Epigenetic modifications of histone H3 during the transdifferentiation of Thy-1(+) Lin(-) bone marrow cells into hepatocytes

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Abstract. The epigenetic modifications during the transdifferentiation of adult stem cells remain to be fully elucidated. In the present study, the histone H3 modifications during the transdifferentiation of rat Thy-1(+) Lin(-) bone marrow cells into hepatocytes *in vitro* were examined, which involved performing hepatocyte growth factor-mediated transdifferentiation of bone marrow Thy-1(+) Lin(-) cells into hepatic lineage cells. Subsequently, the hepatocyte-specific markers, cytokeratin-18 (CK-18), albumin (ALB) and α-fetoprotein (AFP) were examined by immunofluorescence staining or reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Changes in the key pluripotency factor, octamer-binding transcription factor 4 (OCT4) and histone modifications, including the dimethylation and acetylation of H3 at lysine 9 (H3K9me2 and H3K9ac), lysine 14 (H3K14me2 and H3K14ac) and lysine 27 (H3K27me2 and H3K27ac), were also investigated by RT-qPCR, immunofluorescence staining or western blot analysis The mRNA expression levels of AFP and ALB were detected in the bone marrow stem cell-derived hepatic lineage cells on days 7 and 14 following induction, and CK-18 was detected on day 14 following induction. During the transdifferentiation of the bone marrow Thy-1(+) Lin(-) cells into hepatocytes, the mRNA expression of OCT4 was significantly reduced, and the levels of H3K9me2, H3K9ac, H3K14me2, H3K14ac, H3K27me2 and H3K27ac were increased significantly, compared with the levels at baseline ($P<0.05$). Therefore, the results of the present study demonstrated that histone H3 modifications at lysine 9, 14 and 27 are involved in the regulation of transcription during the transdifferentiation of bone marrow stem cells to hepatic lineage cells.

Introduction

Adult stem cells are self-renewing, pluripotent cells, which can cross lineage boundaries to transdifferentiate into different types of tissues (1,2). Since the late 1990s, the number of reports regarding the transdifferentiation of adult stem cells has increased (3). Our previous study demonstrated the differentiation of mouse Thy-1(+) Lin(-) bone marrow cells into hepatocyte-like cells (4). It was further demonstrated that the transplantation of autologous bone marrow-derived liver stem cells in patients with hepatitis B virus-induced liver cirrhosis exhibit improved liver function and structure (5).

The amino-terminal domains of histones are susceptible to enzymatic modifications, including acetylation, methylation, phosphorylation, ubiquitination and SUMOylation (6,7). Epigenetic modification of histones may result in altered chromatin conformation and affect the binding of transcription factors to chromatin. Histone acetylation can induce a reduction in DNA binding to histones and the relaxation of chromatin, providing binding sites for proteins, which are crucial for transcriptional activation (7). Histone methylation refers to the addition of between one and three methyl groups to the nitrogen atoms of lysine, arginine or histidine (8). Histone methylation regulates transcription through providing specific binding sites, which are attractive to effector protein complexes, promoting the recruitment and stabilization of regulatory proteins (9). It has been reported that the acetylation and methylation of histone H3 is important in the regulation of transcription (10,11).

The epigenetic histone modification of embryonic stem cells during differentiation into hepatocytes, or hepatocyte-like cells, has been reported (11-13). However, histone modification during the transdifferentiation of adult stem cells into hepatocyte, or hepatocyte-like cells, has not been reported. Furthermore, the dynamics of epigenetic modifications during transdifferentiation remain to be fully elucidated. It has been

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previously demonstrated that the histone deacetylase inhibitor, valproic acid, induces the hepatic differentiation of mouse bone marrow stromal stem cells *in vitro* and *in vivo*, suggesting histone acetylation during the transdifferentiation of adult stem cells to hepatocytes (14). The present study aimed to examine he epigenetic modifications of histone H3 during the transdifferentiation of Thy-1(+) Lin(-) bone marrow cells into hepatocytes *in vitro*, to provide insight into the role of histone modification in the transdifferentiation of bone marrow stem cells.

