Regulation of mesenchymal stem cell chondrogenesis by glucose through protein kinase C/transforming growth factor signaling

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| Summary |
| Objective: Effective induction of human mesenchymal stem cell (hMSC) differentiation for regenerative medicine applications remains a great challenge. While much research has studied hMSC activity during differentiation, it is unclear whether pre-differentiation culture can modulate differentiation capacity. We investigate the effect of glucose concentration in pre-differentiation/expansion culture on modulating chondrogenic capacity of hMSCs, and explore the underlying molecular mechanism. |
| Design: The extent of chondrogenesis of hMSCs previously cultured with different concentrations of glucose was evaluated. Transforming growth factor-beta (TGF-β) signaling molecules and protein kinase C (PKC) were analyzed to identify the role of these molecules in the regulation of glucose on chondrogenesis. In addition, hMSCs in high-glucose expansion culture were treated with the PKC inhibitor to modulate the activity of PKC and TGF-β signaling molecules. |
| Results: High-glucose maintained hMSCs were less chondrogenic than low-glucose maintained cells upon receiving differentiation signals. Interestingly, we found that high-glucose culture increased the phosphorylation of PKC and expression of type II TGF-β receptor (TGFβRII) in pre-differentiation hMSCs. However, low-glucose maintained hMSCs became more responsive to chondrogenic induction with increased PKC activation and TGFβRII expression than high-glucose maintained hMSCs during differentiation. Inhibiting the PKC activity of high-glucose maintained hMSCs during expansion culture upregulated the TGFβRII expression of chondrogenic cell pellets, and enhanced chondrogenesis. |
| Conclusion: Our findings demonstrate the effect of glucose concentration on regulating the chondrogenic capability of pre-differentiation hMSCs, and provide insight into the mechanism of how glucose concentration regulates PKC and TGF-β signaling molecules to prime pre-differentiation hMSCs for subsequent chondrogenesis. |

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Introduction

Human mesenchymal stem cells (hMSCs) represent a promising component of regenerative medicine due to their extensive proliferative capacity and multipotency 1–3. The cells can be harvested from various tissues including bone marrow, fat or cord blood 4–6. With proper induction signals, hMSCs can differentiate into osteoblasts, adipocytes or chondrocytes 7. Previous studies have reported that the differentiation capacity of hMSCs can be affected by a variety of factors, such as the chemical composition of culture medium 8,9.

Glucose concentration in culture medium has been shown to have a profound effect on mesenchymal stem cell (MSC) differentiation. For example, high-glucose culture medium enhances adipogenesis of mouse bone marrow-derived MSCs 10 and human adipose tissue-derived MSCs 11, compared to low-glucose medium. Mineral deposition of MSCs during osteogenesis in high-glucose medium is greater than that of MSCs in low-glucose medium 12. During chondrogenic induction, high-glucose medium enhances chondrogenesis of chick mesenchymal cells, in comparison with low-glucose medium 13. These studies focused on investigating the effect of glucose concentration on MSCs during cell differentiation. However, it is likely that glucose concentration in hMSC expansion culture can affect properties of pre-differentiation hMSCs, which further changes their capacity of differentiation in response to...
induction signals. Studies have demonstrated that high-glucose expansion culture reduces the proliferation of hMSCs, but whether the differentiation capacity of hMSCs is regulated by glucose concentration in expansion culture is not well-characterized.

Activation of the transforming growth factor-beta (TGF-β) signaling pathway is critical for chondrogenesis of hMSCs. TGF-β ligand binds to type II TGF-β receptor (TGFβRII) to form a heterodimeric complex with type I TGF-β receptor (TGFβRI), which phosphorylates downstream signaling molecule Smad2/3. Phosphorylated Smad2/3 forms a heteromeric complex with Smad4, acting as a transcriptional activator to regulate the activity of TGF-β-responsive genes, including Sox9 for chondrogenesis. Human MSCs transfected with the TGF-β1 or TGF-β2 gene have been shown to induce chondrogenesis with the production of cartilage-related collagen type II.

Glucose has been shown to regulate the TGF-β signaling pathway to modulate cell activity. High-glucose culture induces hypertrophy of mouse embryonic fibroblasts and rat kidney epithelial cells through the upregulation of TGF-β signaling. High-glucose culture also modulates protein kinase C (PKC) activity to upregulate the expression of TGF-β receptor expression of vascular smooth muscle cells.

