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Role of Histone Deacetylase Inhibitors in the Aging of Human Umbilical Cord Mesenchymal Stem Cells

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ABSTRACT

Mesenchymal stem cells (MSCs) are self-renewing cells that exhibit differentiation capacity and immune regulation ability. These versatile cells have a wide range of potential applications. However, the spontaneous differentiation and aging of MSCs during long-term culturing restrict the amount of cells available for therapies and tissue engineering. Thus, maintaining the biological characteristics of MSCs during long-term culturing is crucial. Chromatic modification via epigenetic regulatory mechanisms (e.g., histone acetylation, deacetylation, and methylation) is crucial in stem cell pluripotency. We investigated the effects of largazole or trichostatin A (TSA), a novel histone deacetylase inhibitor (HDACi), against human umbilical cord (hUC)-MSCs aging. Results show that low concentrations of largazole or TSA can significantly improve hUC-MSCs proliferation and delay hUC-MSCs aging. Largazole can better improve MSCs proliferation than TSA. HDAC is modulate histone H3 acetylation and methylation in the telomerase reverse-transcriptase, octamer-binding transcription factor 4, Nanog, C-X-C chemokine receptor 4, alkaline phosphatase, and osteopontin genes. HDACis can promote hUC-MSCs proliferation and suppress hUC-MSCs spontaneous osteogenic differentiation. HDACis can affect histone H3 lysine 9 or 14 acetylation and histone H3 lysine 4 dimethylation, thus increasing the mRNA expression of pluripotent and proliferative genes and suppressing the spontaneous differentiation of hUC-MSCs. J. Cell. Biochem. 114: 2231–2239, 2013.

KEY WORDS: HISTONE DEACETYLASE INHIBITOR; HUMAN UMBILICAL CORD-MESENCHYMAL STEM CELL; PROLIFERATION; EPIGENETIC MODIFICATION

Mesenchymal stem cells (MSCs) are self-renewing and expandable cells [Li et al., 2011b]. Allogeneic MSCs have low immunogenicity and immunosuppressive properties [Zhao et al., 2010]. MSCs are currently being tested in many clinical trials for regenerative medicine applications. These cells have to be expanded in vitro before transplantation. Several studies demonstrated that long-term culturing results in continuous changes in MSCs, including decreased proliferation rate, increased cell size, and different differentiation potentials [Krampera et al., 2005; Wagner et al., 2010]. Thus, maintaining the biological characteristics of MSCs during long-term culturing is crucial. Octamer-binding transcription factor 4 (OCT4), Nanog, and (sex-determining region Y)-box 2 (Sox2) genes are expressed during the early passages of human umbilical cord (hUC)-MSCs [Greco et al., 2007]. The expression levels of these genes, along with those of the telomerase reverse-transcriptase (TERT) gene and the migrate gene C-X-C chemokine receptor 4 (CXCR4), gradually decrease during long-term culturing of MSCs in vitro [Stewart et al., 2008]. However, the molecular mechanisms involved remain poorly understood. OCT4, Sox2, and Nanog are known key regulators of embryonic stem cell self-renewal [Hattori et al., 2004;

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Freberg et al., 2007]. The chromatin status of pluripotent genes is also crucial for regulating the self-renewability and pluripotency of stem cells [Wagner et al., 2008; Jung et al., 2010]. In some specific environments, MSCs can resume some of their performances. The basic fibroblast growth factor modulates histone H3 acetylation in the Oct4 gene, maintains the expression levels of related genes, and suppresses their spontaneous osteogenic differentiation [Li et al., 2011b]. Trichostatin A (TSA) can promote the expression of pluripotency-associated genes in neurosphere cells and can endow these cells with the ability of hematopoietic cells [Ruau et al., 2008; Sethe and Scutt, 2006]. Thus, histone acetylation level is related to MSC differentiation to a certain extent. In this study, We investigated the effects of largazole or trichostatin A (TSA), a novel histone deacetylase inhibitor (HDACi), against human umbilical cord (hUC)-MSCs aging. The results showed that low HDACi concentrations can modulate histone H3 acetylation and methylation in the TERT, OCT4, Nanog, CXCR4, alkaline phosphatase (ALP), and osteopontin (OPN) genes. Low HDACi concentrations promoted MSCs proliferation, suppressed spontaneous MSCs osteogenic differentiation, and maintained the self-renewability of MSCs.

MATERIAL AND METHODS

hUC-MSC ISOLATION, CULTURING, AND IDENTIFICATION

Umbilical cords were obtained from healthy donors at the Second Affiliated Hospital of Jinan University. All donors provided written informed consents. The procedure was approved by the Hospital Institutional Review Board. hUC-MSCs were harvested and cultured as previously described [Seshareddy et al., 2008]. Passage 3 cells were collected and then incubated with phycoerythrin-conjugated monoclonal mouse anti-human CD19, CD34, CD45, CD90, or CD105 antibodies or monoclonal fluorescein isothiocyanate-conjugated mouse anti-human CD29, CD106, or HLA-DR antibodies (BD Pharmingen, San Diego). Quantifications were performed on a flow cytometer (ALTRA, Beckman Coulter, USA). Data were analyzed using Cell Quest software. After 2 weeks of culturing with an adipogenic induction medium containing 10^{-5} mmol/L dexamethasone and 10 μg/ml insulin, the passage 3 cells were stained with Oil-Red-O to detect the presence of lipids. The osteogenic medium contained 10^{-5} mmol/L dexamethasone, 50 mg/ml ascorbic acid, and 10 mM β-glycerophosphate (Sigma, USA). The medium was replaced twice a week for 2 weeks. The cells were stained with Alizarin Red for calcium deposition.