Materials and methods

Experimental animals. A total of 20 rats were used for this study. The animals were kept in collective cages (four animals per group) at a controlled temperature of 25±1℃ and a 12/12 h light/dark cycle of with access to food and water *ad libitum*. The bone marrow cell donors in the present study comprised 5-6-week-old male inbred Wistar rats, weighing 90-100 g, which were purchased from the Experimental Animal Center of Sun Yet-sen University (Guangzhou, China). The animals were maintained in a pathogen-free environment. All experimental procedures conformed to the European Convention on Animal Care (15). The present study was approved by the Institutional Animal Care and Use Committee of Southern Medical University (Guangzhou, China).

Preparation of the Thy-1(+) Lin(-) bone marrow cells. The rats were sacrificed by cervical dislocation. The femurs and tibias of lower limbs of the rats were collected under sterile conditions, and the marrow cavity was exposed by cutting the spongious end of each bone. The marrow cavity was flushed with DMEM/MCDB201 (Beijing Solarbio Science and Technology Co., Ltd., Beijing, China) three times to collect the bone marrow cells. The red blood cells (RBCs) were removed using RBC lysis buffer (Beijing Solarbio Science and Technology Co., Ltd.). The RBC-free bone marrow cells were filtered through a 400-mesh sieve (30 μm opening); and the cells were counted using a hemocytometer (Beyotime Institute of Biotechnology, Shanghai, China). The cell suspension density was adjusted to 100 M cells/ml. A two-step immunomagnetic cell sorting method was used to obtain the Thy-1(+) Lin(-) bone marrow cells (16). First, the Lin(-) cells were separated from the bone marrow cell suspension (negative selection), following which the Thy-1(+) Lin(-) cells were separated (positive selection). The lineage cell depletion kit and immunomagnetic beads were purchased from Miltenyi Biotec GmbH (Bergish Gladbach, Germany), and used, according to the manufacturer's instructions. Overall, the viability of the obtained Thy-1(+) Lin(-) cells was >95%, which was assessed using trypan blue staining. Briefly, the cell suspension was mixed with 0.4% trypan blue solution (Beyotime Institute of Biotechnology) at a 1:1 ratio. After 1-2 min incubation at room temperature, the mixture was loaded onto one chamber of hemocytometer and the squares of the chamber were observed under a BX51 light microscope (Olympus, Tokyo, Japan). The viable/live (clear) and non-viable/dead (blue) cells were counted and the viability was calculated using the following formula: (number of live cells counted / total number of cells counted) x 100. Cell detection by flow cytometry (EPICS

Altra; Beckman Coulter, Brea, CA, USA) was conducted within 1 h. The number of Thy-1(+) Lin(-) bone marrow cells was determined by counting the percentage of all cells. Flow cytometry revealed that the cell purity was >98%.

