Biochemical and Biophysical Research Communications 450 (2014) 1593–1599

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Overexpression of TGF-β1 enhances chondrogenic differentiation and proliferation of human synovium-derived stem cells

Yong Il Kim [a], Jae-Sung Ryu [a], Jee Eun Yeo [a], Yun Jin Choi [a], Yong Sang Kim [a], Kinarm Ko [b], Yong-Gon Koh [a,*]

[a] Center for Stem Cell & Arthritis Research, Department of Orthopedic Surgery, Yonsei Sarang Hospital, Seoul, Republic of Korea
[b] Center for Stem Cell Research, Institute of Advanced Biomedical Science, Konkuk University, Seoul 143-701, Republic of Korea

ARTICLE INFO

Article history:
Received 4 July 2014
Available online 15 July 2014

Keywords:
Synovium-derived mesenchymal stem cell
TGF-β
Chondrogenic differentiation
Proliferation

ABSTRACT

Transforming growth factor-beta (TGF-β) superfamily proteins play a critical role in proliferation, differentiation, and other functions of mesenchymal stem cells (MSCs). During chondrogenic differentiation of MSCs, TGF-β up-regulates chondrogenic gene expression by enhancing the expression of the transcription factor SRY (sex-determining region Y)-box9 (Sox9). In this study, we investigated the effect of continuous TGF-β1 overexpression in human synovium-derived MSCs (hSD-MSCs) on immunophenotype, differentiation potential, and proliferation rate. hSD-MSCs were transduced with recombinant retroviruses (rRV) encoding TGF-β1. The results revealed that continuous overexpression of TGF-β1 did not affect their phenotype as evidenced by flow cytometry and reverse transcriptase PCR (RT-PCR). In addition, continuous TGF-β1 overexpression strongly enhanced cell proliferation of hSD-MSCs compared to the control groups. Also, induction of chondrogenesis was more effective in rRV-TGFB-transduced hSD-MSCs as shown by RT-PCR for chondrogenic markers, toluidine blue staining and glycosaminoglycan (GAG)/DNA ratio. Our data suggest that overexpression of TGF-β1 positively enhances the proliferation and chondrogenic potential of hSD-MSCs.

1. Introduction

Articular cartilage is a highly specialized connective tissue that absorbs an enormous mechanical load, allowing almost frictionless motion between the articulating surfaces of diarthrodial joins. Although remarkably durable, damaged articular cartilage has limited intrinsic self-healing capacity [1]. Currently, various tissue-engineering approaches are applied in clinical practice with the goal of inducing the repair of articular cartilage lesions [2,3]. Mesenchymal stem cells (MSCs) have several features that make them an attractive option for potentiating cartilage repair such as multipotency, high proliferation profile, self-renewal ability, and ease of harvest [4–8]. MSCs are multipotent stem cells that can differentiate into the cell types of various mesenchymal tissues such as osteoblasts, chondrocytes, and adipocytes [6]. MSCs have been isolated from various tissues such as fetal liver, umbilical cord blood, bone marrow, synovium, and adipose [9–13]. Among the MSCs derived from these tissues, synovium-derived MSCs (SD-MSCs) have been recently recognized as an excellent source, owing to their superior chondrogenic potential and higher proliferative ability [13]. Also, synovial tissue is conveniently accessible for the acquisition of autologous SD-MSCs because it is ordinary for osteoarthritis patients to undergo an arthroscopic examination [14].

Growth factors play an important role in the proliferation and chondrogenic differentiation of MSCs. Several valuable growth factors are involved during *in vitro* chondrogenesis of MSCs, including members of the transforming growth factor-beta (TGF-β) superfamily (TGF-β1, 2, and 3), several bone morphogenic proteins (BMPs), insulin-like growth factor-1 (IGF-1), fibroblast growth factors (FGFs), and epidermal growth factor (EGF), among others [15]. Specifically, the TGF-β superfamily proteins play a critical role in proliferation, differentiation, and other functions in MSCs. Numerous studies have shown that TGF-β efficiently induces chondrogenic differentiation [16,17]. TGF-β up-regulates various molecules associated with prechondrogenic condensation [18] and chondrogenic gene expression. Among the TGF-β superfamily, TGF-β1 is capable of inducing chondrogenesis in SD-MSCs [19]. Also, stimulation of TGF-β1 during culture expansion was reported to be required to promote *in vitro* proliferation of MSCs [20,21]. Furthermore, recent research has demonstrated that TGF-β gene transfer into MSCs could enhance chondrogenesis *in vitro* [22,23].

* Corresponding author. Address: Center for Stem Cell & Arthritis Research, Department of Orthopedic Surgery, Yonsei Sarang Hospital, 478-3, Bangbae-dong, Seocho-gu, Seoul, Republic of Korea. Fax: +82 2 2023 5584.
E-mail address: yonseranglab@daum.net (Y.-G. Koh).

http://dx.doi.org/10.1016/j.bbrc.2014.07.045
0006-291X/

In the present study, we hypothesized that continuous TGF-β1 overexpression could enhance the proliferation and chondrogenic potential of human SD-MSCs (hSD-MSCs). Therefore, we used an rRV vector containing the human TGF-β1 gene transfer into hSD-MSCs and examined the effects of continuous TGF-β1 overexpression on their phenotype, proliferation rate, and differentiation potential of hSD-MSCs *in vitro*. Consequently, proliferation and chondrogenic differentiation of hSD-MSC was enhanced by continuous TGF-β1 overexpression.