EFFECT OF HDACi ON THE PROLIFERATION AND APOPTOSIS OF hUC-MSCs

Largazole (synthesized by our research group) and TSA (Sigma) were prepared in dimethylsulfoxide (DMSO); all DMSO concentrations were below 0.01% for all assays. Passage 4 cells were plated on 96-well plates (100 μl/cell) at a density of 2.0×10^4/ml in Dulbecco's modified Eagle's medium (DMEM)-F12 containing 10% fetal bovine serum (FBS) and different concentrations of largazole (100 nm/ml, 10 nm/ml, 1,000 pm/ml, 100 pm/ml, or 10 pm/ml) or TSA (100, 50, 20, 10, or 5 nm/ml). The cells were then cultured for 4 days at 37°C under 5% CO_2. The effects of largazole and TSA on MSCs proliferation were determined by a Cell Counting Kit-8 (CCK-8; Dojindo, Japan) assay according to the manufacturer's instructions. Then 1×10^5 MSCs suspension was stained with 5 μl of Annexin V-FITC and 1 μl of PI. The apoptosis was analyzed by flow cytometry (ALTRA, Beckman coulter).

The passage 4 cells were placed in 96-well plates at a density of 5.0×10^3/ml. The control group comprised 10% FBS and DMEM-F12 (Hyclone, USA). The TSA group comprised 10% FBS, DMEM-F12, and 10 nM/ml TSA. The largazole group comprised 10% FBS, DMEM-F12, and 10 pM/ml largazole. The cells were cultured for 7 days at 37°C under 5% CO_2. All groups were transferred in triplicate wells daily for CCK-8 detection. The average values were used for the growth curve.

Cumulative population doubling levels (CPDLs) were also measured at passages 1–18. The hUC-MSCs were placed on a 10 cm plate at a density of 5×10^3/ml and then incubated for 4 days. Cell counting was performed at least three times; the average values were used for the CPDL plot.

RNA EXTRACTION AND REAL-TIME POLYMERASE CHAIN REACTION (PCR)

Passage 3 cells were cultured in a 10 cm Petri dish. After the cells adhered to the dish, the culture medium that contained the optimal largazole or TSA concentration was added to the dish. The cells were divided into the control [10% FBS and DMEM-F12 (Hyclone)], TSA (10% FBS, DMEM-F12, and 10 nM/ml TSA), and largazole (10% FBS, DMEM-F12, and 10 pM/ml largazole) groups. The cells were cultured at 37°C under 5% CO_2 until passage 16.

Passage 4, 8, 12, and 16 cells were subjected to real-time PCR. Total RNA was extracted using TRIzol (Invitrogen, USA). cDNA was synthesized using reverse transcription with the PrimeScript Buffer (TaKaRa, Japan) and Oligo dT Prime. Real-time PCR was performed using SYBR Premix ExTaqTM II (TaKaRa) on an ABI 7500 QPCR system. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was quantified parallel to the target genes as an internal control. The $^{\Delta\Delta}C_t$ method [Wu et al., 2007] was used to calculate the normalization and fold changes. Changes in the mRNA expression levels of the pluripotent genes (TERT, OCT4, and Nanog), the migratory gene (CXCR4), and the differentiated genes (ALP and OPN) were monitored. The primer sets are as follows: CXCR4 forward 5′-AGACCACAGTCATCCTCATCCT-3′, CXCR4 reverse 5′-TGTTCTCAAACTCACACCCTTG-3′; TERT forward 5′-CTCCCATTTCATCAGCAAGTTT-3′, TERT reverse 5′-CTTGGCTTTCAGGATGGAGTAG-3′; Oct4 forward 5′-GGGGTTCTATTTGGGAAGGTAT-3′, Oct4 reverse 5′-TACTGGTTCGCTTTCTCTTTCG-3′; Nanog forward 5′-GAACTCTCCAACATCCTGAACC-3′, Nanog reverse 5′-AGTAAAGGCTGGGGTAGGTAGG-3′; ALP forward 5′-CAGGGGAGAAACTCACTTATGG-3′, ALP reverse 5′-CGCTCACTACCTGTCTTTTGTC-3′; OPN forward 5′-ATGCTACAGACGAGGACATCAC-3′, OPN reverse 5′-ATTGCTCTCATCATTGGCTTTC-3′; GAPDH forward 5′-AGAAGGCTGGGGCTCATTTG-3′, GAPDH reverse 5′-AGGGGCCATCCACAGTCTTC-3′.

CHROMATIN IMMUNOPRECIPITATION (ChIP) ASSAYS

The experiment was divided into three groups as previously described. Passage 4, 8, 12, and 16 cells were analyzed by CHIP assay. CHIP was performed using an EZ-ChIP™ Chromatin Immunoprecipitation Kit (Millipore, USA) according to the manufacturer's instructions [Qi et al., 2011]. The cells were incubated in 1% formaldehyde for 10 min

at 37°C. After the media were removed, the cells were washed twice with cold phosphate-buffered saline buffer containing protease inhibitors. The cells were resuspended in a sodium dodecyl sulfate lysis buffer. The lysate was sonicated (JYD-L150, China) to shear DNA to an average length of 200–1,000 bp. After dilution, each sample group was immunoprecipitated with histone H3 acetylate or methylate antibodies and then incubated overnight. Salmon sperm DNA/protein A agarose was used to collect the immunoprecipitated complexes, which were then eluted with elution buffer after extensive washing. Crosslinking was reversed by the addition of NaCl at 65°C. DNA was purified using spin columns and then measured by quantitative fluorescent PCR analysis.

