation and differentiation potential after several passages (Yoon et al., 2011). Furthermore, they enter the stages of senescence, characterized by enlarged and irregular cell shape, and cessation of cell division occurs after several passages (Hayflick, 1965).

The key transcription factors *Oct4* and *Sox2* have been extensively studied in embryonic stem cells (ESCs). *Oct4* is a critical transcription factor for regulating the self-renewal and differentiation properties of ESCs, and is a POU domain transcription factor expressed in ESCs and germ cells (Nichols, et al., 1998). Overexpression of *Oct4* causes ESC differentiation into primitive endoderm and mesoderm. Repression of *Oct4* expression induces loss of ESC pluripotency and dedifferentiation to trophectoderm (Niwa et al., 2000). The transcription factor *Sox2* is a member of the SRY-related HMG box family (Masui et al., 2007). It is expressed in pluripotent, embryonic, and extraembryonic cells and plays an important role in embryonic development and in maintaining the pluripotency and self-renewal ability of ESCs (Lefebvre et al., 2007; Niwa, 2007). *Oct4* and *Sox2* were shown to be critical factors for reprogramming somatic cells into induced pluripotent stem cells (Takahashi and Yamanaka, 2006). However, whether *Oct4* or *Sox2* genes might improve the growth and plasticity properties of BMSCs is still unknown.

In the current study, *Oct4* and *Sox2* genes, essential for the pluripotency and self-renewal of ESCs, were introduced into BMSCs of Xiaomeishan porcine, and their overexpressions were evaluated to determine whether they could improve the proliferation and differentiation abilities of BMSCs.

MATERIAL AND METHODS

The experimental protocol was approved in accordance with the Guide for the Care and Use of Laboratory Animals prepared by the Ethics Committee of Nanjing Agricultural University.

Isolation and culture of BMSCs

Fresh bone marrow was extracted from Meishan porcine (2 weeks of age) femurs under sterile conditions. BMSCs were isolated by bone marrow aspiration by centrifugation (20 min, 1500 g) at room temperature. Then, cells were cultivated under standard culture conditions with Dulbecco's modified Eagle's medium with low glucose (L-DMEM; Gibco, USA) supplemented with 15% fetal bovine serum (FBS; Gibco), 100 U/mL penicillin, 100 µg/mL streptomycin, and 2 mM glutamine. Cultures were maintained at 37°C in a humidified atmosphere containing 5% carbon dioxide. The medium was replaced after the first 72 h and then twice a week thereafter. Approximately 90-95% confluence cells were subcultured at 3-5-day intervals by trypsinization using 0.25% trypsin and 0.02% EDTA, and stored in freezing medium in liquid nitrogen.

Flow cytometric analysis

Phenotypic characterization of BMSCs was performed by flow cytometric analysis. Cells were trypsinized and washed with phosphate-buffered saline (PBS; Gibco) and incubated at 4°C for 30 min with the following cell-specific antibodies: CD29 (Abcam, USA), CD34 (Abcam), CD44 (AbD, England), CD45 (Abcam), and CD90 (Abcam). Cells were first permeabilized with cold methanol/PBS for 2 min at -20°C. After washing with cold PBS, cells were incubated with primary antibody (1:500) at 4°C for 30 min, followed by staining with a fluorescein isothiocyanate (FITC)-linked secondary antibody (1:100) for an additional 30 min. Labeled cell analysis was performed with an FACS Calibur cytometer (Becton Dickinson Immunocytometry System), and data were analyzed using the FlowJo software (Tree Star, Inc.)

Lentivirus production and transduction

293FT cells (Invitrogen, USA) were plated at 80% confluence and were transfected with the lentiviral vector of either Oct4 or Sox2 along with packaging plasmids, using Lipofectamine 2000 (Invitrogen), following manufacturer instructions. The resulting supernatant was collected 48 h after transfection and filtered through a 0.45-µm filter (Millipore, USA). The viruses were precipitated at 1500 g and resuspended with Opti-MEM medium (Invitrogen). Then, BMSCs were infected with either of the viruses to achieve *Oct4* or *Sox2* overexpression. BMSCs transfected with the empty vector, which was not inserted with *Oct4* or *Sox2*, were used as controls. The expression of *Oct4* and *Sox2* was detected by Western blotting.