2. Materials and methods

2.1. Isolation and culture of hSD-MSCs

hSD-MSCs were obtained from the synovium of osteoarthritis patients in accordance with the regulations of the Ethics Committee of Yonsei Sarang Hospital. hSD-MSCs were isolated following a standardized procedure [14]. Briefly, Synovial tissue digested in 0.2% collagenase (Worthington) in high glucose Dulbecco's modified Eagle's medium (DMEM; Hyclone) containing 1× penicillin/streptomycin (Hyclone) at 37 °C for 1 h. Single cell suspensions were then centrifuged, and the supernatant was removed. The cells were resuspended in pre-warmed high glucose DMEM supplemented with 10% fetal bovine serum (FBS) (Gibco) and 1× penicillin/streptomycin, and then plated onto a culture dish. Once the attached cells proliferated and reached confluence, the cells were trypsinized, re-plated for expansion, and established as hSD-MSCs.

2.2. Construction of recombinant retroviral vectors and cell infection

cDNA of human *TGFB1* was amplified by RT-PCR using total RNA isolated from the hSD-MSCs. The cDNA was then cloned into the pMX retroviral vector (Addgene) by recombination. The recombinant plasmid was verified by DNA sequencing. Recombinant retrovirus was transduced into HEK-293T cells using Superfectene Pro (RD TECH). After filtration of the medium from the HEK-293T cell cultures, retroviral particles were concentrated by centrifugation with the Retro X concentrator (Clonetech) for 1 h at 1500×*g* at 4 °C. The retroviral particles were then resuspended in 5% FBS/DMEM medium and stored at −80 °C. For viral infection, passage 3 hSD-MSCs were seeded in six well plates at a concentration of 5×10^4 cells per well. The next day, hSD-MSCs were transduced with either rRV-TGFB1 or rRV-GFP at a multiplicity of infection (MOI) of 10 in the presence of 4 μg/mL polybrene (Sigma). The efficiency of transduction was evaluated via fluorescence microscopy (Leica DMI 3000B). ELISA analysis (R&D Systems) was performed to detect the production of TGF-β1 in culture medium on the days 1, 3, 6, 10, and 15 after transduction.

2.3. Flow-assisted cell sorting (FACS) analysis

After trypsinization, non-transduced or rRV-transduced hSD-MSCs were resuspended in ice-cold PBS containing 0.5% bovine serum albumin (BSA, Sigma) at a concentration of 1×10^6 cells/mL. The cells were then incubated for 20 min on ice with FITC-conjugated anti-human CD14, PE-conjugated anti-human CD31, APC-conjugated anti-human CD34, PerCP-conjugated anti-human CD90, or PerCP-conjugated anti-human CD105 (BD Biosciences). Analyses were performed by flow cytometry (FACS, BD Biosciences) using the CellQuest software (BD Biosciences).

2.4. In vitro differentiation assay

For chondrogenic differentiation, non-transduced or rRV-transduced hSD-MSCs were cultured to obtain adequate cells for 21 days. After expansion, the cells were trypsinized and seeded at 2.5×10^6 cells/mL in 15-mL polypropylene tubes. The cells were centrifuged for 5 min at 1500 rpm for use in an aggregate culture system. The cell aggregates were prepared and kept in chondrogenesis medium that consisted of 1× Insulin-Transferrin-Selenium premix (Gibco BRL), 50 mM ascorbate-2-phosphate (Sigma) and 100 nM dexamethasone (Sigma) without exogenous recombinant TGF-β at 37 °C. The aggregates formed into a free-floating mass within the first 24 h of culture. The medium was replaced every 3 days for 21 days.

2.5. Histology

Aggregates were fixed in 10% paraformaldehyde for 10 min and rinsed twice with PBS or distilled water. Aggregates were embedded in paraffin and cut into 5-μm sections. Sections were stained with toluidine blue (for the staining of matrix proteoglycans) and hematoxylin & eosin (H&E) stain according to routine protocols. Samples were examined via light microscopy (Leica DMI 3000B).

2.6. Viability assay

The cells of all groups were seeded at a density of 5×10^3 cells/well in 24-well plates. Cell proliferation was determined by the established 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-based assay on the first, third, fifth, seventh, tenth and fifteenth day after transduction. Each well was incubated with MTT solution (Sigma) for 4 h, and the absorbance of each well was measured at 590 nm using a spectrophotometer.

For the cell counting assay, hSD-MSCs (3×10^4 cells) were seeded onto six-well plates after transduction and cultured in high-glucose DMEM supplemented with 10% FBS and 1% streptomycin/penicillin. The cells were trypsinized every 4 days, stained with trypan blue and counted using a hemocytometer under a light microscope. Cells were then reseeded into new culture plates for expansion.

2.7. RNA isolation and RT-PCR

Total RNA was isolated from samples using a total RNA extraction kit (iNtRON) according to the manufacturer's recommended protocol. Synthesis of cDNA and polymerase chain reaction (PCR) was conducted using AccuPower RT PreMix (Bioneer) and Ex Taq (Takara), respectively, according to the manufacturer's recommended protocol. PCR analysis was performed with gene specific primers (Supplementary Table 1). *GAPDH* was amplified as a control. The thermal cycle profiles for PCR involved 30 cycles of 30 s at 94 °C, 1 min at 59 °C, and 1 min at 72 °C, followed by a final 7 min extension at 72 °C. The PCR products were separated on a 1% agarose gel. The relative intensity of the individual PCR products within the gels was determined from digital images using the Image Lab software.

2.8. Measurement of GAG content

After chondrogenic induction for 21 days, all aggregates were digested for 18 h at 65 °C with 125 μg/mL papain in PBE buffer (10 mM EDTA, 100 mM sodium phosphate, pH 6.5) containing 5 mM L-cysteine–HCl, using 500 μL of enzyme per sample. Chondrogenic potential was quantified by measuring sulfated glycosaminoglycans (S-GAG) production using a 1,9-dimethylmethylene blue (DMMB) assay according to the manufacturer's recommended protocol (Blyscan™ Glycosaminoglycan Assay Kit). The absorbance at 656 nm was measured using the microplate immunoreader (Sunrise™, TECAN). To quantify cell density, the amount of DNA in the papain digests was analyzed using a

Quant-iT PicoGreens dsDNA Assay kit (Invitrogen) according to the manufacturer's recommended protocol. Measurements (excitation at 485 nm; emission at 538 nm) were conducted using CytoFluors Series 4000 (Applied Biosystems). The GAG content was normalized to the total DNA content.