The primer sets are as follows: CXCR4 forward 5′-GGCTTATTTGCTGGTTTCTCC-3′, CXCR4 reverse 5′-CACTCATTCATTCTCACAACACC-3′; TERT forward 5′-CTCCATTTCCCACCCTTTCT-3′, TERT reverse 5′-GCCCGTCATTTCTCTTTGC-3′; Oct4 forward 5′-GACGGGGTTTCATAGTGTTAGC-3′, Oct4 reverse 5′-GGTGATTTGGCTCATCCATACT-3′; Nanog forward 5′-GCCCTATCCAAATCCTATCACTT-3′, Nanog reverse 5′-GGTCAGCACAAAATACAGGTCA-3′; ALP forward 5′-TGTTGACAGACACAGAGACAGACG-3′, ALP reverse 5′-GTCGGCATCTTCCTTCTGCG-3′; OPN forward 5′-AGGCAAGAGTGGTTGCAGATA-3′, OPN reverse 5′-CCCATGAAAAAGGGAGAAAGT-3′. The percent input method (Invitrogen) was used to analyze the data.

STATISTICAL ANALYSIS

All experiments were conducted at least in triplicate ($n = 3$). The results are expressed as mean ± SD. Statistical analysis was conducted by ANOVA, followed by Duncan's multiple range tests or student's t test. A value of $P < 0.05$ was considered significant.

RESULTS

hUC-MSCs ISOLATION AND CULTURE

The confluence time of primary culture cells was 5–7 days in cells harvested with enzyme digestion. They were initially polygonal or spindle-shaped adherent cells (Fig. 1A). After purification, the majority of cells retained their spindle shapes (Fig. 1B). Fluorescence-activated cell sorting showed that the hUC-MSCs were strongly positive for the lineage cell markers CD29 (99.86%), CD90 (99.94%), and CD105 (99.94%) but were negative for CD19 (2.69%), CD45 (1.23%), CD34 (1.45%), CD106 (3.50%), and HLA-DR (1.02%; Fig. 1C). These results showed that the hUC-MSCs exhibited typical hUC-MSC immunophenotypic features [Seshareddy et al., 2008]. After 2 weeks, the cells submitted to differentiation induction in the appropriate media was able to demonstrate adipogenic and osteogenic differentiation assessed by the appropriate staining protocol. adipogenic differentiation was stained with Oil-Red-O to detect the presence of lipids (Fig. 1D). osteogenic differentiation could be identified by calcium deposits (Fig. 1E).

EFFECTS OF DIFFERENT HDACI CONCENTRATIONS ON THE PROLIFERATIVE AND APOPTOSIS OF hUC-MSCs

The hUC-MSCs were cultured with different concentrations of largazole or TSA in 96-well culture plates for 4 days, and proliferation was determined by Cell Counting Kit-8 assay. Treatment with 10 pm/ml largazole or 10 nm/ml TSA exhibited a distinct proliferative ability on cells (Fig. 2A,B). The hUC-MSCs were cultured with 10 pm/ml largazole or 10 nm/ml TSA, The apoptosis of hUC-MSCs were analyze by Flow cytometry. The apoptosis percent of TSA group, largazole group and control group was very little (0.55 ± 0.05%, 0.42 ± 0.03%, and 0.48 ± 0.04%). These were not significantly differences ($P > 0.05$). The growth curve (Fig. 2C) consisted of a lag phase (1–2 days), a logarithmic growth phase (2–5 days), and a stagnant phase. The proliferative abilities of the TSA (10 nm/ml) and largazole (10 pm/ml) groups during the logarithmic phase were higher than that of the control group ($P < 0.01$). CPDL was then measured. The CPDL was 15 at passage 12 for the control group, 19 at passage 14 for the TSA group, and 22 at passage 16 for the largazole group. These results indicated that low HDACi concentrations can improve the proliferative ability of hUC-MSCs. The proliferative ability of the largazole group was stronger than that of the TSA group ($P < 0.05$; Fig. 2D).

EFFECTS OF HDACi ON THE PLURIPOTENT, MIGRATORY, AND DIFFERENTIATED GENES OF hUC-MSCs

The hUC-MSCs were cultured in the growth medium and exhibited morphological changes with successive passages. In general, the cells increased in size. The hUC-MSCs at passage 8 started to age. The mRNA expressions of the pluripotent genes (OCT4 and Nanog), TERT, and CXCR4 started to decrease. Cells at passages 12 and 16 showed distinct reductions. The mRNA expressions of ALP and OPN progressively increased with successive cell passages, indicating enhanced spontaneous hUC-MSCs differentiation into osteoblasts (Fig. 3A). In the TSA and largazole groups, the expression levels of OCT4, Nanog, TERT, and CXCR4 started to increase at passage 4 and then slightly decreased at passages 8–12. However, the levels remained higher than that in the control group. The gene expression level in the largazole group was slightly higher than that in the TSA group at successive passages. The expression levels of ALP and OPN in the TSA and largazole groups decreased compared with those in the control group at successive cell passages (Fig. 3B–G). The two HDACis increased the expression levels of pluripotent and migratory genes but reduced the expression levels of osteogenic differentiation genes.