In this study, we hypothesize that the glucose concentration in hMSC expansion culture is able to modulate the chondrogenic capacity of pre-differentiation hMSCs through the regulation of PKC activity and TGF-β signaling. To test our hypothesis, hMSCs were maintained in culture medium with different glucose concentrations, and then induced in cell pellets for chondrogenesis. Cartilage-related markers were analyzed to determine the extent of chondrogenesis of hMSCs between different groups. PKC activity and TGF-β signaling were studied to identify the underlying mechanism.

Materials and methods

Human MSC isolation and expansion

Human bone marrow-derived MSCs were isolated from femoral heads of three patients between 25 and 50 years of age who underwent total hip arthroplasty. The protocol was approved by the Institutional Review Boards of University of Washington and University of Wisconsin–Madison. Briefly, 5 mL bone marrow was harvested from the interior compartment of the femoral neck and head, and thoroughly mixed with 30 mL Dulbecco’s Modified Eagle Medium (DMEM) (Gibco, Carlsbad, CA). A syringe with an 18-gauge needle was used to filter out bone debris. The bone marrow was then centrifuged at 1,200 rpm for 5 min. After removing the supernatant, the cell pellet was reconstituted in Hank’s Balanced Salt Solution (Invitrogen, Carlsbad, CA), gently added into a conical tube containing the Ficoll gradient solution (GE Health, Pittsburgh, PA), and centrifuged at 600 g for 30 min. Mononuclear cells were collected, reconstituted in culture medium composed of low-glucose DMEM supplemented with 10% fetal bovine serum (FBS) (Gibco) and antibiotics, plated in cell culture flasks (Corning, Corning, NY), and maintained at 37°C in a humidified, 5% CO2 atmosphere. The cells were trypanized using 0.05% trypsin/EDTA (Gibco) after reaching 70–80% density confluence, and re-plated at a seeding density of 1,000 cells/cm². Culture medium was replaced every 3 days. At passage 2, hMSCs were divided into two independent groups. One group was maintained in low-glucose (1.0 mg/ml) DMEM and termed as low-glucose maintained cells (LGMCs), and the other group was maintained in high-glucose (4.5 mg/ml) DMEM and termed as high-glucose maintained cells (HGMCs). Each assay was carried out using a single donor’s cells and then repeated using different donors’ cells to confirm the reproducibility of experimental data. The results presented in this study are representative results based on one donor’s cells.

Differentiation of different glucose concentration-maintained hMSCs

Human MSCs were collected and made into high-density cell pellets for chondrogenesis in a 96-well plate following the previously reported method with modifications. Briefly, 250,000 hMSCs were centrifuged in a well containing chondrogenic medium and then maintained in a cell culture incubator. The chondrogenic medium was composed of high-glucose DMEM, 1% ITS+ (BD Biosciences, San Diego, CA), 0.9 mM sodium pyruvate, 50 µg/ml l-ascorbic acid-2-phosphate, 10⁻² M dexamethasone (Sigma–Aldrich, St. Louis, MO), and 40 µg/ml l-proline (Fluka, St. Louis, MO), supplemented with 10 ng/ml TGF-β1 (R&D System, Minneapolis, MN). For osteogenesis and adipogenesis, hMSCs were trypsinized and replated in cell culture flasks at the density of 5,000 or 10,000 cells/cm², respectively. The cells were induced in osteogenic medium composed of low-glucose DMEM, 10% FBS, 50 µg/ml l-ascorbic acid-2-phosphate, 10⁻² M dexamethasone, and 10 mM β-glycerophosphate (Sigma–Aldrich), or in adipogenic medium composed of high-glucose DMEM, 10% FBS, 10⁻⁶ M dexamethasone, and 1 µg/ml insulin (Sigma–Aldrich). Culture medium was replaced every 3 days.

Flow cytometric analysis

Human MSCs were trypsinized and resuspended in ice-cold PBS containing 1% bovine serum albumin and 0.1% sodium azide (Sigma–Aldrich). The cells were incubated with antibody against cell surface antigens CD73, CD90, CD105, CD34, and CD45 (BD Biosciences), fixed with 1% paraformaldehyde solution, and then analyzed by flow cytometry (BD Biosciences). Data were analyzed using the FlowJo software (TreeStar Inc., Ashland, OR).

Cell proliferation analysis

Human MSCs during expansion culture were harvested and digested with Proteinase K (Sigma–Aldrich) to extract DNA. Cell proliferation was determined by measuring the total amount of DNA using the PicoGreen Assay (Invitrogen).