2.9. Statistical analysis

All data are presented as the mean ± SD. Comparisons of multiple groups were performed by one-way analysis of variance (ANOVA) and two-way ANOVA, followed by pairwise comparisons with a Bonferroni post hoc test. Differences were considered statistically significant at $P < 0.05$. All data were analyzed using the GraphPad Prism software, version 5.00 (Graph-Pad software).

3. Results

3.1. Efficient and sustained overexpression of TGF-β1 by rRV transduction in monolayer culture of hSD-MSCs

To determine the efficiency of retroviral gene transduction into hSD-MSCs, rRV-GFP was transduced into hSD-MSCs at various MOIs. GFP-positive hSD-MSCs were quantified under a fluorescence microscope 3 days after transduction. We found that transduction efficiency was adequate (about 89%) when the rRV-GFP was transduced into hSD-MSCs at an MOI of 10 (Fig. 1A). Considering this transduction efficiency, an MOI of 10 was selected for the transduction of rRV-TGFB1. RT-PCR analysis detected strong expression of *TGF-β1* in rRV-TGFB1-transduced hSD-MSCs (Fig. 1B). Also, TGF-β1 protein levels increased in a time-dependent fashion in culture medium of rRV-TGFB1-transduced hSD-MSCs (Fig. 1B). To investigate immunophenotype of rRV-TGFB1-transduced hSD-MSCs, we characterized MSC-specific markers via FACS and RT-PCR analysis. All groups revealed the same typical immunophenotype ($CD14^-$, $CD31^-$, $CD34^-$, $CD90^+$, $CD105^+$) and surface marker gene expression ($CD45^-$, $CD133^-$, $CD44^+$, $CD73^+$, $CD90^+$) of MSC whether they were cultured with or without continuous TGF-β1 overexpression for 21 days (Fig. 1C). These results indicate that continuous TGF-β1 overexpression in hSD-MSCs did not influence the immunophenotype and surface marker gene expression of MSCs.

3.2. Retroviral-mediated transduction of TGFB1 enhance cell proliferation

To characterize the influence of continuous TGF-β1 overexpression on hSD-MSC viability, we measured cell viability using an MTT assay. The MTT assay revealed that cell numbers were not