EFFECTS OF HDACi ON HISTONE H3 ACETYLATION AND METHYLATION OF hUC-MSCs

To determine the molecular mechanism underlying the HDACi-induced maintenance of the proliferation and self-renewability of hUC-MSCs, the changes in gene expression and epigenesis were monitored by CHIP assays. The control group showed a gradual decrease in histone H3 lysine 9 or 14 (H3K9/K14) acetylation and histone H3 lysine 4 (H3K4) dimethylation of the gene (OCT4, Nanog, TERT, and CXCR4) promoters, as well as a gradual increase in H3K9 trimethylation at passages 4–16. By contrast, H3K9/K14 acetylation and H3K4 dimethylation in the ALP and OPN promoters increased, whereas H3K9 trimethylation decreased (Fig. 4 and Supplementary Fig. 1).

The expression levels of H3K9/K14 acetylation and H3K4 dimethylation in the OCT4, Nanog, TERT, and CXCR4 gene promoters were higher in the TSA and largazole groups than in the control group ($P < 0.01$). These levels increased with the cell subculture until passage 16. The H3K9/K14 acetylation and H3K4 dimethylation expression levels in the largazole group were higher than those in the

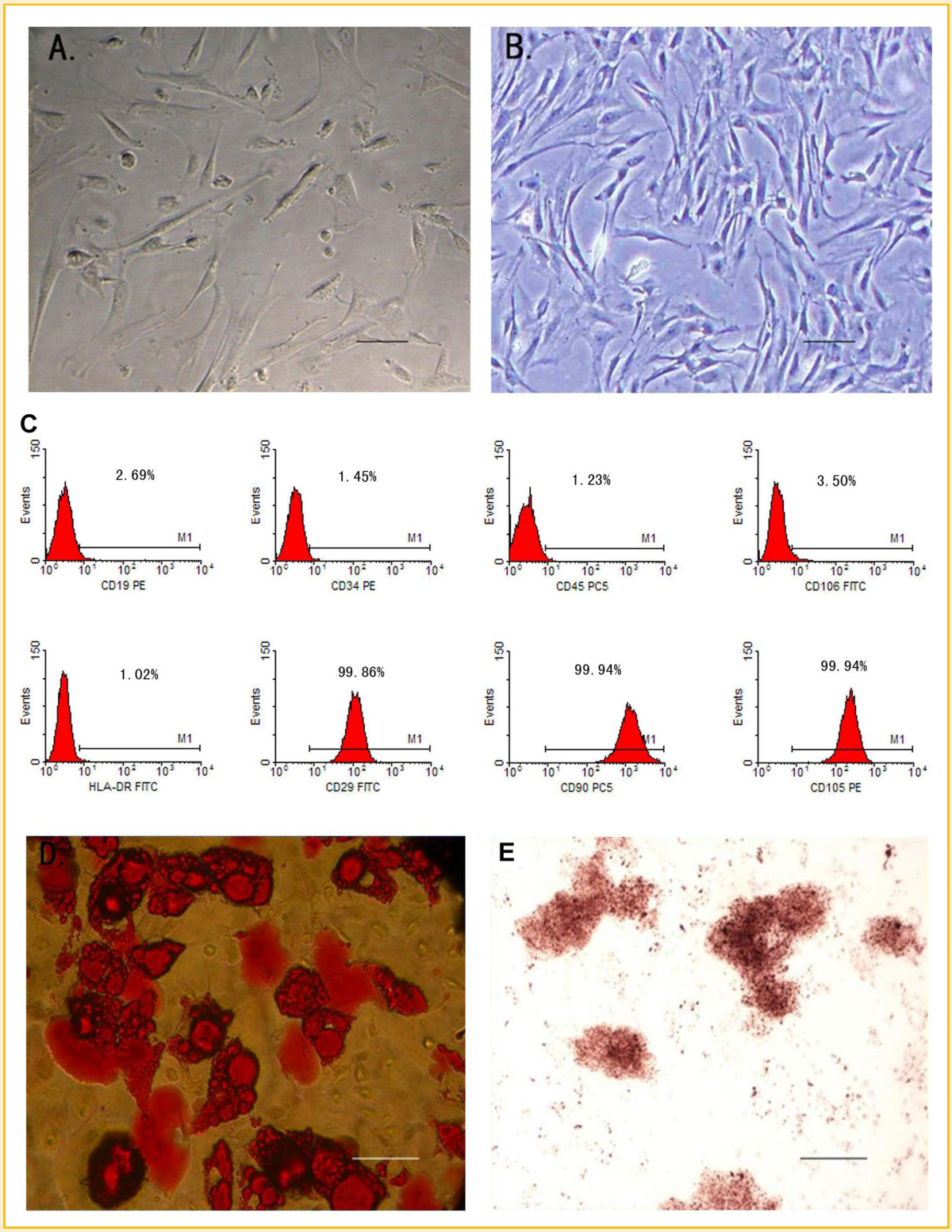

Fig. 1. Mesenchymal stem cells (MSCs) isolated from the human umbilical cord in a growth medium and then cultured in the appropriate induction medium: (A) primary cells; (B) phase-contrast micrograph of human umbilical cells at passage 4; (C) Fluorescence-activated cell sorting (FACS) analysis of MSCs. Flow cytometry analysis demonstrated a homogenous MSC polulation. As expected, MSCs were negative for reactivity to antigens CD14, CD34, CD45, CD106, and HLA-DR, but positive for reactivity to antigens CD29, CD90, and CD105; (D) hUC-MSCs differentiated into adipocytes that accumulated lipid vacuoles, which were demonstrated by the morphology and oil red stains; (E) osteoblasts produced mineralized matrices, which were stained by alizarin red (Scale bar = 50 μm).