RNA extraction and mRNA expression analysis

For quantitative reverse transcription polymerase chain reaction (RT–PCR) analysis, total RNA was isolated using the RNeasy Mini Kit (Qiagen, Hilden, Germany). First-strand cDNA was reverse-transcribed using the SuperScript III First-Strand Synthesis System Kit (Invitrogen). Quantitative PCR analysis was performed using the iQ SYBR Green Supermix (BioRad, Hercules, CA) with the primers listed in Table I. The relative expression level of each target mRNA was determined by referencing to the internal control glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using the 2⁻ΔΔCT method.

Histological analysis and glycosaminoglycan (GAG) quantification

After 21 days of chondrogenic induction, cell pellets were fixed in 4% paraformaldehyde solution, dehydrated, infiltrated with xylene, and embedded in paraffin. Eight-µm sections were cut using a microtome, and then deparaffinized, rehydrated, and stained using hematoxylin & eosin (H&E) and Alcian blue (Polysciences, Warrington, PA).

Cell pellets were collected after 21 days of chondrogenic induction and papain-digested at 60°C. Total sulfated GAG was then
quantified by the 1,9-dimethylmethylene blue-based Blyscan GAG Assay Kit (Biocolor, Carrickfergus, United Kingdom). The result was normalized with the total amount of DNA determined separately by the PicoGreen Assay.

Protein extraction and western blot analysis

Cell lysates were prepared using RIPA buffer composed of 50 mM Tris—HCl (pH 7.5), 1% Nonidet P-40, 0.25% Na-deoxycholate, 150 mM NaCl, 1 mM EDTA, and complete protease inhibitor cocktail (Roche, Indianapolis, IN). After being centrifuged, the supernatant was collected and the protein concentration was determined using the BCA Protein Assay Kit (Pierce, Rockford, IL). A 50 μg protein sample was loaded into each lane of a 10% polyacrylamide gel for electrophoresis, and the separated proteins on the gel were then blotted onto a polyvinylidene fluoride membrane. Primary antibodies for PKC pan-isofrom (Pierce), phospho-PKC pan-isofrom, type I TGF-β receptor, type II TGF-β receptor, Smad3, phospho-Smad3, and GAPDH (Cell Signaling, Danvers, MA) were used to detect the target proteins. The immuno-detected membrane was labeled with the secondary horseradish peroxidase-conjugated antibody (Cell Signaling), visualized using the SuperSignal West Pico Chemiluminescent Substrate (Pierce), and documented by a digital image system (Kodak, Rochester, NY). The signal intensity of each target protein expressed in gels was quantified using the 1,9-dimethylmethylene blue-based Blyscan GAG Assay Kit (Biocolor, Carrickfergus, United Kingdom). The result was normalized with the total amount of DNA determined separately by the PicoGreen Assay.

PKC activity regulation

PKC activity was suppressed using the PKC inhibitor, Gö6983 (Sigma–Aldrich). Specifically, hMSCs were cultured in high-glucose culture medium with or without the addition of 70 nM of Gö6983 and analyzed for the effect of PKC activity on the regulation of TGF[RII expression.

 soluble TGF-β1 quantification

Culture medium was collected 15 min, 30 min, 1 h, 2 h and 4 h after fresh medium change from hMSC expansion culture. TGF-β1 in the collected culture medium was quantified using the human-specific TGF-β1 enzyme-linked immunosorbent assay (ELISA) Kit (R&D Systems).
suggesting that both TGFβRII and PKC are similarly affected by glucose concentration.

Glucose concentration surge is not a factor causing the different regulation of PKC activity and TGF-β receptor between HGMC and LGMC pellets

We tested whether the different PKC activity and TGFβRII expression in HGMC and LGMC pellets was associated with a glucose concentration surge from expansion culture to chondrogenic culture. To compare with the experiment using the gold standard protocol illustrated in Fig. 1(A), we set up another experiment in which we induced chondrogenesis of HGMC pellets in high-glucose medium, and chondrogenesis of LGMC pellets in low-glucose medium [Fig. 2(C)]. The results showed that the phosphorylation of PKC and TGFβRII expression level of HGMC pellets were decreased compared to those of LGMC pellets [Fig. 2(D)], which is similar to what is shown in Fig. 2(A and B). This suggests that a glucose concentration surge is not involved in regulating PKC activity and TGFβRII expression between HGMCs and LGMCs during chondrogenesis.