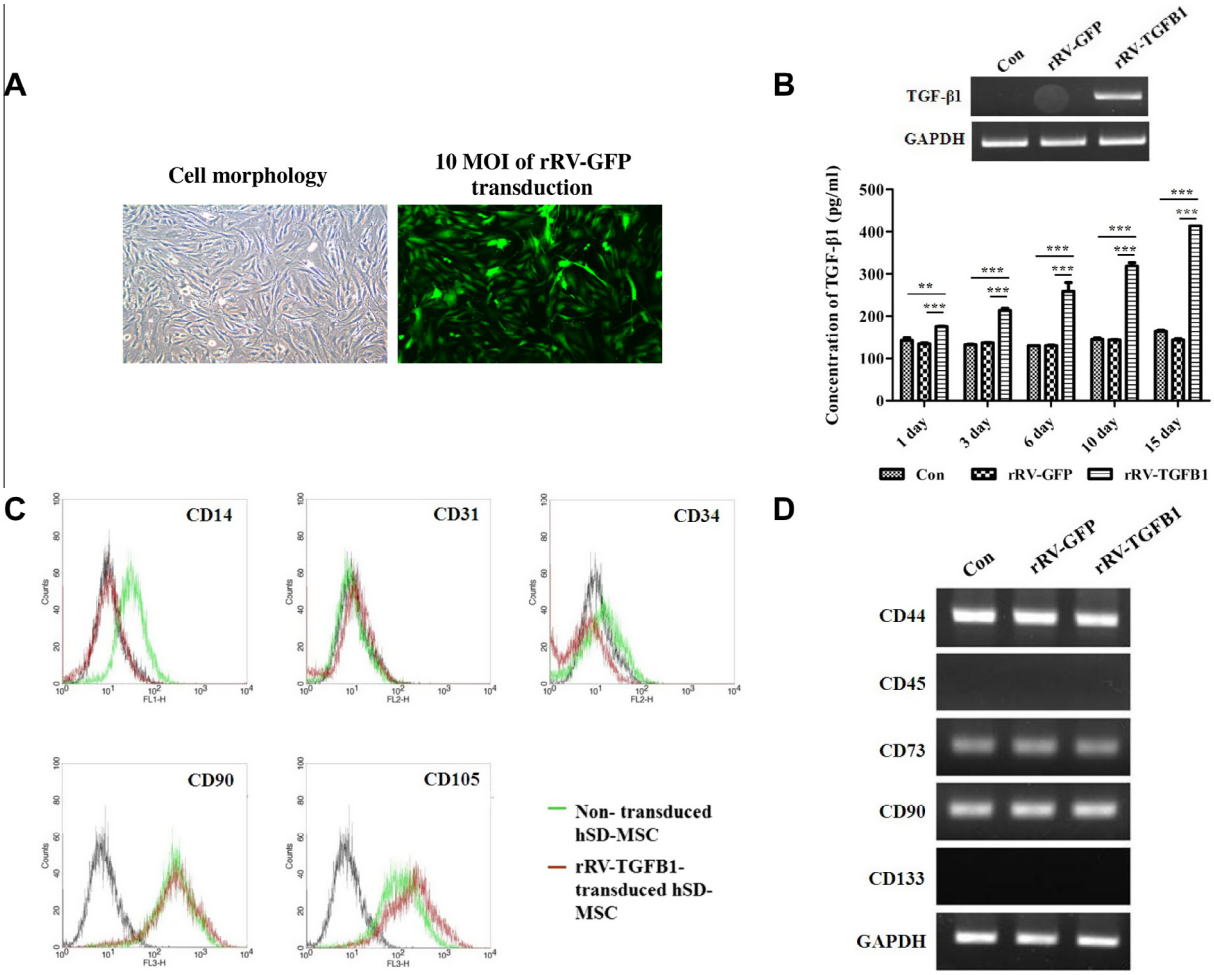

Fig. 1. Continuous overexpression of TGF-β1 in hSD-MSCs and phenotypic characterization after transduction. (A) Fluorescence microscope examination showing the efficiency after transduction with MOI of 10. (B) TGF-β1 mRNA and protein were evaluated by RT-PCR and ELISA analysis after transduction with MOI of 10. ELISA analysis was performed to detect TGF-β1 expression in culture medium 1, 3, 6, 10 and 15 days after transduction. (C) Immunophenotypic analysis of hSD-MSCs cultured for 21 days after rRV-TGFB1 transduction was performed by FACS. (D) RT-PCR analysis of hSD-MSCs cultured for 21 days after rRV-TGFB1 transduction was performed for the gene expression of surface marker. The values shown are the means ± SEM from three independent experiments. $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with the non-transduced or rRV-GFP-transduced groups.

significantly different by day 3. However, cell viability of rRV-TGFB1-transduced hSD-MSCs gradually increased from day 5 to day 15 relative to non-transduced or rRV-GFP-transduced hSD-MSCs (Fig. 2A). Next, the proliferation-inducing activity of continuous TGF-β1 overexpression was examined to determine whether it could lead to an increase in the cell numbers. Continuous TGF-β1 overexpression significantly increased the cell numbers of hSD-MSCs in a time-dependent manner compared with non-transduced or rRV-GFP-transduced hSD-MSCs (Fig. 2B). Also, when we compared cumulative population doublings (cPDs) over each consecutive passage, it revealed a pattern similar to the results of MTT and cell counting assays. Although cPDs were not significantly different during passage 1–2, cPDs level after the third passage gradually were increased in the rRV-TGFB1-transduced group compared with the control groups (Fig. 2C). Therefore, retroviral-mediated transduction into hSD-MSCs did not affect cytotoxicity, and specifically, TGF-β1 overexpression in hSD-MSCs enhanced cell proliferation.

3.3. Differentiation of hSD-MSCs after retroviral-mediated gene transfer of TGFB1

Firstly, we investigated adipogenic and osteogenic potential of rRV-TGFB1-transduced hSD-MSCs in differentiation inducing medium. However, overexpression of endogenous TGF-β1 did not influence the adipogenic or osteogenic potential of hSD-MSCs, as demonstrated by Oil Red O or Von Kossa's staining and expression patterns of adipogenic and osteogenic markers (Supplementary Fig. 1). Next, we used the aggregate culture system to investigate the chondrogenic potential of rRV-TGFB1-transduced hSD-MSCs. After chondrogenic induction for 7, 14, and 21 days, aggregates of all groups were subjected to histological examination by toluidine blue (matrix proteoglycans) and H&E staining. Interestingly, aggregates from rRV-TGFB1-transduced hSD-MSCs had a greater amount of toluidine blue staining in cartilage matrix; in contrast, aggregates from non-transduced and rRV-GFP-transduced hSD-MSCs had a small amount of cartilage matrix. Furthermore, rRV-TGFB1-transduced hSD-MSCs successfully formed successful aggregates at early time points (day 14) (Fig. 3A) and aggregate size was slightly increased in rRV-TGFB1-transduced hSD-MSCs compared with control groups (Fig. 3B). Next, we investigated expression of chondrogenic specific marker genes, such as aggrecan, Type II collagen, and SOX9 (Fig. 3C). The comparative analysis of gene expression showed a pattern similar to that of the histological results. The expression levels of aggrecan and SOX9 increased continuously from day 14 to day 21 in rRV-TGFB1-transduced group. Also, an increase in type II collagen expression was clearly observed at day 21. Although non-transduced or rRV-GFP-transduced groups had elevated SOX9 expression levels at day 21, aggrecan and type II collagen mRNA expression was unchanged. Furthermore, the chondrogenic potential of rRV-TGFB1-transduced hSD-MSCs showed similar results to that of the non-transduced hSD-MSCs with exogenous recombinant TGF-β1 protein (Supplementary Fig. 2). These results suggest that continuous overexpression of