Western blot analysis

Total cellular protein content of BMSCs with overexpressed *Oct4*, *Sox2*, and con-

trol, and BMSCs at passage 3, were extracted using RIPA lysis buffer and incubated on ice for 30 min. The extract was centrifuged at 12,000 *g* for 10 min at 4°C, the supernatant was collected, and the total protein content was measured using the bicinchoninic acid (BCA) assay. These proteins were separated using 0.1 g/mL polyacrylamide sodium dodecyl sulfate (SDS) gel electrophoresis and transferred to polyvinylidene fluoride (PVDF) membranes, which were then incubated with a blocking buffer (Tris-buffered saline with 0.05 g/mL milk and 0.1% (v/v) Tween-20, pH 7.5) for 1 h at room temperature, and incubated overnight at 4°C with a monoclonal antibody against Oct4 (1:100; Abcam) or Sox2 (1:100; Abcam). The mouse monoclonal antibody against α -tubulin (1:2000; Santa Cruz) was used as a house-keeping control gene. The treated membranes were washed 3 times and incubated with the horseradish peroxidase-linked secondary antibody, which was diluted (1:8000) in the blocking buffer for 1 h. Then, the blots were rinsed 3 times and detected for immunoreactivity by chemiluminescence detection, and were used to expose radiographic films to visualize immunoreactive signals. Finally, the blots were quantified by computerized densitometry using the Adobe Photoshop software (Adobe, Mountain View, CA, USA). Values were obtained using the anti-Oct4 or anti-Sox2 antibody, which were normalized for the α -tubulin present in the sample.

Cell proliferation analysis

The BMSCs with overexpressed *Oct4* or *Sox2* and the control were respectively seeded on 24-well plates (1×10^4 cells/well) at passage 12. Then, the cells were harvested by trypsinization at 24-h intervals for 7 days. Cell numbers were counted daily with a hemocytometer, and cell population growth was determined by calculating the population doubling time.

Multipotential differentiation of BMSCs

The BMSCs with the overexpressed *Oct4* or *Sox2* and the control were cultured in L-DMEM, containing 10% FBS until they reached 60-70% confluence, at which point they were induced by appropriate media to determine their multipotential differentiation abilities. To induce osteogenic differentiation, the BMSCs with overexpressed *Oct4* and the control were each treated with osteogenic differentiation medium on 6-well plates (5×10^5 cells/well) for 2 weeks. The osteogenic differentiation medium consisted of DMEM supplemented with 10% FBS, 0.1 mM dexamethasone (Sigma, USA), 10 mM glycerol-2-phosphate (Sigma), and 50 μ g/mL ascorbic acid (Sigma). The BMSCs with overexpressed *Sox2* were induced by osteogenic differentiation medium supplemented with basic fibroblast growth factor (bFGF). The culture medium was replaced with fresh medium twice a week. Adipogenic differentiation was induced on 6-well plates (5×10^5 cells/well) for 3 weeks by adipogenic differentiation medium consisting of DMEM supplemented with 10% FBS, 1 mM dexamethasone (Sigma), 0.5 mM 3-isobutyl-1-methylxanthine (IBMX; Sigma), 10 mg/mL insulin (Sigma), and 0.1 mM indomethacin (Sigma). The culture medium was replaced with fresh medium twice a week. Differentiation of the BMSCs with overexpressed *Oct4* or *Sox2* and the control were evaluated by staining and real-time reverse transcription polymerase chain reaction (RT-PCR). Osteogenesis was stained with Alizarin red, and adipogenesis was examined with Oil Red O solution (Sigma).

RT-PCR analysis

The differentiated BMSCs were harvested and washed once in PBS. Their total RNA was extracted using the PureLink RNA Mini kit (Invitrogen) according to manufacturer instructions, and reverse-transcribed using the Prime Script RT reagent kit (Takara, China). The mRNA levels were assayed with the SYBR Premix Ex Taq kit (Takara) on an ABI 7300 PRISM system (Applied Biosystems, USA). The amplification program consisted of an initial denaturation at 95°C for 30 s followed by 40 cycles at 95°C for 5 s, annealing at 60°C for 31 s, and extension at 72°C for 7 min. RT-PCR was performed using the primers listed in Table 1. Each PCR analysis was performed in duplicate, with each set of assays repeated three times. To minimize the effects of unequal quantities of starting RNA and to eliminate potential sources of inconsistency, the relative expression levels of each gene were normalized to GAPDH using the $2^{-\Delta\Delta Ct}$ method.

Table 1. Osteogenic and adipogenic marker genes.