Induction of the differentiation of Thy-1(+) Lin(-) bone marrow cells into hepatocytes. The induction of differentiation of Thy-1(+) Lin(-) bone marrow cells into hepatocytes has been previously described (17). Briefly, the Thy-1(+) Lin(-) bone marrow cells were seeded into 6-well plates coated with 25 ng/ml fibronectin (1x10 cells/ml; Sigma-Aldrich, St. Louis, MO, USA) in DMEM/F-12 (Gibco Life Technologies, Carlsbad, CA, USA) containing 10% fetal bovine serum (GE Healthcare Life Sciences, Logan, UT, USA), 20 mM HEPES (Sigma-Aldrich), 10 nM dexamethasone (Sigma-Aldrich), penicillin and streptomycin. Coverslips were placed in the wells. Following cell adhesion, the medium was replaced with DMEM/F-12 containing 2.5% cholestatic rat serum (Gibco Life Technologies), 25 ng/ml hepatocyte growth factor (PeproTech EC, Ltd., London, UK), 20 mM HEPES, 10 nM dexamethasone, penicillin and streptomycin (Gibco Life Technologies). The cells were induced for 14 days consecutively At 37℃, and the medium was replaced once every 3 days. Subsequently, cell growth and morphology were observed, and the cells were collected on days 0, 7 and 14 for analysis. Cell growth and morphology were observed by a BX51 light microscope. Five visual fields were selected randomly.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The mRNA expression levels of the octamer-binding transcription factor 4 (OCT4), α-fetoprotein (AFP) and albumin (ALB) genes during the differentiation of bone marrow stem cells were detected using one-step RT-qPCR. Total RNA was extracted using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA). The 1-3 μg mRNA was reverse transcribed using a QuantiTect Reverse Transcription kit (Takara Bio, Inc., Otsu, Japan). The mRNA expression levels the of OCT4, AFP and ALB genes were determined 0, 7 and 14 days following induction using a Takara One Step RNA PCR kit (Takara Biotechnology, Co., Ltd., Dalian, China). The following primers were used: ALB, forward 5'-atcctgaaccgtctgtgtgt-3' and reverse 5'-cag ttatccttgtcggcagc-3'; AFP, forward 5'-gcgcatccatttccttcctt-3' and reverse 5'-tctaaacacccatcgccagt-3'; OCT4, forward 5'-agt ttgccaagctgctgaaa-3'and reverse 5'-cactcgaaccacatccctct-3'; β-actin, forward 5'-gagaaattgtgcgtgacatca-3' and reverse 5'-cctgaacctctcattgcca-3'. All primers were purchased from Sangon Biotech (Beijing, China). Each qPCR reaction was performed using a final volume of 50 μl, containing 24 μl nuclease-free water, 5 μl 10X One Step RNA PCR buffer, 1 μl of each 0.4 μM forward and reverse primer, 10 μl 5 mM MgCl$_2$, 5 μl 1 mM deoxynucleoside triphosphate mixture, 1 μl 0.8 U/μl RNase inhibitor, 0.1 U/μl AMV reverse transcriptase (RTase) XL (Sigma-Aldrich) and AMV-Optimized Taq (Sigma-Aldrich). Expression levels of different genes were validated by RT-qPCR analysis using the ABI 7500 sequence detector system according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA).The thermocycling conditions were as follows: 50℃ for 30 min for RT, 94℃ for 2 min for RTase denaturation, 94℃ for 30 sec, 25-30 cycles

of 65°C for 30 sec; 72°C for 10 min, 50°C for 15 min, 94°C for 2 min, 94°C for 30 sec and 60°C for 30 sec (28 cycles). β-actin was used as a positive control. Normal rat Thy-1(+) Lin(-) bone marrow cells, which had not undergone induction, were used as a negative control. Each qPCR reaction was repeated three times. Agarose gel electrophoresis was used to verify RNA integrity, and the quantity and quality of RNA samples were measured with the NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The ΔΔCt method was used for quantification.