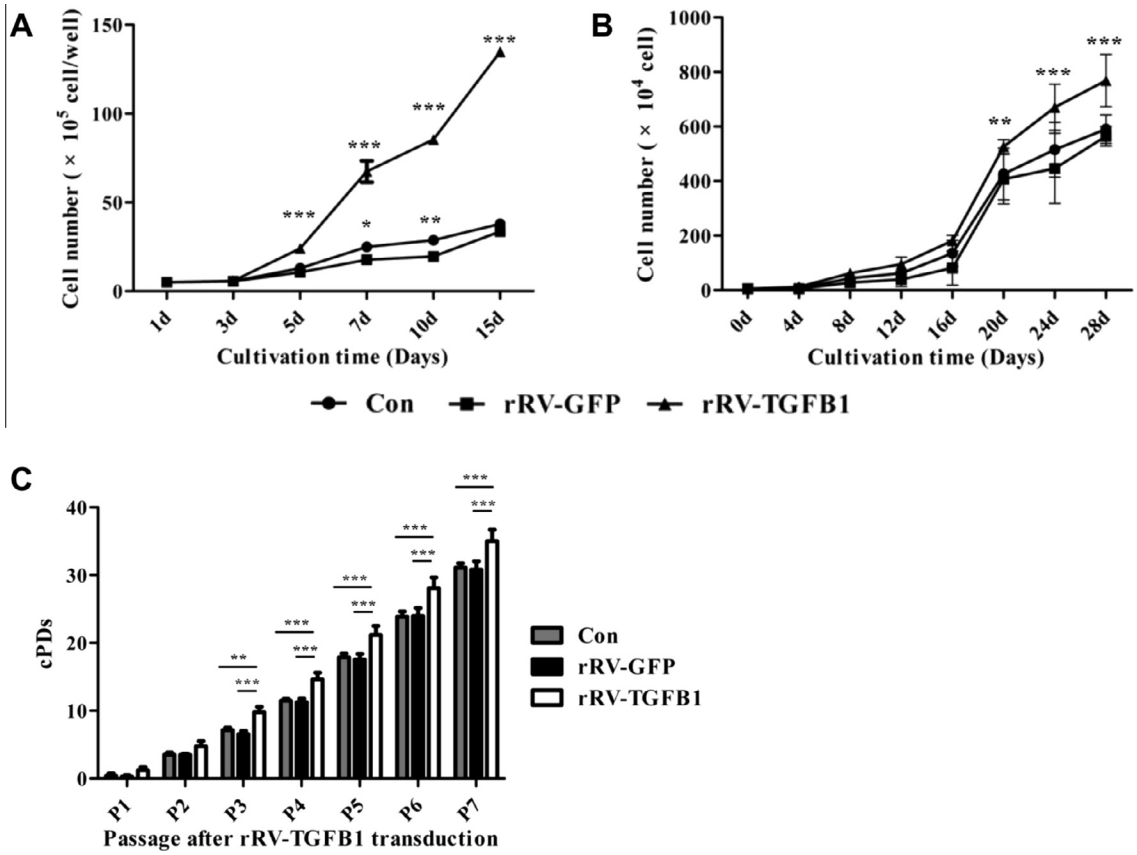

Fig. 2. Continuous overexpression of TGF-β 1 enhances hSD-MSCs proliferation. (A) Non-transduced or rRV transduced hSD-MSCs were seeded at a density of 5×10^3 cells/well in 24-well plates. Cell viability was estimated at 1, 3, 5, 7, 10 and 15 days after transduction using the MTT assay. (B) For the cell counting assay, hSD-MSCs (3×10^4 cells) were seeded onto six-well plates after transduction and cultured in normal culture media for 28 days. Cells were trypsinized stained with trypan blue, and counted every 4 days using a hemocytometer using a light microscope. (C) Comparison of cumulative population doublings (cPD) for the first seven passages after transduction revealed gradually increase in rRV-TGFB1-transduced hSD-MSCs. The values are the means ± SEM from 3 independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with the non-transduced or rRV-GFP-transduced groups.