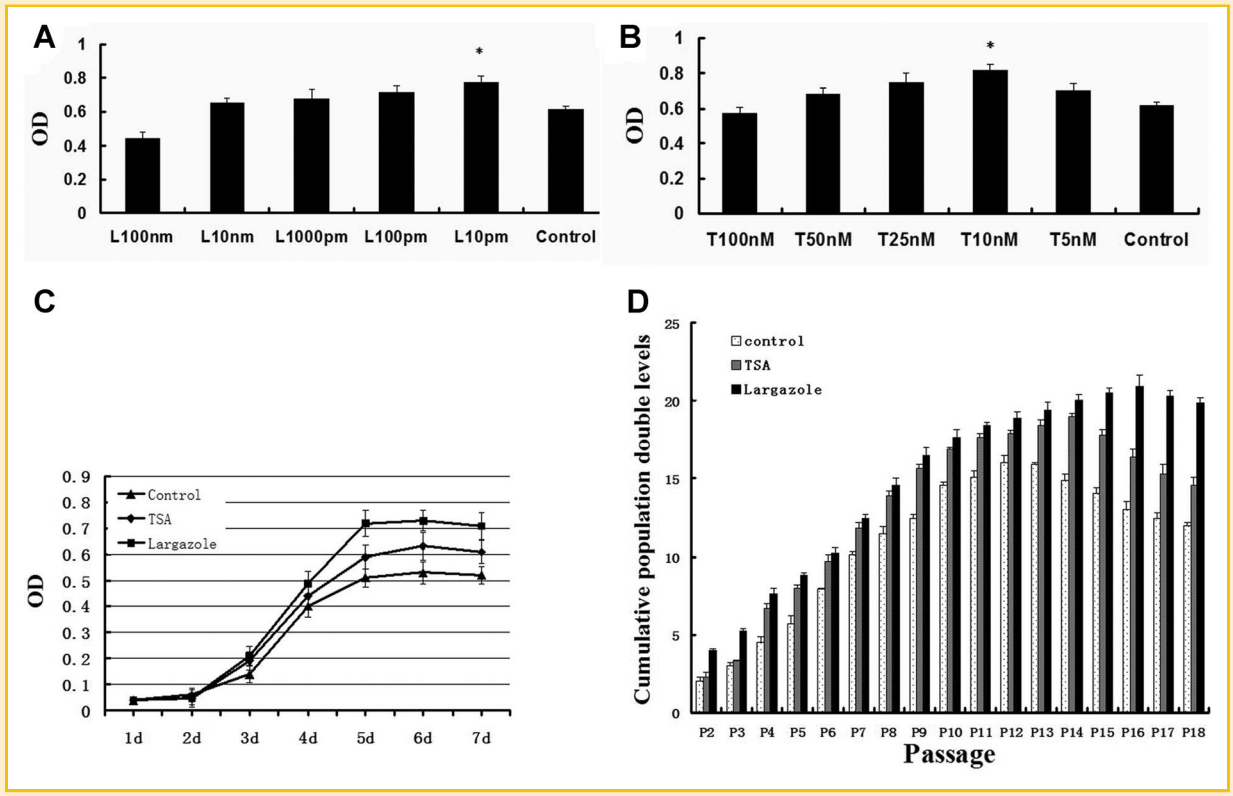

Fig. 2. Proliferative potential of largazole- and TSA-treated hUC-MSCs: (A,B) proliferation of treated hUC-MSCs, as determined by the CCK8 method; (C) growth curves of hUC-MSCs cultured in the growth medium supplemented without (control) and with largazole (10 pm) or TSA (10 nm); and (D) CPDL of hUC-MSCs grown in the control, largazole (10 pm), and TSA (10 nm) media at different passages. Each bar represents the mean ± SD of three replicated experiments. $^{*}P < 0.01$ versus control.

TSA group. However, H3K9 trimethylation in the three groups displayed the same level. The expression levels of H3K9/K14 acetylation and H3K4 dimethylation in the ALP and OPN promoters were lower in the TSA and largazole groups than in the control group; these levels increased as cell subculturing progressed. H3K9 trimethylation in the three groups displayed the same level. These results indicated that TSA and largazole can affect the expression levels of H3K9/K14 acetylation and H3K4 dimethylation in the OCT4, Nanog, CXCR4, and TERT promoters. HDAC is can also promote the mRNA expression of these genes while suppressing the spontaneous osteogenic differentiation of MSCs.