Immunofluorescence staining. Histone modification, including dimethylation and acetylation of H3 at lysine 9 (H3K9me2 and H3K9ac), dimethylation and acetylation of H3 at lysine 14 (H3K14me2 and H3K14ac) and dimethylation and acetylation of H3 at lysine 27 (H3K27me2 and H3K27ac), during the differentiation of bone marrow stem cells was determined using immunofluorescence staining. The cells were collected on days 0, 7, and 14 following induction and seeded into 6-well plates ($1x10^5$ cells/well). Cells were incubated for 30 min with the cytokeratin-18 (CK-18) primary antibody at 37°C. Following cell adhesion, the medium was discarded and the cells were rinsed with phosphate-buffered saline (PBS). Subsequently, the cells were fixed in paraformaldehyde (Sigma-Aldrich) at room temperature for 20 min and rinsed with PBS (three times, 10 min each). The cells were then blocked with PBS containing 10% normal goat serum for 1 h. The cells were then incubated with the following antibodies against histone modification: Anti-histone H3 (dimethyl K9; rabbit monoclonal; 1:100 dilution; cat. no. ab32521; Abcam, Cambridge, MA, USA), anti-histone H3 (dimethyl K14; rabbit monoclonal; 1:1,000 dilution; cat. no. ab202416; Abcam), anti-histone H3 (dimethyl K27; rabbit polyclonal; 1:100 dilution; cat. no. ab194690; Abcam), anti-histone H3 (acetyl K9; rabbit polyclonal; 1:500 dilution; cat. no. ab61231; Abcam), anti-histone H3 (acetyl K14; rabbit polyclonal; 1:100 dilution; cat. no. ab82501; Abcam) or anti-histone H3 (acetyl K27; rabbit monoclonal; 1:200 dilution; cat. no. ab45173; Abcam) (Abcam, Cambridge, MA, USA). The sample was incubated at 4°C overnight. After incubation with the primary antibodies, cells were washed in PBS three times and then incubated with their specific secondary antibodies (anti-rabbit; cat. no. ZDR-5306; Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China) for 1 h at room temperature. For the assessment of CK-18, the primary antibody used was anti-CK18 rabbit anti-rat antibody (cat. no. sc-24603; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) and the samples were incubated at 37°C for 30 min. The secondary antibody used for detection was HRP-labeled anti-rabbit (cat. no. ZF-0513; Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd.). Following incubation, the sample was rinsed with PBS containing 0.1% Tween-20 (three times, 10 min each). The cells were then incubated with either fluorescein isothiocyanate (FITC)-labeled goat anti-rabbit IgG (1:1,000; cat. no. ZF-0511) or FITC-labeled goat anti-mouse IgG (1:2,000; cat. no. ZF-0512) (Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd.) for the detection of histone modification or the CK-18, respectively. The cells were incubated for 1 h or 30 min, respectively at 37°C. Following secondary antibody removal, diamidino-2-phenylindole staining solution Invitrogen Life Technologies) was added and

the sample was incubated at room temperature for 15 min. The sample was then rinsed with PBS containing 0.1% Tween-20 (three times, 10 min each). The stained cells were then observed under a fluorescent microscope (ECLIPSE TE2000-U, Nikon Corporation, Tokyo, Japan), and images were captured using a Universal Hood II gel imaging system (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Western blot analysis. The histone modification expression levels of H3K9me2, H3K14me2, H3 K27me2, H3K9ac, H3K14ac and H3 K27ac during the differentiation of bone marrow stem cells were detected using western blot analysis. Total protein was extracted from the cells using a nuclear and cytoplasmic protein extraction kit (Beyotime Institute of Biotechnology, Haimen, China), according to the manufacturer's instructions. Protein concentrations were measured using the Bradford method. The methods of the western blot assay used were those previously described (18). Briefly, the blots were incubated with the same corresponding first and secondary antibodies used for the above-mentioned immunofluorescence staining. Subsequently, the samples were detected using an enhanced chemiluminescence kit (PerkinElmer, Inc, Waltham, MA, USA). Normal rat Thy-1(+) Lin(-) bone marrow cells, which did not undergo induction, were used as a negative control. The rats received an intravenous injection of heparin and the liver was cannulated. The liver was perfused through the portal vein, first with perfusion buffer for 10 min and then with collagenase buffer for 12 min at a flow rate of 25 ml/min. Cells were separated from the digested liver using a 100 μm cell strainer (BD Biosciences, Franklin Lakes, NJ, USA) to obtain a cellular suspension. The cell suspension was centrifuged for 3 min at 200 x g. The upper layer was discarded and 10 ml of washing buffer was added. Rat hepatocytes, which were freshly separated, were used as a positive control. β-actin was used as a loading control. All analyses were repeated three times. The intensity of bands was determined by using Quantity One software (Bio-Rad Laboratories, Inc.), and the gray-scale value of each target protein vs. that of β-actin was quantitatively analyzed. All analyses were repeated three times.