Glucose concentration modulates PKC activity and TGFβRII expression of pre-differentiation hMSCs, and changes their responsiveness to chondrogenic induction

To investigate whether the effect of glucose on the regulation of TGFβRII and PKC activity occurred prior to the initiation of chondrogenesis, we analyzed HGMCs and LGMCs harvested from expansion culture and chondrogenic pellets. Interestingly, the expression levels of TGFβRII in both the cells from expansion culture were lower than those from chondrogenic pellets [Fig. 3(A)], suggesting that the process of chondrogenic induction increases TGFβRII of both HGMCs and LGMCs but with a greater extent in LGMCs. This result indicates that HGMCs are less responsive to chondrogenic induction in the regulation of TGFβRII. Together with the result shown in Fig. 2(A), this suggests that glucose concentration in expansion culture can regulate hMSCs to alter their responsiveness to chondrogenic induction through the modulation of TGFβRII, thus affecting the activation of TGF-β signaling upon induction.

We further analyzed total and phosphorylated PKC in HGMCs and LGMCs to determine the PKC activity of pre-differentiation hMSCs. The result showed that PKC of HGMCs was increasingly phosphorylated compared to that of LGMCs [Fig. 3(B)], which was different from the PKC activity of cell pellets during chondrogenesis shown in Fig. 2(B). These results suggest that the glucose concentration in pre-differentiation culture medium modulates the responsiveness of hMSCs to chondrogenic induction. To test whether PKC is an upstream molecule regulating the expression of TGFβRII, we treated HGMCs in expansion culture with the PKC inhibitor, Gö6983, and analyzed the expression of TGFβRII. The results showed that TGFβRII expression of HGMCs treated with the PKC inhibitor was downregulated compared to that of the cells without PKC inhibition [Fig. 3(C)], suggesting that glucose concentration in pre-differentiation culture modulates the expression of TGFβRII through the regulation of PKC activity.
We also measured the amount of active TGF-β1 ligands released in expansion culture medium to determine whether glucose concentrations affect the amounts of endogenous TGF-β1 production. ELISA results showed that the concentrations of active TGF-β1 ligands in both HGMC- and LGMC-conditioned media collected 15 or 30 min after fresh medium change were lower than 5 pg/ml [Fig. 3(D)], and those collected 1, 2, or 4 h after medium change were undetectable, suggesting that glucose concentration does not affect the production of endogenous TGF-β1 ligands. Moreover, our study also showed that without the induction of TGF-β1, the mRNA expression levels of cartilage-related markers of HGMC and LGMC pellets were comparable during chondrogenesis (Fig. S2). These
results collectively suggest that endogenously produced TGF-β1 ligands are not involved in the regulation of glucose concentration in expansion culture on hMSC chondrogenesis.

PKC inhibition in expansion culture upregulates the chondrogenic capacity of hMSCs

We further investigated whether the modulation of PKC activity of hMSCs using a PKC inhibitor during expansion culture regulates subsequent chondrogenesis of cell pellets. HGMCs treated with or without the PKC inhibitor for two passages expressed similar spindle cell morphology and size [Fig. 4(A)]. No significant difference in cell proliferation was found between the two culture groups [Fig. 4(B)], suggesting that PKC inhibition does not affect cell morphology and proliferation. On the other hand, 12 h after chondrogenic induction, cell pellets consisting of HGMCs previously treated with the PKC inhibitor in expansion culture showed increased PKC phosphorylation and TGFβRII expression compared to those consisting of HGMCs without PKC inhibition [Fig. 4(C)], suggesting that inhibition of PKC activity in HGMCs during pre-differentiation culture can increase PKC activity and TGFβRII expression of cell pellets during chondrogenesis. After 14 days of chondrogenesis, cell pellets consisting of HGMCs previously treated with the PKC inhibitor in expansion culture expressed significantly higher levels of cartilage-related markers, aggrecan and collagens type II and IX but a comparable level of Sox5, compared to cell pellets consisting of HGMCs without PKC inhibition [Fig. 4(D)]. This demonstrates that chondrogenesis of HGMC pellets can be enhanced by inhibiting the PKC activity of cells in expansion culture.