DISCUSSION

Numerous studies reported that MSCs undergo replicative senescence at around passage 10 in vitro. MSCs are currently being tested in many clinical trials for application in regenerative medicine. These cells have to be expanded in vitro prior to MSC-based therapies and tissue engineering because of their scarcity in the bone marrow and in other tissues [Baxter et al., 2004]. Long-term culturing or high concentrations of HDACi results in continuous changes in MSCs, including reduced proliferation rates, as well as changes in migration ability and differentiation potential [Schellenberg et al., 2011; Wagner et al., 2010]. After transplantation, MSCs can migrate into the infarcted myocardium to enhance myocardial repair and improve cardiac function [Mangi et al., 2003; Lee et al., 2009]. However, the therapeutic effect of MSCs is positively correlated to a certain extent with the number of MSCs in the damaged tissue. MSCs spontaneously differentiate into osteoblasts in vitro. Instead of adopting a stable chondrogenic phenotype, MSCs pellets that are transplanted to ectopic sites in severe combined immunodeficiency mice undergo changes that are associated with endochondral ossification [Ip et al., 2007]. This phenomenon can seriously affect cardiac contractility. Therefore, differentiation prevention is crucial.

The development of methods that can maintain the biological characteristics of stem cells during in vitro expansion has become a hot topic in stem cell research [Pelttari et al., 2006]. Studies show that the epigenetic modification of proteins have a key function in gene transcription regulation [Nishida et al., 2006]. Treatment of bone marrow progenitor cells with 5-Aza-dC and TSA induces the expression of the pluripotent genes OCT4, Nanog, and Sox2. This treatment also converts bone marrow progenitor cells into multipotent cells, which can then differentiate into myocyte progenitors. The transplantation of these modified progenitor cells into infracted mouse hearts improves the left ventricular function [Lomvardas and Thanos, 2001]. Therefore, we used largazole (an HDACi) synthesized by our research group to study the biological characteristics of hUC-MSCs by the epigenetic modification of proteins. Largazole isolated from the marine cyanobacterium *Symploca* sp. exhibits potent inhibitory activity against many cancer cell lines. However, largazole