Statistical analysis. Friedman one-way analysis of variance was used to detect differences in the mRNA expression levels of the OCT4, AFP and ALB genes; as well as the expression of the histone modifications, including H3K9me2, H3K9ac, H3K14me2, H3K14ac, H3K27me2 and H3K27ac, between groups. A Wilcoxon rank sum test was used for pair-wise comparisons. Statistical analyses were performed using SAS 9.2 (SAS Institute Inc., Cary, NC, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

Differentiation of Thy-1(+) Lin(-) bone marrow cells into hepatocytes in vitro. The freshly separated Thy-1(+) Lin(-) bone marrow cells were uniform in size and they exhibited a lymphocyte-like round shape. Prior to induction, the shape of the adherent cells was variable, resembling spindle-, bipolar-, and fibroblast-like cells. On day 7 post-induction, the morphology of the adherent cells had diversified, and resembled short-spindle,
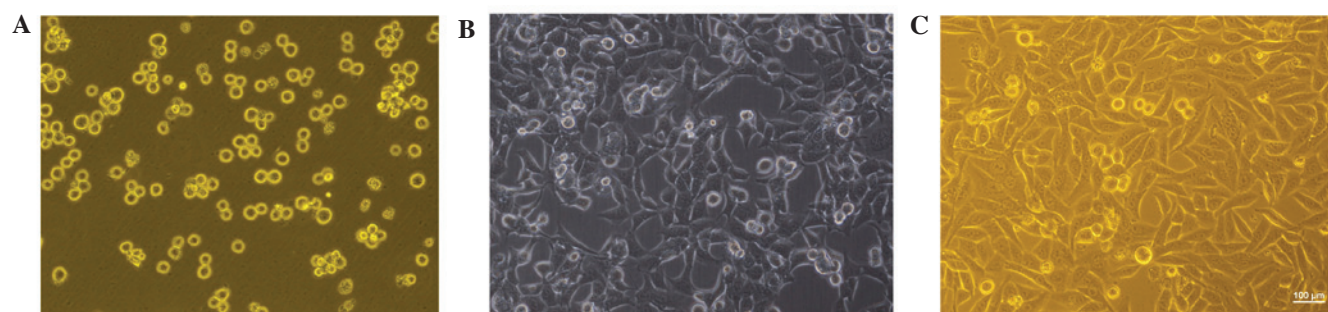
Figure 1. Immunofluorescence shows the morphology of the Thy-1(+) Lin(-) bone marrow cells during differentiation into hepatocytes on (A) day 0, (B) day 7 post-induction and (C) day 14 post-induction (magnification, x20).
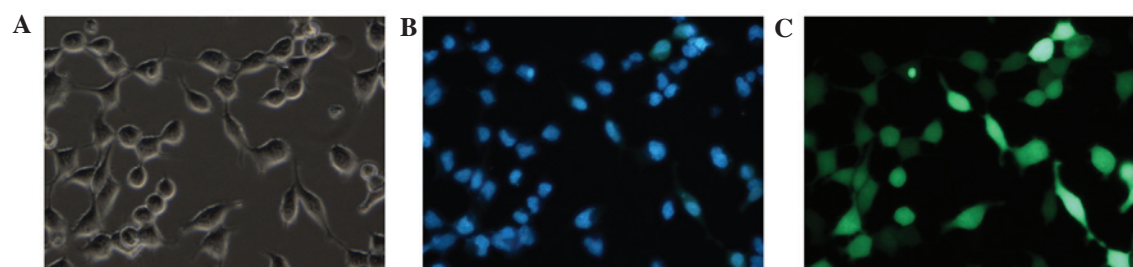

Figure 2. Analysis of Thy-1(+) Lin(-) bone marrow cells. (A) Light microscopy shows the morphology of Thy-1(+) Lin(-) bone marrow cells which induced to differentiate into hepatocytes on day 14. (B) Immunofluorescence shows DAPI and (C) cytokeratin-18 positive cells. Magnification, x20.
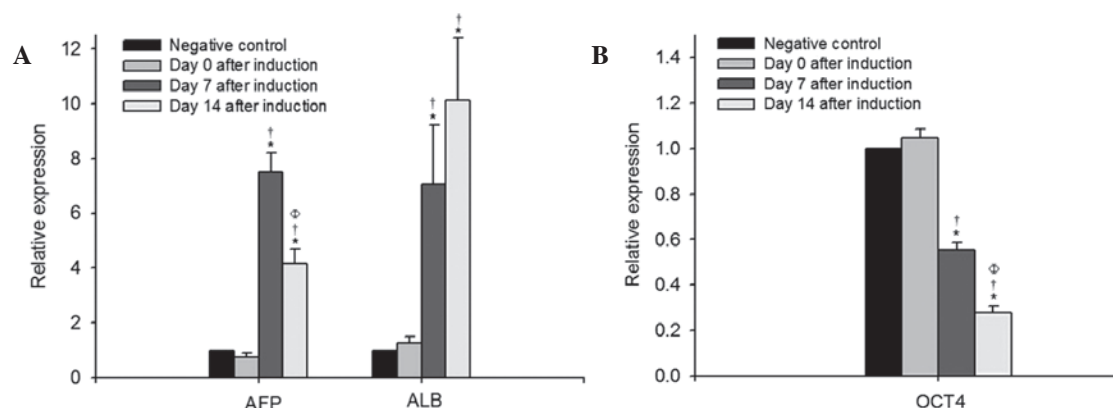