High-glucose culture upregulates the adipogenic capacity of hMSCs through PKC activity

To investigate whether high-glucose culture promotes hMSCs to differentiate into other cell lineages, we induced HGMCs and LGMCs for osteogenesis and adipogenesis. After 21 days of osteogenic induction, the results showed that the mRNA expression levels of bone-related markers, Cbfα1, alkaline phosphatase, and osteocalcin, of HGMCs and LGMCs were comparable [Fig. 5(A)]. On the other hand, mRNA levels of adipose-related markers, peroxisome proliferator-activated receptor gamma 2 and lipoprotein lipase (LPL), of HGMCs were significantly higher than those of LGMCs after 21 days of adipogenic induction [Fig. 5(B)], suggesting that high-glucose expansion culture increases the adipogenic capacity of hMSCs. Moreover, we also found that the mRNA expression level of LPL of HGMCs treated with the PKC inhibitor during pre-differentiation culture was significantly downregulated during adipogenesis, compared to those without PKC inhibition [Fig. 5(C)], suggesting that PKC activity is associated with an upregulated adipogenic capacity of HGMCs.

Discussion

Our study demonstrates that hMSCs expanded in high-glucose culture prior to differentiation show decreased chondrogenesis. We also demonstrate that while chondrogenic induction upregulates the expression of TGFβRII of hMSCs, high-glucose expansion culture reduces the responsiveness of hMSCs to chondrogenic induction, resulting in less TGFβRII on HGMCs than LGMCs and thereby decreasing the activation of downstream signaling molecules during chondrogenesis. Based on our findings, we propose a molecular mechanism that illustrates how glucose concentration in expansion culture regulates hMSCs to modulate PKC and TGFβRII prior to and during chondrogenesis (Fig. 6).

The significance of our findings is that glucose concentration in expansion culture is capable of modulating the chondrogenic capacity of hMSCs through regulation of PKC and TGFβRII before chondrogenesis. Previous studies focused on studying the effect of glucose concentration during the process of chondrogenesis. For example, Han et al. demonstrated that high-glucose chondrogenic culture promotes chondrogenesis of chick mesenchymal cells13, and Mobasheri et al. found that high-glucose chondrogenic culture
is essential for maintaining matrix structural integrity. Our study takes one step further to examine the effect of glucose concentration on MSCs in pre-differentiation culture and shows that glucose concentration of the expansion medium has a remarkable influence on cell behavior and subsequent chondrogenesis. This finding suggests that glucose concentration plays a critical role in priming hMSCs during pre-differentiation culture for chondrogenesis upon receiving induction signals. A previous study by Cramer et al. has shown that chondrogenesis of hMSCs maintained in high-glucose medium and subsequently induced in low-glucose culture is downregulated compared to that of hMSCs maintained and induced in low-glucose medium, which is consistent with our findings. Moreover, we identify the regulatory mechanism involved in the glucose concentration-mediated modulation. Increasing clinical research evidence has revealed that joint degeneration diseases, such as osteoarthritis, may be associated with hyperglycemia. Our finding that high-glucose expansion culture decreases the chondrogenic capacity of hMSCs provides an in vitro model to elucidate the mechanism of how hyperglycemia affects hMSC chondrogenesis and the production of cartilaginous matrix, thus leading to disrupted cartilage homeostasis observed in osteoarthritis.

The extent of cell signaling stimulated by growth factors is associated with the amount of available ligand receptors. We demonstrate that the amount of TGF-β receptors on hMSCs is modulated by glucose concentration prior to chondrogenesis, thus affecting the activation extent of TGF-β signaling molecules upon induction. Our results also show that the modulation of TGF-β receptor expression in expansion culture is not regulated by endogenously produced TGF-β1 ligands. Though we cannot rule out the possibility that glucose stimulates hMSCs to produce endogenous TGF-β1 ligands that bind to extracellular matrix after being released, or to produce other TGF-β isoforms, it is evident that soluble endogenous TGF-β1 ligands are not involved in the regulation of TGF-β signaling.

We show that high-glucose culture increases the activity of PKC and the amount of TGFβRII in hMSCs, consistent with previous studies using different cell types. We also identify the mechanism by which glucose concentration regulates TGFβRII of hMSCs through the modulation of PKC activity to affect chondrogenesis. Similarly, Ryu et al. have reported that high-glucose culture regulates the TGF-β1 expression of hMSCs through the modulation of

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**Fig. 5.** Osteogenesis and adipogenesis of HGMCs and LGMCs, and adipogenesis of HGMCs with PKC inhibition. A. The mRNA expression levels of Cbfa1, alkaline phosphatase (ALP), and osteocalcin (OC) of HGMCs and LGMCs after 21 days of osteogenic induction. B. The mRNA expression levels of peroxisome proliferator-activated receptor gamma 2 (PPARγ2) and lipoprotein lipase (LPL) of HGMCs and LGMCs after 21 days of adipogenic induction. C. The mRNA expression levels of PPARγ2 and LPL of HGMCs and PKC inhibitor-treated HGMCs (HGMC + I) after 14 days of adipogenic induction. The mRNA transcript expression of bone- and adipose-related markers was determined using quantitative RT-PCR and referenced to that of the control GAPDH. Data are presented as the fold change relative to the group of HGMCs. The mean was calculated based on three replicate samples (n = 3) for each group.