Gene	Accession No.	Oligonucleotides (5'→3') (up/down)	Product size (bp)	Annealing temperature (°C)
<i>GAPDH</i>	AF017079	CTCAACGACCACTTCGTCAA TCTGGGATGGAAACTGGAAG	233	58
<i>PPARγ</i>	AF059245	TGACCCAGAAAGCGATGC ATGGCACTTGGTAGTCCTG	285	58
<i>CEBP-α</i>	AF103944	TGGACAAGAACA TTGTCACCTGGTCAGCTCCAG	130	56
<i>osteocalcin</i>	AW346755	TCAACCCCGACTGCGACGAG TTGGAGCAGCTGGGATGATGG	204	62
<i>osteonectin</i>	NM001031794	CCATCGGCGAGTTTGAG GCTTCTGCTTCTCGGTCA	270	62
<i>type Iα1</i>	AF201723	CCAAGAGGAGGGCCAAGAAGAAGG GGGGCAGACGGGGCAGCACTC	232	58

Statistical analysis

All experiments were repeated at least three times. Values are reported as means \pm standard error of the mean (SE). The data were subjected to the independent sample Student *t*-test in the software package SPSS 17.0. Differences between means were determined by the Duncan multiple range test. Statistical differences were to be considered significant when $P < 0.05$.

RESULTS

Characterization of BMSCs *in vitro*

In this study, BMSCs of Xiaomeishan porcine were successfully established. Most of the nonadherent cells were removed at the first media replacement after 72 h. Fibroblast-like cells attached to the plastic were evident at 3-5 days after initial seeding (Figure 1A). Most cell lines were composed of cells with a characteristic spindle shape, whereas the other lines had cells with a polygonal morphology (Figure 1B). The cells increased in number progressively and reached 80% confluence by 12-14 days after seeding. However, after several passages, BMSCs entered senescence, characterized by enlarged and irregular cells (Figure 1C).

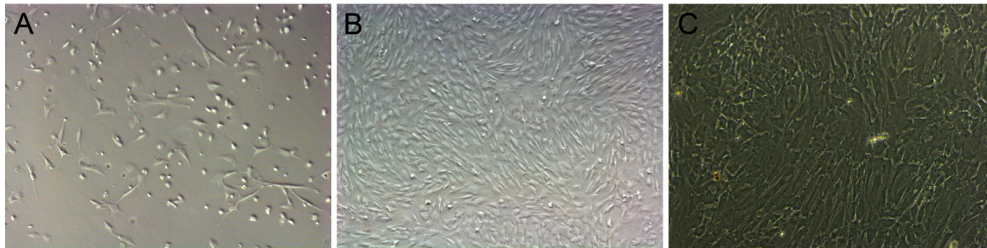


Figure 1. Morphology of Xiaomeishan porcine BMSCs. **A.** Cells exhibited a stretched fibroblast-like phenotype, mixed with some round-shaped erythrocytes and nonadherent cells at 6 days. **B.** Subcultivated cells, expanded up to at passage 3, presented a stable fibroblast-like morphology. **C.** BMSCs showed characteristic morphology changes after several passages, and became elongated in shape (100X).

To verify cell identity, Xiaomeishan porcine BMSCs were routinely examined by flow cytometry analysis at passage 3. Cells were positive for the stromal markers CD29, CD44, and CD90, but not for the hematopoietic markers CD34 and CD45 (Figure 2).