Figure 3. mRNA expression levels following the induced differentiation of Thy-1(+) Lin(-) bone marrow cells into hepatocytes. (A) Relative mRNA expression levels of AFP and ALB in the Thy-1(+) Lin(-) bone marrow cells during differentiation into hepatocytes. (B) Relative mRNA expression levels of OCT4 in the Thy-1(+) Lin(-) bone marrow cells during differentiation into hepatocytes. Normal rat Thy1(+) Lin(-) bone marrow cells, which did not undergo induction, were used as a negative control. *P<0.05, compared with the negative control; †P<0.05, compared with day 0; ΦP<0.05, compared with day 7. AFP, α-fetoprotein; ALB, albumin; OCT4, octamer-binding transcription factor 4.

oval- and polygonal-like cells. On day 14 post-induction, the cell morphology had changed further, with cells exhibiting an oval, round or polygonal shape with an epithelioid shape (Fig. 1). On day 14 post-induction, the bone marrow stem cells, which were derived from the hepatic lineage cells, expressed the hepatocyte-specific marker, CK-18 (Fig. 2). The results of the RT-qPCR analysis demonstrated that the mRNA expression levels of hepatocyte-specific AFP and ALB increased significantly on day 7 and day 14 following induction, compared with the negative controls or those on day 0 (Fig. 3A).

Epigenetic modifications in Thy-1(+) Lin(-) bone marrow cells during differentiation into hepatocytes. The mRNA expres-

sion levels of the key pluripotency factor, OCT4, in the bone marrow stem cell-derived hepatic lineage cells was significantly reduced 7 days following induction, compared with that on day 0, followed by a more marked reduction on day 14 post-induction (P<0.05; Fig. 3B). Changes in histone modification, indicated by the expression levels of H3K9me2, H3K9ac, H3K14me2, H3K14ac, H3K27me2 and H3K27ac, are shown in Fig. 4. On days 7 and 14 following induction, the levels of H3K9me2, H3K9ac, H3K14me2, H3K14ac, H3K27me2 and H3K27ac increased significantly, compared with the levels in the negative controls or those on day 0 (P<0.05). The level of H3K27ac on day 14 post-induction was higher than that observed on day 7 post-induction (P<0.05).
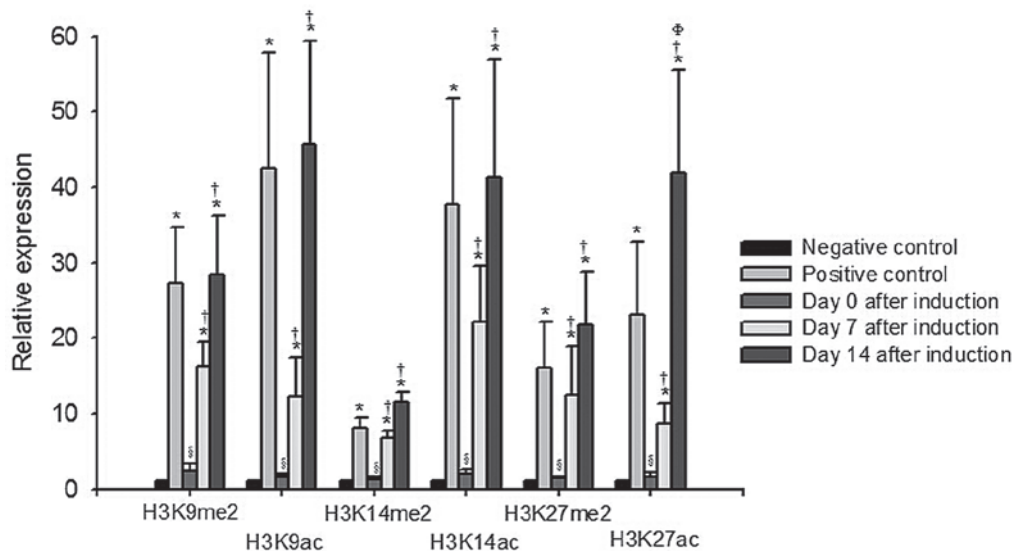
Figure 4. Relative expressions levels of H3K9me2, H3K9ac, H3K14me2, H3K14ac, H3K27me2 and H3K27ac following the induced differentiation of Thy-1(+) Lin(-) bone marrow cells into hepatocytes. The expression levels were normalized to β-actin. *P<0.05, compared with the negative control. §P<0.05, compared with the positive control; †P<0.05, compared with day 0; ΦP<0.05, compared with day 7.