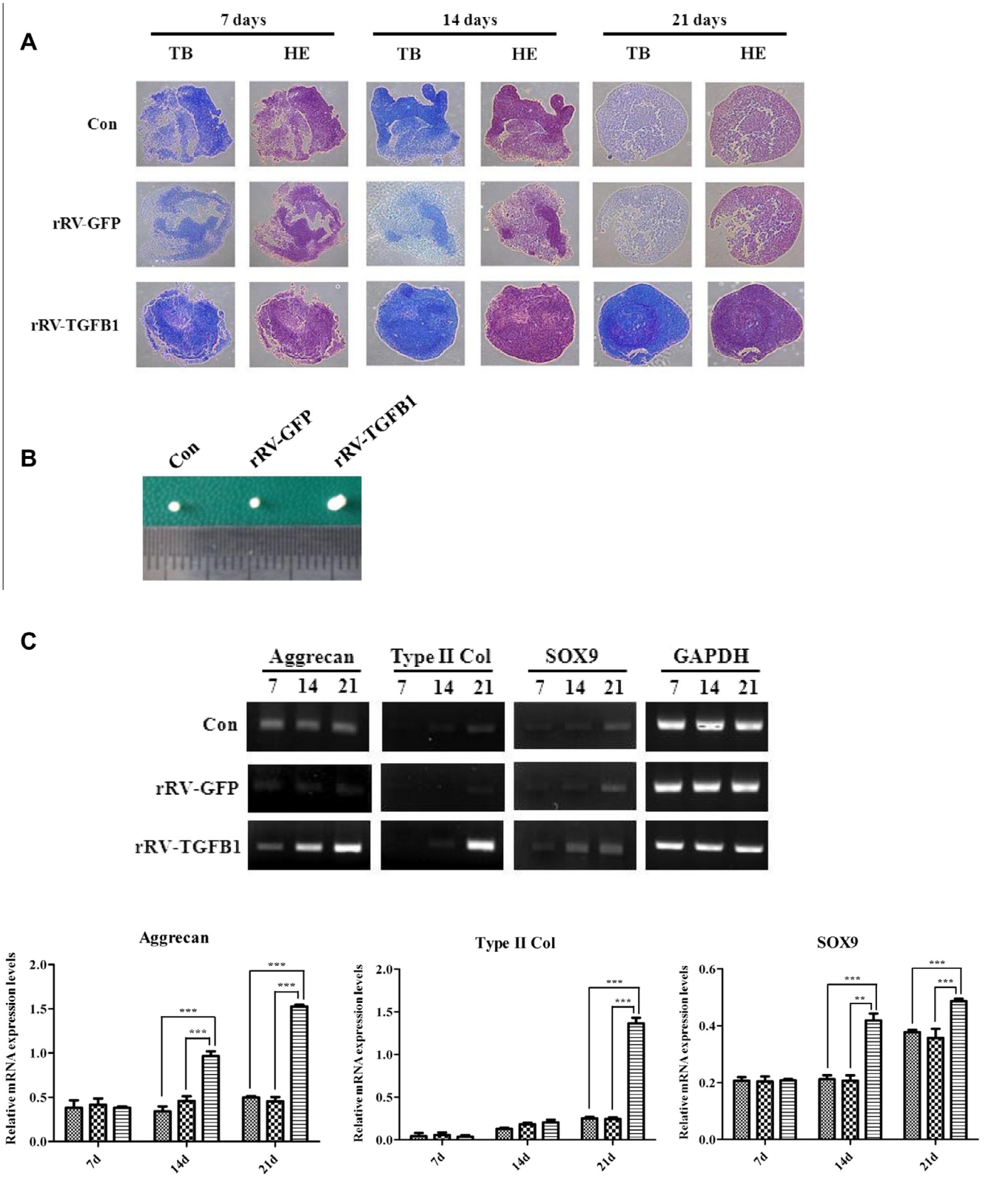

Fig. 3. Chondrogenic differentiation of hSD-MSCs in aggregate culture following transduction. (A) Non-transduced or rRV-transduced hSD-MSCs were expanded for 21 days, cultured into aggregates, and maintained in chondrogenic differentiation medium without the addition of exogenous recombinant TGFβ protein for 7, 14, or 21 days. Aggregates of all groups were stained with H&E stain and toluidine blue for histological characterization. (B) Aggregate sizes of a representative for each group with a 0.5-mm scaled ruler. (C) For each treatment group and time point indicated, the chondrogenic-specific marker genes aggrecan, type II collagen and SOX9 were determined by RT-PCR (7, 14, and 21 days). mRNA expression levels were normalized to the housekeeping gene *GAPDH*. The values shown are the means ± SEM from three independent experiments. $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with the non-transduced or rRV-GFP-transduced groups.

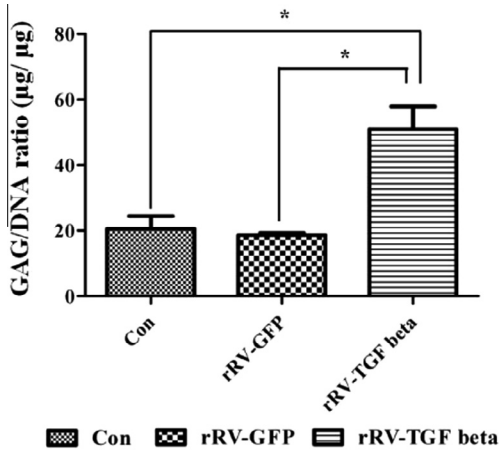

Fig. 4. Biochemical analysis for the assessment of chondrogenic differentiation index. After 21 days of chondrogenic induction, aggregates were digested in papain and sulfated glycosaminoglycans (S-GAG) and DNA content was measured. The GAG content was normalized to the total DNA content of each sample. The values shown are the means ± SEM from three independent experiments. $^{*}P < 0.05$ compared with the non-transduced or rRV-GFP-transduced groups.

TGF-β1 in hSD-MSCs could stimulate and accelerate chondrogenesis without exogenous recombinant TGF-β1 protein.

Finally, we measured the accumulation of glycosaminoglycans (GAGs) normalized to total DNA in aggregates of all groups at day 21 after chondrogenic induction. GAG/DNA ratios (in μg/μg) were 20.55 ± 3.91 for the control group, 18.63 ± 0.64 for the rRV-GFP-transduced group, and 50.97 ± 6.94 for the rRV-TGFB1-transduced group. These results reached statistical significance only for the rRV-TGFB1-transduced group (Fig. 4). These findings correspond to the results of histological analysis and correlate with the expression level of chondrogenic markers during chondrogenesis.

4. Discussion

Transplantation of MSCs possessing chondrogenic potential represents a new alternative approach for damaged articular cartilage repair. These MSCs are able to differentiate into osteoblasts, chondrocytes, and adipocytes and expand to numbers relevant to clinical application. Also, growth factors such as TGF-β play an essential role in the chondrogenic differentiation of MSCs. Previous studies have demonstrated that supplementation of TGF-β1 initiated and improved chondrogenic differentiation of MSCs in a time- and dose-dependent manner [24].

Several studies have investigated the role of TGF-β on cell fate in human cells. Stimulation of the TGF-β1 signaling pathway induces premature senescence in human skin fibroblasts [25]. Additionally, increased *TGFB2* mRNA expression by long-term culture induced cellular senescence in hSD-MSCs and down-regulation of *TGFB2* expression inhibited cellular senescence [26]. In contrast, treatment with TGF-β1 during cell growth enhanced cell proliferation in MSCs [20,21,27,28]. Specifically, treatment of bone marrow derived MSCs with TGF-β1 enhanced cell proliferation via the rapid nuclear translocation of β-catenin in a SMAD3-dependent manner [20]. Walenda et al. have recently reported that exogenous TGF-β1 significantly enhanced proliferation of MSCs and did not induce senescence [21]. However, most studies have investigated the proliferation effects of treatment with exogenous recombinant TGF-β1 protein during MSC culture. In this study, we demonstrated that endogenous TGF-β1 overexpression by genetic modification enhanced hSD-MSC proliferation, similar to results from previous reports that examined treatment with exogenous recombinant TGF-β1 [20,21,27,28]. In this regard, gene transfer is an attractive alternative for the delivery of recombinant protein. As the half-life of exogenous recombinant TGF-β1 protein is short, usually their repeated supplementation requires prolonged *in vitro* culture periods. This feature can be avoided by the continuous overexpression of endogenous TGF-β1 through application of gene transfer. However, the underlying mechanisms behind the enhancement of hSD-MSC proliferation by endogenous TGF-β1 overexpression are not clearly understood and will require further investigation.