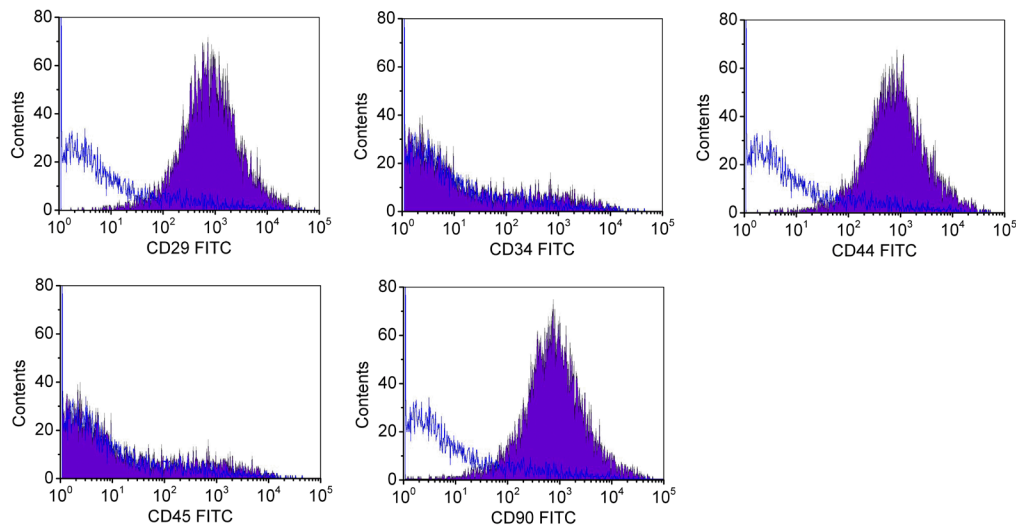


Figure 2. Flow cytometry histograms of three passaged Xiaomeishan porcine BMSCs for hematopoietic (CD34 and CD45) and stromal (CD29, CD44 and CD90) markers. BMSCs labeled with fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies. BMSCs were positive for CD29, CD44 and CD90, but negative for CD34 and CD45. The open areas stand for antibody isotype as control, and the shaded areas for the expression of the indicated markers.

***Oct4* or *Sox2* expression in BMSCs**

In order to achieve high efficiency of introduction and subsequent stable expression of *Oct4* or *Sox2* in BMSCs, a lentiviral vector was employed. The red fluorescent protein was used as a reporter gene, so that expression of the construct was easily detectable during the cell

culture. The translation efficiency of *Oct4* and *Sox2* was high in BMSCs, and the fluorescent signal appeared strong under a fluorescence microscope (Figure 3). Western blotting results showed that the Oct4 protein content in *Oct4*-overexpressed BMSCs and the Sox2 protein content in *Sox2*-overexpressed BMSCs at passage 3 were significantly higher ($P < 0.01$) than protein contents in control or normal BMSCs. However, there was no difference in Oct4 or Sox2 protein content between control and normal BMSCs ($P > 0.05$) (Figure 4). *Oct4* or *Sox2* was expressed effectively in the cells transfected with Oct4 or Sox2.

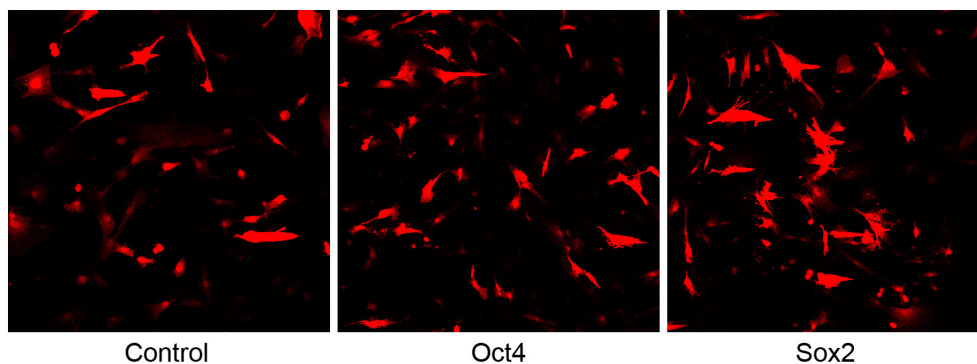


Figure 3. Intensive red fluorescence was observed by fluorescence microscopy after lentivirus infecting for 48 h (100X).