Discussion

The *in vitro* induction of the differentiation of mesenchymal stem cells derived from bone marrow, adipose tissue, skin, placenta, and umbilical cord into endodermal cells have been reported (19-22). Epigenetic reprogramming is critical in the differentiation of stem cells, and is essential for the differentiation process, in which the cells overcome lineage borders and attain the specialized functions of a new tissue (23). In the present study, the differentiation of Thy-1(+) Lin(-) bone marrow cells into hepatocytes was induced *in vitro*. The bone marrow derived epithelioid cells demonstrated morphological characteristics of primary hepatocytes, and expressed the hepatocyte-specific marker cytoskeletal protein, CK18, on day 14 post-induction, and the mRNA expression of AFP and ALB were observed on days 7 and 14 post-induction, indicating a hepatic phenotype of the bone marrow stem cell-derived epithelioid cells. In addition, histone modifications, including significant increases in the levels of H3K9me2, H3K9ac, H3K14me2, H3K14ac, H3K27me2 and H3K27ac were observed on days 7 and 14 following induction, compared with their levels on day 0 or in the negative control.

Embryonic stem cell investigations have progressed by identifying three key transcription factors, Oct4, sex determining region Y-box 2 (Sox2) and NANOG (24,25), the interplay of which leads to the formation of regulatory circuitry for the maintenance of the exclusive features of embryonic stem cells (26). It is now well recognized that Oct4, Sox2 and NANOG are essential for the pluripotency of embryonic stem cells *in vivo* and *in vitro* (27). Pluripotent stem cells have been induced from somatic cells through the overexpression of four transcription factors, including Oct4 and Sox2 (28). Previous studies have demonstrated the downregulation of Oct4 during the differentiation of embryonic stem cells; with *de novo* DNA methylation of the Oct4 regulatory region occurring during differentiation (29). Kim *et al* reported that the transcription of Oct4 is elevated in pluripotent human embryonic stem cells

and reduces significantly during the differentiation of embryonic stem cells into hepatocytes *in vitro* (12). In menstrual blood-derived stem cells that can differentiate into functional hepatocyte-like cells following induction, expression of the Oct 4 pluripotency marker gene has also been detected (30). The present study demonstrated that, during the transdifferentiation of bone marrow stem cells into hepatocytes, a similar decrease in the transcription of the Oct 4 pluripotency marker gene was observed.