The TGF-β signaling pathway is important for chondrogenic differentiation of MSCs [24]. A previous study revealed that the TGF-β signal was transmitted into the nucleus through the SMAD pathway, which then activated the SOX9 transcription factor for cartilage gene expression such as Type II collagen and aggrecan [29]. In present study, we found that aggregates of rRV-TGFB1-tranduced hSD-MSCs exhibited increased levels of toluidine blue-positive matrix production. Also, continuous overexpression of TGF-β1 not only led to larger aggregate sizes, greater cellularity and successful aggregate formation at an early time point (day 14), but also stimulated the expression of chondrogenic marker genes after aggregate culture. Additionally, GAG/DNA ratios were higher in the rRV-TGFB1-transduced group than control groups. Similarly, previous studies have reported that human TGF-β1 induced chondrogenesis in rabbit bone marrow stromal cell *in vivo* with increased chondrogenic marker gene expression [30]. Other studies have shown that TGF-β3 can promote the expression of aggrecan, Type II collagen, and cartilage oligomeric matrix protein and *in vitro* chondrogenic differentiation of porcine SD-MSCs [22], and rabbit SD-MSCs co-cultured with TGFB3-transduced chondrocytes enhanced expression of cartilage markers and the production of GAG and Collagen II, successfully inducing chondrogenesis [31]. However, most studies utilized an adenoviral-associated vector system to induce transient target gene expression [22,23,30–32]. Therefore, these previous studies immediately induced chondrogenic differentiation within 48 h after the transduction of the gene encoding TGF-β into target cells. However, we were able to acquire enough hSD-MSC numbers through cell culture for 21 day after retroviral gene transduction and confirmed enhancement of chondrogenic potential.

In conclusion, we demonstrate that continuous TGF-β1 overexpression in hSD-MSCs does not alter their phenotype, even after expansion for 21 days following transduction. Furthermore TGF-β1 overexpression enhanced cell proliferation and chondrogenic potential. Although additional studies are needed, such as insertional mutagenesis, the confirmation of the safety of the retroviral vector, and the use of regulatory mechanisms for effective target gene expression, our results demonstrate the possibility that continuous overexpression of TGF-β1 in hSD-MSCs may be a useful strategy to obtain adequate cell density for the clinical application of hSD-MSC-based cartilage regeneration.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.07.045.

References

[1] J.A. Buckwalter, H.J. Mankin, A.J. Grodzinsky, Articular cartilage and osteoarthritis, Instr. Course Lect. 54 (2005) 465–480.
[2] E.B. Hunziker, Articular cartilage repair: basic science and clinical progress. A review of the current status and prospects, Osteoarthritis Cartilage 10 (2002) 432–463.
[3] A. Getgood, R. Brooks, L. Fortier, N. Rushton, Articular cartilage tissue engineering: today's research, tomorrow's practice?, J Bone Joint Surg. Br. 91 (2009) 565–576.
[4] D.C. Colter, I. Sekiya, D.J. Prockop, Identification of a subpopulation of rapidly self-renewing and multipotential adult stem cells in colonies of human marrow stromal cells, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 7841–7845.