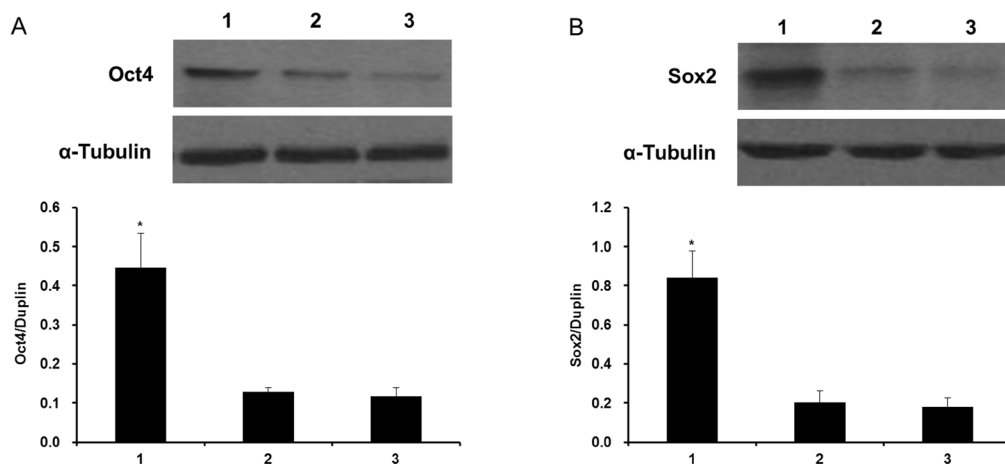


Figure 4. Detection of Oct4 (A) and Sox2 (B) protein expression by Western blot analysis and digital image analysis. Total cellular protein was extracted from Oct4- or Sox2-overexpressed BMSCs. The blot was first stained with the primary antibodies and then with horseradish peroxidase (HRP)-conjugated secondary antibody (goat anti-mouse IgG). The level of α -tubulin protein was used as a loading control. Then, following immunoblot analysis, the intensities of Oct4 and Sox2 immunoreactive bands were determined by digital image analysis. Data (ratio to α -tubulin) represent means \pm SE. Asterisks indicate a statistically significant difference. $P < 0.01$ vs 1 and 2 groups. Lane 1 = Oct4 or Sox2 overexpressed BMSCs; lane 2 = control at passage 3; lane 3 = BMSCs at passage 3.

Growth properties of *Oct4* or *Sox2* overexpression on BMSCs

The proliferative ability of the *Oct4*- or *Sox2*-overexpressed BMSCs at passage 12 was compared with that of the control, which showed that *Sox2*-overexpressed cells had a higher proliferation potential in the presence of bFGF. However, the *Oct4*-overexpressed cells showed higher proliferation potential in the absence of bFGF (Figure 5A). During passage, the *Oct4*- and *Sox2*-overexpressed cells were relatively small and grew very well, whereas the control cells became elongated in shape (Figure 5B). Moreover, *Sox2*-overexpressed cells show higher proliferation potential in the presence of bFGF (Go et al., 2008). These observations suggested that *Oct4* or *Sox2* overexpression not only promoted the proliferation of BMSCs, but also maintained the morphology of the cells.

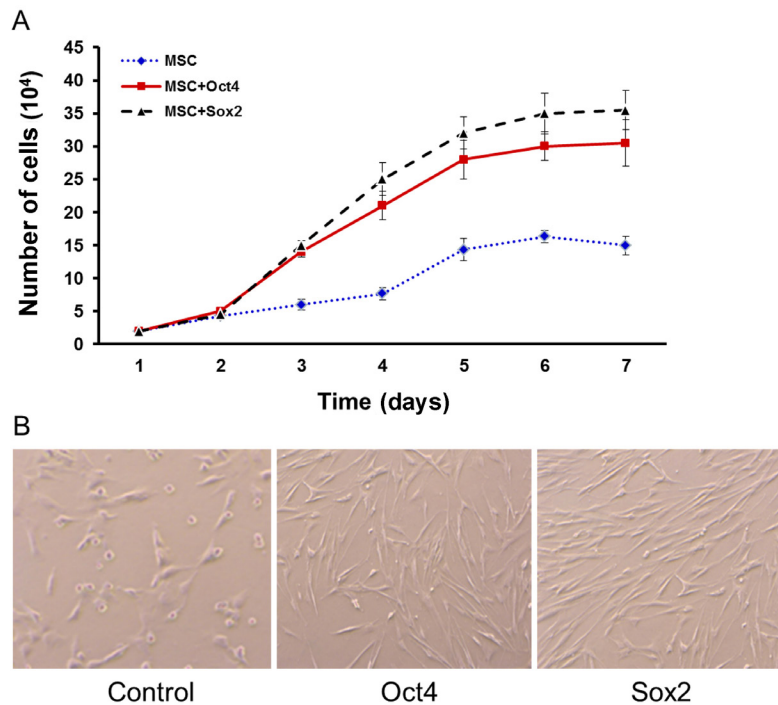


Figure 5. Characterization of *Oct4* and *Sox2* overexpressing bone mesenchymal stem cells (BMSCs) and effects of their overexpression on proliferation of BMSCs. **A.** Proliferation curves of *Oct4*- or *Sox2*-overexpressed BMSCs compared with control. The time points represent the mean values from duplicate measurements and their standard deviation. *Oct4* or *Sox2* overexpression promoted cell growth of BMSCs. **B.** *Oct4* or *Sox2* overexpression maintained BMSCs morphology. At passage12, control became flat and gradually lost mesenchymal stem cell (MSC) morphology whereas *Oct4* and *Sox2* overexpression retained MSC morphology.