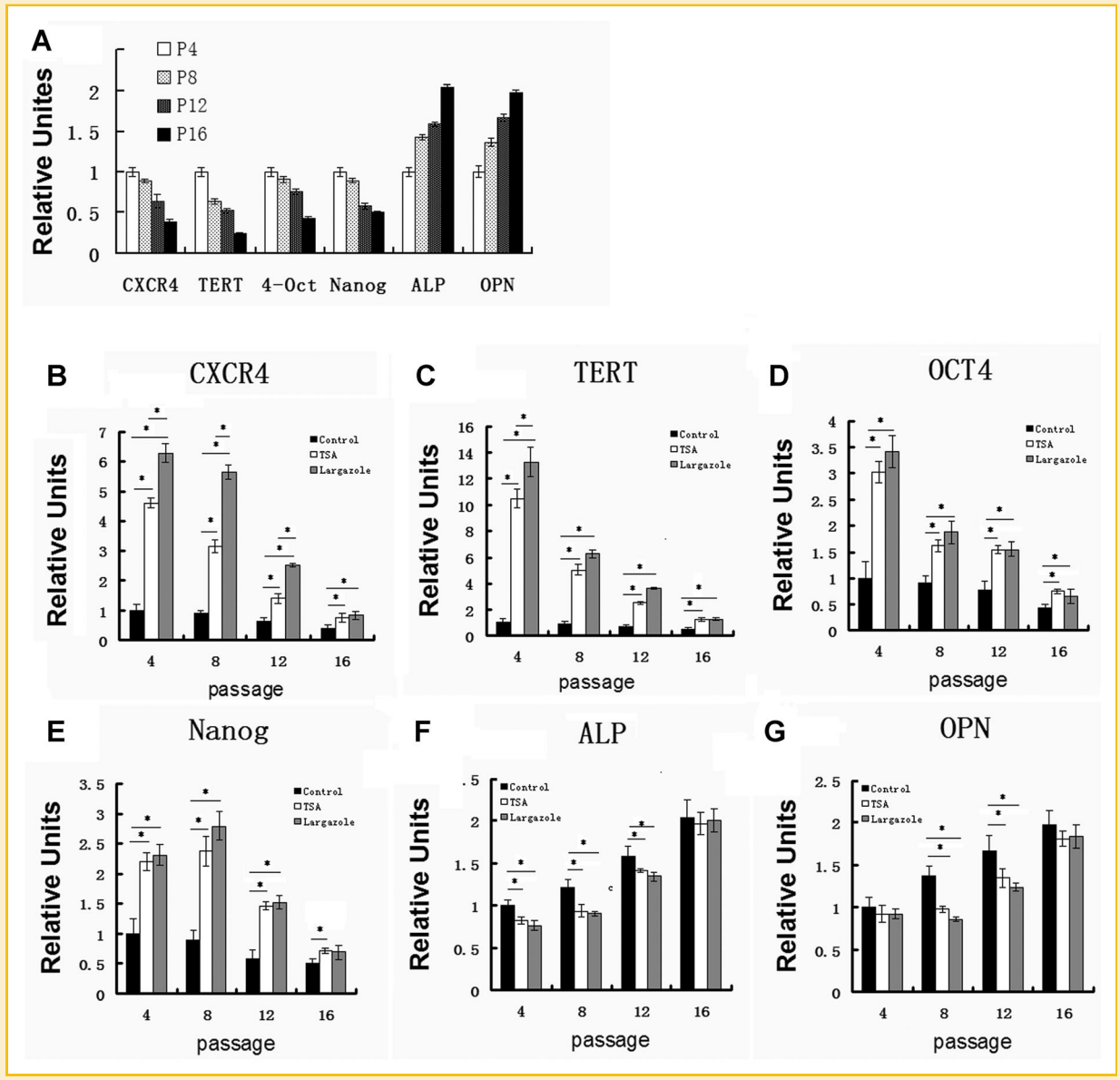

Fig. 3. Gene expression in early- and late-passage hUC-MSCs: (A) real-time PCR analysis of CXCR4, OCT4, Nanog, ALP, and OPN expression levels at culture passage (P) 4, P8/12, and P16 in the growth medium; (B) CXCR4 gene expression during P4, P8/12, and P16 in different growth media; (C–E) multipotent gene (TERT, OCT4, and Nanog) expression levels during the early and late passages in different growth media; and (F,G) osteogenic gene (ALP and OPN) expression levels during the early and late passages in different growth media ($^{*}P < 0.01$).

does not show antiproliferative activities against normal cells [Nasveschuk et al., 2008; Rajasingh et al., 2011]. TSA is obtained from *Streptomyces* metabolites [Ying et al., 2008; Li et al., 2011a]. In this study, hUC-MSCs were cultured with different largazole or TSA concentrations. The results showed that low concentrations of largazole (10 pm/ml) or TSA (10 nm/ml) can improve hUC-MSC proliferation and not induce apoptosis. The hUC-MSCs growth curve showed that the CPDL values of the hUC-MSCs at different passages in the largazole and TSA media were higher than those in the control medium. As a new HDACi, largazole exhibited noticeable pharmacological activity. The proliferative ability of the largazole group was significantly higher than that of the TSA group at low concentrations. Flow cytometry experiments were performed to evaluate the effect of passage (P4 and P12) on the expression levels of eight cell surface markers in the three groups (data not shown). No significant changes were observed in the expression levels of surface markers during the early and late passages by HDACi, indicating that HDACis did not affect the surface markers of the stem cells in the growth medium.

During the long-term culturing of hUC-MSCs in vitro, the expression levels of the "stemness" genes OCT4, Nanog, and TERT and of the migratory gene CXCR4 significantly decreased. The expression levels of the osteogenic differentiation genes ALP and