**Fig. 6.** Diagram of the working model illustrating the effect of high-glucose pre-differentiation culture on the regulation of PKC, TGFβRII, and TGF-β signaling molecules of hMSC pellets during chondrogenesis. During ex vivo chondrogenesis in pellet culture, hMSCs previously cultured with high-glucose medium in expansion culture are less responsive to chondrogenic induction. The high-glucose exposure decreases PKC activity, thus downregulating the expression of TGFβRII in cell pellets. The reduced TGFβRII expression results in decreased TGF-β signaling upon the activation of TGF-β ligand, further leading to reduced chondrogenesis.
PKC and several other signaling molecules. It is known that high-glucose culture has a global impact on cellular machinery. Thus, it is possible that other mechanisms besides the PKC/TGF-β signaling pathways are also involved in the chondrogenic regulation by high-glucose medium. Nonetheless, since the TGF-β signaling pathways is the dominant mechanism for chondrogenesis, and PKC activity is closely associated with glucose concentration, this study was designed to identify the role of the PKC/TGF-β signaling pathways regulated by glucose concentration in the modulation of hMSC chondrogenesis.

One of our interesting findings is that chondrogenic induction upregulates the expression of TGFβRII of both HGMCs and LGMCs. During chondrogenic induction, hMSCs are made into cell pellets and induced in serum-free medium supplemented with TGF-β1. Previous reports suggest that the expression of growth factor receptors is differentially regulated in two-dimensional (2D) and three-dimensional (3D) culture. For example, activation of epidermal growth factor receptor II of human breast cancer cells was greater in 3D culture than in 2D culture, and the amount of TGFβRII in microvascular endothelial cells is significantly lower in 3D than 2D culture. A previous study demonstrates that TGF-β1 induction or serum deprivation upregulates the expression of TGF-β receptor, suggesting the chemistry of chondrogenic medium also contributes to the regulation of TGFβRII. However, the details of how these factors work together to upregulate the expression of TGFβRII in hMSCs are not clear, and should be the focus of further investigation.

Our results demonstrate that while decreasing the chondrogenic capacity of hMSCs, high-glucose expansion culture increases the adipogenic capacity of the cells. Other research groups have shown similar results using adipose tissue- or muscle-derived stem cells, suggesting that high-glucose culture drives hMSCs toward the adipogenic lineage. Our results also show that suppressing the PKC activity of HGMCs can reduce the upregulated adipogenesis and increase the downregulated chondrogenesis, suggesting that while losing the adipogenic potential, these cells regain their chondrogenic potential. These findings together suggest that the PKC activity of pre-differentiation hMSCs plays a critical role in mediating other cellular activities associated with the commitment of cell fates. Thus, it seems beneficial to reduce PKC activity of hMSCs in expansion culture before differentiation to enhance subsequent chondrogenesis in cell pellets. Based on our findings, we propose that treating hMSCs with a PKC inhibitor in expansion culture may be a practical approach to prime hMSCs for enhancing chondrogenesis.

In summary, we demonstrate that the chondrogenic capacity of pre-differentiation hMSCs is modulated by glucose concentration of medium in expansion culture through the regulation of PKC activity and TGF-β receptor expression. Moreover, chondrogenesis induced in subsequent pellet culture can be enhanced by modulating PKC activity of hMSCs before differentiation. Our findings uncover the importance of maintaining low-glucose concentration in both in vitro and in vivo environments to support chondrogenesis of hMSCs, which can potentially benefit the research of cartilage tissue engineering and cartilage diseases, such as osteoarthritis induced by hyperglycemic disorders and offer a viable option to enhance hMSC chondrogenesis using a pharmacological approach for cartilage regeneration applications.

Author contributions

T-L. Tsai participated in the study design, carried out cell culture, data collection, analysis and interpretation, and drafted the manuscript. PA Manner harvested bone marrow, and participated in data interpretation, and drafting the manuscript. W-J. Li designed the experiment, coordinated the study, interpreted data, and drafted the manuscript.

Conflict of interest

None to declare.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.joca.2012.11.001.

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