Adipogenesis of *Oct4* or *Sox2*-overexpressed BMSCs

BMSCs can differentiate into adipocytes and osteoblasts. Therefore, the differentiation potential for adipoblasts of *Oct4* or *Sox2*-expressing cells was examined. Oil Red O staining showed that *Oct4* overexpression increased the number of lipid droplets at 21 days

compared to the control (Figure 6A). Consistent with the staining result, *Oct4* overexpression increased the adipogenic marker *PPAR γ* by 1.94-fold and *CEBPA* by 1.82-fold compared with the control (Figure 6B). Similar to *Oct4* overexpression, Oil Red O staining in adipogenesis showed that the adipogenesis of the *Sox2*-overexpressed BMSCs resulted in stronger lipid deposits than the control (Figure 6A). In the presence of bFGF, *Sox2*-overexpressed BMSCs raised the expression level of *PPAR γ* by 2.25-fold and *CEBPA* by 2.63-fold (Figure 6B). These observations indicated that *Oct4* or *Sox2* overexpression improved adipogenesis of BMSCs.

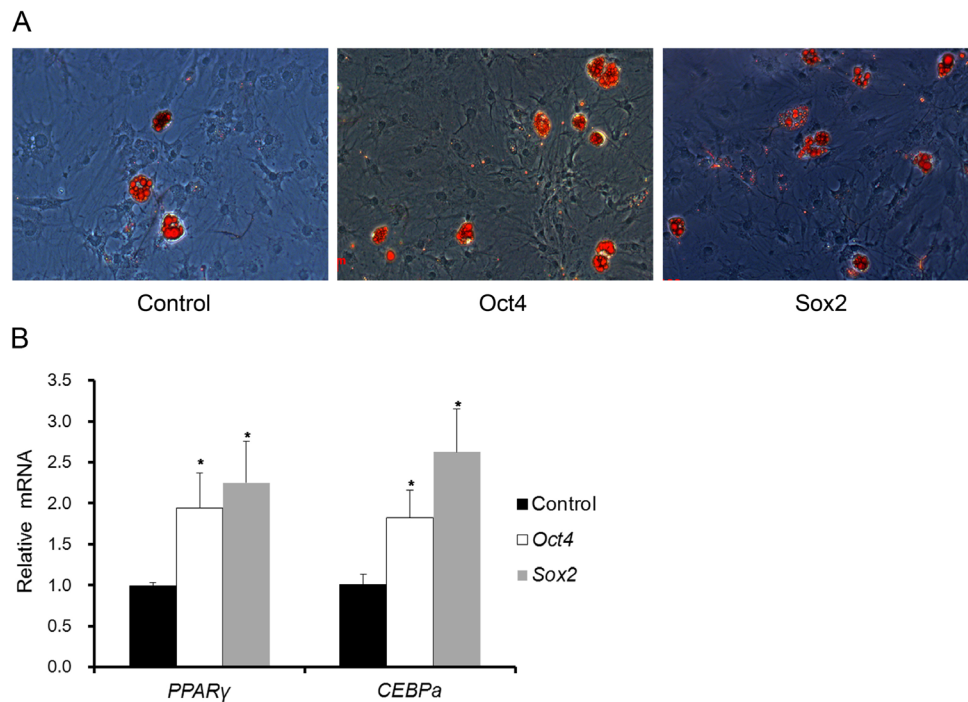


Figure 6. Adipogenic activities of *Oct4*- and *Sox2*-overexpressed BMSCs compared to control. **A.** Cells cultured in the adipoinductive medium for 3 weeks were stained by alkaline phosphatase activity. *Oct4* and *Sox2* overexpression improved adipogenesis of BMSCs. **B.** Cells cultured in adipoinductive medium for 3 weeks were collected and analyzed for the expression of *PPAR γ* and *CEBPA* transcripts using RT-PCR. Asterisks indicate significant differences compared to control ($P < 0.05$).