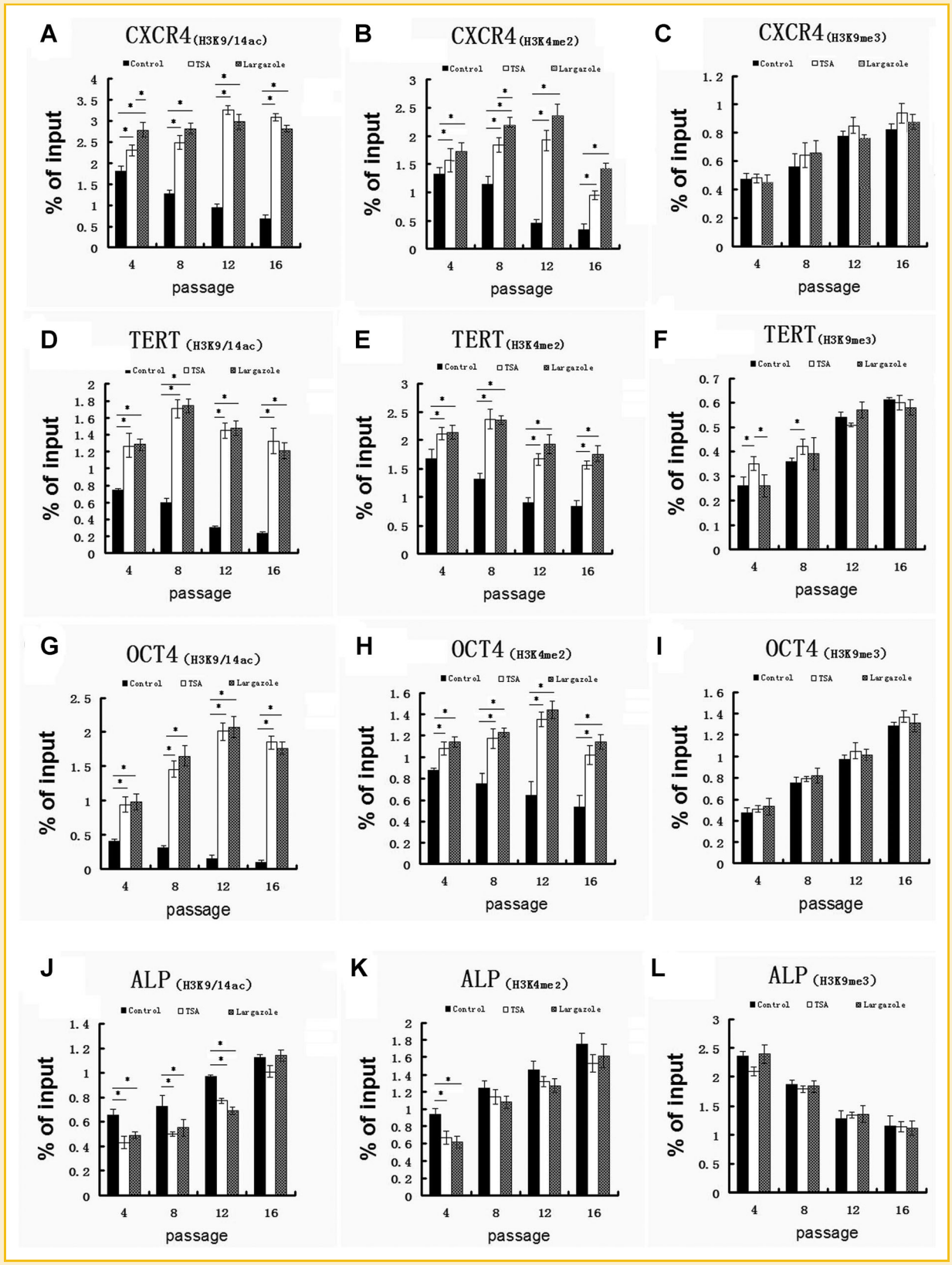

Fig. 4. Histone acetylation and methylation in early- and late-passage hUC-MSCs, as determined by chromatin immunoprecipitation: (A–L) CXCR4, TERT, OCT4, ALP, and histone H3K9ac, H3K4me2, and H3K9me3 in different passages and growth media (* $P < 0.01$).

OPN gradually increased. However, The molecular mechanisms underlying MSC spontaneous differentiation and aging are unclear. Low concentrations of vinyl phosphonic acid can promote the self-renewability and proliferation of hemopoietic stem cells [Bug et al., 2005]. The loss of OCT4 and TERT genes is partially reversed by the addition of an HDACi and a demethylating agent during the differentiation of the human embryonic stem [Stewart et al., 2008]. In this study, the low largazole and TSA concentrations for hUC-MSCs treatment were identified. The results showed that the mRNA expression levels of OCT4, Nanog, CXCR4, and TERT were higher than those of the control group at passage 4. Meanwhile, the mRNA expression levels of the osteogenic differentiation genes ALP and OPN significantly decreased. These changes in gene expression levels may be associated with epigenetic changes. Genome-wide histone H3-K9 acetylation levels at gene promoters in human bone marrow-derived MSCs coincide well with the overall mRNA expression levels [Tan et al., 2008]. This finding suggests that MSCs aging is associated with changes in gene promoter histone acetylation level.

Epigenetic changes are defined as modifications of DNA or chromatin that do not involve alterations of the DNA sequence or genetic deletions [Jenuwein and Allis, 2001]. The BPCs treated with TSA in a dose-dependent manner activated the pluripotent genes Oct4, Nanog, and Sox2, and resulted in the expression of these proteins. These cells did not result in the formation of teratomas [Rajasingh et al., 2011]. In this study, relevant chromosomal aberrations were not detected by karyotyping in the three groups. Nishida et al. [2006] and Hu et al. [2012] found that the nucleosome density is relatively low near the transcription start site that is flanked by the significantly acetylated H3K9 promoter in liver hepatocellular carcinoma cells. The repression of hTERT expression in hUC-MSCs is due to promoter-specific histone hypoacetylation coupled with low RNA polymerase II and transcription factor IIB trafficking. This repression is overcome by treatment with TSA, an HDACi that increases promoter-specific histone acetylation and RNA polymerase II and transcription factor IIB tracking, TSA then promotes TERT gene transcription [Serakinci et al., 2006]. In this study, the epigenetic changes in histone H3 acetylation and methylation were also investigated. The changes in H3K9ac and H3K4me2 in the OCT4, Nanog, TERT, CXCR4, ALP, and OPN gene promoter levels closely coincided with the corresponding gene expression levels in successive hUC-MSCs passages. This suggest that histone H3 acetylation and methylation is an important mechanism in regulating MSC aging and differentiation. We know that post-translational histone modifications participate in modulating the structure and function of chromatin and are critical in regulating gene transcription. Promoters of transcribed genes are enriched with hyperacetylation on the N-terminal tail of histone H3 [MacDonald and Howe, 2009]. Acetylation of K9 and K14 in histone H3 is required for the recruitment of TFIID [Agalioti et al., 2002], and TFIID binding to the promoter causes DNA bending and downstream translocation of the SWI/SNF-modified nucleosome, thus allowing the initiation of transcription [Lomvardas and Thanos, 2001]. The changes in H3K9/14 acetylation and H3K4 dimethylation in the largazole group were higher than those in the TSA group. Low concentrations of HDACi can modulate histone H3 acetylation and methylation in the TERT, OCT4, Nanog, CXCR4, ALP, and OPN genes; promote MSCs proliferation; and suppress spontaneous MSCs osteogenic differentiation. The self-renewability of MSCs was also maintained.

CONCLUSIONS

hUC-MSCs can undergo spontaneous differentiation and aging during expansion. Low concentrations of HDACi can improve the proliferative ability of hUC-MSCs and delay their aging. HDACi modulates histone H3 acetylation and methylation in the TERT, OCT4, Nanog, CXCR4, ALP, and OPN genes; promotes hUC-MSCs proliferation; and suppresses the spontaneous osteogenic differentiation of MSCs. The self-renewability of hUC-MSCs is also maintained in vitro. Therefore, a new method for obtaining sufficient amounts of cells for MSC-based therapies and tissue engineering is developed in this study.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Supplementary Fig. S1. Histone acetylation and methylation in early- and late-passage hUC-MSCs, as determined by chromatin immunoprecipitation: (A–F) Nanog, OPN, and histone H3K9ac, H3K4me2, and H3K9me3 in different passages and growth media ($^{*}P < 0.01$).