[5] K. Nishimura, L.A. Solchaga, A.I. Caplan, J.U. Yoo, V.M. Goldberg, B. Johnstone, Chondroprogenitor cells of synovial tissue, Arthritis Rheum. 42 (1999) 2631–2637.
[6] M.F. Pittenger, A.M. Mackay, S.C. Beck, R.K. Jaiswal, R. Douglas, J.D. Mosca, M.A. Moorman, D.W. Simonetti, S. Craig, D.R. Marshak, Multilineage potential of adult human mesenchymal stem cells, Science 284 (1999) 143–147.
[7] X. Shi, Y. Wang, R.R. Varshney, L. Ren, F. Zhang, D.A. Wang, In-vitro osteogenesis of synovium stem cells induced by controlled release of bisphosphate additives from microspherical mesoporous silica composite, Biomaterials 30 (2009) 3996–4005.
[8] F.H. Chen, K.T. Rousche, R.S. Tuan, Technology insight: adult stem cells in cartilage regeneration and tissue engineering, Nat. Clin. Pract. Rheumatol. 2 (2006) 373–382.
[9] A.J. Friedenstein, K.V. Petrakova, A.I. Kurolesova, G.P. Frolova, Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues, Transplantation 6 (1968) 230–247.
[10] W. Wagner, F. Wein, A. Seckinger, M. Frankhauser, U. Wirkner, U. Krause, J. Blake, C. Schwager, V. Eckstein, W. Ansorge, A.D. Ho, Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood, Exp. Hematol. 33 (2005) 1402–1416.
[11] A.J. Katz, A. Tholpady, S.S. Tholpady, H. Shang, R.C. Ogle, Cell surface and transcriptional characterization of human adipose-derived adherent stromal (hADAS) cells, Stem Cells 23 (2005) 412–423.
[12] S. Kern, H. Eichler, J. Stoeve, H. Kluter, K. Bieback, Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue, Stem Cells 24 (2006) 1294–1301.
[13] J. Fan, R.R. Varshney, L. Ren, D. Cai, D.A. Wang, Synovium-derived mesenchymal stem cells: a new cell source for musculoskeletal regeneration, Tissue Eng. B Rev. 15 (2009) 75–86.
[14] C. De Bari, F. Dell'Accio, P. Tylzanowski, F.P. Luyten, Multipotent mesenchymal stem cells from adult human synovial membrane, Arthritis Rheum. 44 (2001) 1928–1942.
[15] A.F. Steinert, S.C. Ghivizzani, A. Rethwilm, R.S. Tuan, C.H. Evans, U. Noth, Major biological obstacles for persistent cell-based regeneration of articular cartilage, Arthritis Res. Ther. 9 (2007) 213.
[16] J. Hao, R.R. Varshney, D.A. Wang, Engineering osteogenesis and chondrogenesis with gene-enhanced therapeutic cells, Curr. Opin. Mol. Ther. 11 (2009) 404–410.
[17] J.S. Park, D.G. Woo, H.N. Yang, H.J. Lim, H.M. Chung, K.H. Park, Heparin-bound transforming growth factor-beta3 enhances neocartilage formation by rabbit mesenchymal stem cells, Transplantation 85 (2008) 589–596.
[18] J. Chimal-Monroy, L. Diaz de Leon, Expression of N-cadherin, N-CAM, fibronectin and tenascin is stimulated by TGF-beta1, beta2, beta3 and beta5 during the formation of precartilage condensations, Int. J. Dev. Biol. 43 (1999) 59–67.
[19] M. Pei, D. Chen, J. Li, L. Wei, Histone deacetylase 4 promotes TGF-beta1-induced synovium-derived stem cell chondrogenesis but inhibits chondrogenically differentiated stem cell hypertrophy, Differentiation 78 (2009) 260–268.
[20] H. Jian, X. Shen, I. Liu, M. Semenov, X. He, X.F. Wang, Smad3-dependent nuclear translocation of beta-catenin is required for TGF-beta1-induced proliferation of bone marrow-derived adult human mesenchymal stem cells, Genes Dev. 20 (2006) 666–674.
[21] G. Walenda, K. Abnaof, S. Joussen, S. Meurer, H. Smeets, B. Rath, K. Hoffmann, H. Frohlich, M. Zenke, R. Weiskirchen, W. Wagner, TGF-beta1 does not induce senescence of multipotent mesenchymal stromal cells and has similar effects in early and late passages, PLoS One 8 (2013) e77656.
[22] Y. Yao, F. Zhang, R. Zhou, K. Su, J. Fan, D.A. Wang, Effects of combinational adenoviral vector-mediated TGF beta 3 transgene and shRNA silencing type I collagen on articular chondrogenesis of synovium-derived mesenchymal stem cells, Biotechnol. Bioeng. 106 (2010) 818–828.
[23] G.D. Palmer, A. Steinert, A. Pascher, E. Gouze, J.N. Gouze, O. Betz, B. Johnstone, C.H. Evans, S.C. Ghivizzani, Gene-induced chondrogenesis of primary mesenchymal stem cells in vitro, Mol. Ther. 12 (2005) 219–228.
[24] M. Pei, F. He, G. Vunjak-Novakovic, Synovium-derived stem cell-based chondrogenesis, Differentiation 76 (2008) 1044–1056.
[25] F. Debacq-Chainiaux, C. Borlon, T. Pascal, V. Royer, F. Eliaers, N. Ninane, G. Carrard, B. Friguet, F. de Longueville, S. Boffe, J. Remacle, O. Toussaint, Repeated exposure of human skin fibroblasts to UVB at subcytotoxic level triggers premature senescence through the TGF-beta1 signaling pathway, J. Cell Sci. 118 (2005) 743–758.
[26] T. Ito, R. Sawada, Y. Fujiwara, Y. Seyama, T. Tsuchiya, FGF-2 suppresses cellular senescence of human mesenchymal stem cells by down-regulation of TGF-beta2, Biochem. Biophys. Res. Commun. 359 (2007) 108–114.
[27] K. Koli, M.J. Ryynanen, J. Keski-Oja, Latent TGF-beta binding proteins (LTBPs)-1 and -3 coordinate proliferation and osteogenic differentiation of human mesenchymal stem cells, Bone 43 (2008) 679–688.
[28] F. Ng, S. Boucher, S. Koh, K.S. Sastry, L. Chase, U. Lakshmipathy, C. Choong, Z. Yang, M.C. Vemuri, M.S. Rao, V. Tanavde, PDGF, TGF-beta, and FGF signaling is important for differentiation and growth of mesenchymal stem cells (MSCs): transcriptional profiling can identify markers and signaling pathways important in differentiation of MSCs into adipogenic, chondrogenic, and osteogenic lineages, Blood 112 (2008) 295–307.
[29] W.M. Kulyk, J.L. Franklin, L.M. Hoffman, Sox9 expression during chondrogenesis in micromass cultures of embryonic limb mesenchyme, Exp. Cell Res. 255 (2000) 327–332.
[30] B.W. Qi, A.X. Yu, S.B. Zhu, M. Zhou, G. Wu, Chitosan/poly(vinyl alcohol) hydrogel combined with Ad-hTGF-beta1 transfected mesenchymal stem cells to repair rabbit articular cartilage defects, Exp. Biol. Med. (Maywood) 238 (2013) 23–30.
[31] R.R. Varshney, R. Zhou, J. Hao, S.S. Yeo, W.H. Chooi, J. Fan, D.A. Wang, Chondrogenesis of synovium-derived mesenchymal stem cells in gene-transferred co-culture system, Biomaterials 31 (2010) 6876–6891.
[32] I. Garza-Veloz, V.J. Romero-Diaz, M.L. Martinez-Fierro, I.A. Marino-Martinez, M. Gonzalez-Rodriguez, H.G. Martinez-Rodriguez, M.A. Espinoza-Juarez, D.A. Bernal-Garza, R. Ortiz-Lopez, A. Rojas-Martinez, Analyses of chondrogenic induction of adipose mesenchymal stem cells by combined co-stimulation mediated by adenoviral gene transfer, Arthritis Res. Ther. 15 (2013) R80.