Osteogenesis of *Oct4* or *Sox2*-overexpressed BMSCs

The differentiation potentials for osteoblasts of *Oct4* or *Sox2*-overexpressed cells were examined. The *Oct4*-overexpressed cells and the *Sox2*-overexpressed cells both showed higher osteogenic potentials compared to the control, as revealed by Alizarin red staining and by RT-PCR. Alizarin red staining showed that *Oct4* overexpression raised the quantity of calcium deposition at 14 days compared to the control (Figure 7A). Consistent with the staining results, *Oct4* overexpression increased the expression level of *type Ia1* by 2.84-fold, *osteonectin* by 1.56-fold, and *osteocalcin* by 3.82-fold (Figure 7B). Therefore, both analyses showed

that deposition of the mineralized matrix was enhanced by *Oct4* overexpression (Figure 7A). These phenomena were also observed as a result of *Sox2* overexpression in which *type Ia1*, *osteonectin*, and *osteocalcin* expressions were upregulated by 3.83-, 1.97-, and 5.93-fold, respectively (Figure 7B). The *Sox2*-overexpressed cells showed higher osteogenesis potential in the presence of bFGF (Go et al., 2008). These observations indicated that *Oct4* or *Sox2* overexpression also improved the osteogenesis of BMSCs.

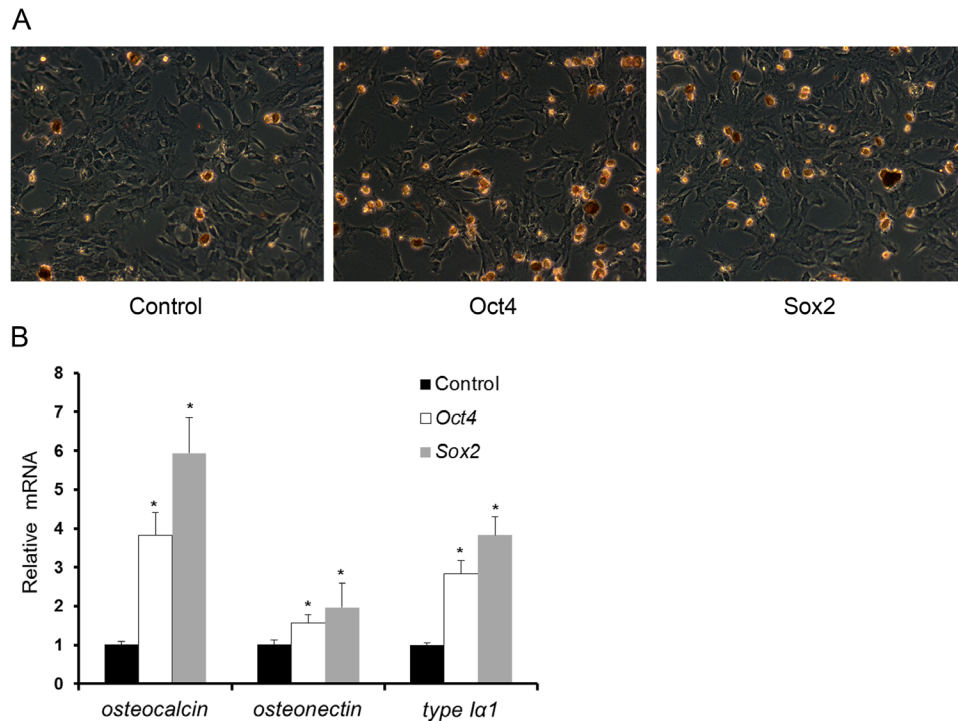


Figure 7. Osteogenic activities of *Oct4*- and *Sox2*-expressed BMSCs compared to control. **A.** Cells cultured in the osteoinductive medium for 2 weeks were stained by Alizarin red. *Oct4* and *Sox2* overexpression improved osteogenesis of BMSCs. **B.** Cells cultured in the osteoinductive medium for 2 weeks were collected and analyzed for the expression of *osteocalcin*, *osteonectin*, and *type Ia1* transcripts using RT-PCR. Asterisks indicate significant differences compared to control ($P < 0.05$).

DISCUSSION

Mononuclear cells separated from porcine bone marrow were able to produce an adherent layer of cells when cultured in L-DMEM containing 15% FBS in our culture system. These adherent cells were observed to be spindle and fibroblast-like under a phase-contrast light microscope. They were positive for the BMSC markers CD90, CD29, and CD44, and negative for CD45 and CD34, all of which were previously reported as MSC-related antigens (Pittenger et al., 1999).