It has been suggested that histone tail modifications, including acetylation, methylation, phosphorylation/ubiquitination/sumoylation, ADP-ribosylation and glycosylation can alter chromatin structure and subsequent gene expression (31-33). In the present study, increases in histone H3 modification*s*, including H3K9me2, H3K9ac, H3K14me2, H3K14ac, H3K27me2 and H3K27ac were observed during the transdifferentiation of bone marrow stem cells into hepatocytes *in vitro*. To the best of our knowledge, histone modification during the transdifferentiation of adult stem cells has not been reported previously, and investigations on epigenetic modifications during the transdifferentiation of adult stem cells have been limited. It has been demonstrated that the chromatin remodeling agent trichostatin A induces the transdifferentiation of human mesenchymal stem cells into hepatocyte-like cells (34) and dimethylsulfoxide enhances the transdifferentiation of human adipose tissue-derived stromal cells into hepatic lineage cells (35). DNA methyltransferase inhibitors have also been observed to induce transdifferentiation (36-38). However, reports regarding epigenetic modifications during the transdifferentiation of adult stem cells remain minimal, and further investigations are required.

There are previous reports describing epigenetic modification during the differentiation of embryonic stem cells (11-13). Well-acknowledged epigenetic modifications associated with transcriptionally active chromatin include DNA hypomethylation, the acetylation of histone H3 and H4 and the methylation of H3K4, H3K36 and H3K79 (13). Representative epigenetic

modifications associated with the repressive chromatin state include the hypoacetylation of H3 and H4 and methylation of H3K9, H4K20, H3K27 or H4K20 (11). During the differentiation of embryonic stem cells into hepatocytes *in vitro*, permissive histone modifications, including H3K4me3 and H3K9ac within the promoter regions of the OCT4, SOX2, and NANOG pluripotency marker genes and hepatocyte marker genes are rich in embryonic stem cells prior to induction, and then decreased rapidly in definitive endoderm cells and hepatocytes (12). By contrast, repressive histone modifications, including H3K27me3, H3K9me2 and H3K9me3 in the promoter regions of the pluripotency marker genes and hepatocyte marker genes increase gradually during the differentiation of embryonic stem cells into hepatocytes (12). These findings suggest that, during the differentiation of embryonic stem cells, a combination of specific histone modifications can modulate gene transcription. However, further investigations are required to compare the histone code (histone signature) during the differentiation of embryonic stem cells in the transdifferentiation of adult stem cells into hepatocytes.

In the present study, the hepatic characteristics of bone marrow-derived hepatocyte-like cells at a morphological, RNA and protein level were demonstrated. A limitation of the investigation was the lack of functional assays available to examine the glycogen uptake and urea synthesis of the bone marrow-derived hepatocyte-like cells. Urea synthesis is one of the unique functions of mature hepatocytes (39,40). However, the *in vivo* transplantation of stem cell-derived hepatocytes in immunodeficient animal models with liver injury is essential for validating functional hepatic behavior (41). In our previous study, the same induction method was used to induce the differentiation of bone marrow stem cells into hepatocytes, and the hepatocytes generated were transplanted *ex vivo* into animal models and patients (42-44). In those investigations, the function of the stem cell-derived hepatocytes had been supported previously (4,5). The focus of the present study was to investigate the levels of histone H3 modifications during the differentiation of bone marrow stem cells into hepatocytes, and the changes associated with these histone modification, with respect to key lineage-specific genes during transdifferentiation, require further examination. Future results may improve current understanding of the intrinsic mechanism governing transdifferentiation-inducing signals.

In conclusion, the present study demonstrated that, during the transdifferentiation of Thy-1(+) Lin(-) bone marrow cells into hepatocytes *in vitro*, the mRNA expression of the key pluripotency factor, OCT4, was significantly reduced. This change resembled that, which occurs during the differentiation of embryonic stem cells into hepatocytes. The levels of histone H3 modifications, including H3K9me2, H3K9ac, H3K14me2, H3K14ac, H3K27me2 and H3K27ac increased during the transdifferentiation of the Thy-1(+) Lin(-) bone marrow cells into hepatocytes. Future investigations are required to examine the changes in histone modification, which are pertinent to key lineage-specific genes during the transdifferentiation of adult stem cells. The results may assist in understanding the similarity and discrepancy in the epigenetic modifications during the transdifferentiation of adult stem cells, and the differentiation of embryonic stem cells, into hepatocytes.

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