MSCs derived from bone marrow have significant therapeutic potential with extensive applications for regenerative therapy. Based on previous observations, we speculated that

BMSCs might represent a useful cell in clinical practices (Hu et al., 2010; Griffin et al., 2011; Gurudutta et al., 2012). Indeed, the advantage of employing large animals, such as pigs, rather than small animal models, is based on several factors, including disease complexity, effective cell dosage, cell survival after transplantation, as well as tissue related inflammatory and immunological responses to engraftment after transplantation (Poncelet et al., 2007; Kuo et al., 2009). Xiaomeishan porcine BMSCs were chosen for this study based on their similarity to human cells. Therefore, studies using the Xiaomeishan porcine model may offer significant contributions toward the development of cellular therapy strategies for a clinical setting. *In vivo* tracking studies to examine the survival, distribution, and homing of BMSCs after infusion in large animal models should further allow optimization of the clinical use of BMSCs to promote tolerance induction in solid organ transplantation patients. In addition, this is the first study to examine the effect of pluripotent genes on proliferation and differentiation of BMSCs from Xiaomeishan porcine.

The expansion and differentiation potential of these BMSCs *in vitro* is essential for use in various stem cell therapies. However, the cells lose their proliferation and differentiation abilities after several passages. Moreover, little is known about how self-renewal and differentiation are regulated in BMSCs at present.

To this end, the present study focused on the reported expression of the embryonic transcription factors *Oct4* and *Sox2* in BMSCs. *Oct4* and *Sox2* are transcription factors known to be essential for normal pluripotent cell development and maintenance (Dailey and Basilico, 2001; Rodda et al., 2005). *Oct4* is a critical transcription factor for regulating self-renewal and differentiation of ESCs. In the absence of *Oct4*, ESCs revert to the trophoblast lineage *in vivo* and *in vitro*. Increasing the expression of *Oct4* above endogenous levels in ESCs leads to differentiation toward the extraembryonic mesoderm lineage (Niwa et al., 2000). Based on previous observations, we observed cell growth and found that overexpression of *Oct4* promoted the proliferative rate and differentiation potential. In addition, *Oct4*-overexpressed BMSCs were relatively small with a spindle-shaped morphology and grew very well. Therefore, *Oct4* overexpression played a role in maintaining the stemness of Xiaomeishan porcine BMSCs.

Sox2 is transcription factor involved in the regulation of stem cell development and in the determination of cell fate. Recently, a novel function for *Sox2* was identified in a mouse osteoblastic lineage: the maintenance of the self-renewal of cells. In addition, *Sox2*-knock-out cells could not form colonies, and their population growth was arrested with a senescent phenotype (Basu-Roy et al., 2010). In another study, most *Sox2*-overexpressed BMSCs were small and showed high proliferative and osteogenic capabilities from human BMSCs (Go et al., 2008). Knockdowns of *Sox2* and *Msx2* using RNA interference, showed a significant decrease in colony-forming ability. Specifically, a knockdown of *Sox2* significantly inhibited multipotentiality and cell proliferation (Yoon et al., 2011). Overall, our findings were in good accordance with those of previous studies. *Sox2*-overexpressed cells all showed significantly higher viability and differentiation potential compared to control cells, and these cells were smaller with a spindle-shaped morphology. These findings suggested that *Sox2* overexpression maintained the stemness of Xiaomeishan porcine BMSCs and might function in a similar fashion in progenitor BMSCs as in ESCs.

The overexpression of *Oct4* and *Sox2* was detected in BMSCs, but at lower levels than has been observed in ESCs (Wei and Shen, 2011). Moreover, although the expression of *Oct4* was observed in early-passage MSCs, its expression was too low to detect at late-passage

(Greco et al., 2007). Therefore, it is possible that low-level *Oct4* and *Sox2* underwent growth arrest or a program of differentiation. *Oct4* expression could be an indicator of MSC differentiation potential, which could be used as an experimental tool or in clinical diagnostics.

Pluripotent stem cells can be directly generated by reprogramming somatic cells using defined transcription factors, including *Oct4* and *Sox2* (Takahashi and Yamanaka, 2006; Qin et al., 2007). Therefore, *Oct4* and *Sox2* are critical for cell reprogramming (Takahashi and Yamanaka, 2006; Qin et al., 2007) and for altering the cell status. Our observations on the overexpression of *Oct4* or *Sox2* in Xiaomeishan porcine BMSCs were consistent with those of previous studies. We succeeded in maintaining the proliferation and differentiation potentials of cells in senescent passages by introducing *Oct4* or *Sox2*, which could be an effective and feasible way of maintaining high-quality Xiaomeishan porcine BMSCs as stem cells.

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