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PTHrP promotes chondrogenesis and suppresses hypertrophy from both bone marrow-derived and adipose tissue-derived MSCs

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ABSTRACT

During chondrogenesis from mesenchymal stem cells (MSCs), inadequate differentiation and hypertrophic differentiation are two important limitations. The purpose of this study was to test the hypothesis that chondrogenesis is enhanced and unwanted hypertrophic changes are suppressed by treating bone marrow-derived (BMMSCs) and adipose tissue-derived mesenchymal stem cells (ATMSCs) with parathyroid hormone-related peptide (PTHrP). To induce chondrogenesis, in vitro pellet cultures were carried out using 2.5×10^5 MSCs at passage 3 in chondrogenic medium containing 5 ng/ml of TGF-β_2 for BMMSCs, and 5 ng/ml of TGF-β_2 and 100 ng/ml of BMP-7 for ATMSCs. From the 14th day of culture, subsets of pellets were treated with PTHrP [0, 10, 100 ng/ml], and after two more weeks of in vitro culture, pellets were harvested for analysis. The addition of PTHrP dose-dependently increased DNA contents in both BMMSCs and ATMSCs. GAG contents also increased after PTHrP treatment. The gene expression of COL1A1 decreased by three-fourths, while the decrease was not evident in ATMSCs after PTHrP treatment. SOX-9 mRNA increased up to four fold in both BMMSCs and ATMSCs, and COL2A1 gene expression sharply increased to sevenfold in BMMSCs and to 4 fold in ATMSCs. COL10A1 gene expression decreased by a third in both cell types, and Runx-2 expression dropped sharply in both cell types after PTHrP treatment. Safranin-O and immunohistochemistry for type I, II, X collagen and Runx-2 generally paralleled qRT-PCR findings with minor variations. In conclusion, PTHrP was found to promote chondrogenesis and suppress hypertrophy during in vitro chondrogenesis from both BMMSCs and ATMSCs, which supports its use for cartilage tissue engineering.

Mesenchymal stem cells (MSCs) from adults are capable of self-regeneration and differentiation to several connective tissue cell types [1,2]. Thus, they are considered to be an attractive cell source for regenerative medicine [3]. As articular cartilage (AC) has limited potential for self-repair, AC injuries rarely heal spontaneously and usually lead to osteoarthritic changes [4]. Hence, tremendous effort has been dedicated to devise methods whereby functional AC would be regenerated [4]. MSCs have also been extensively investigated regarding their potential for cartilage repair [3,5]. Although bone marrow provides the most universal source of MSCs, other tissues, such as periosteum, muscle, synovial membrane, and adipose tissue also possesses MSCs. Of these tissues, adipose tissue has attracted most attention on account of its easy accessibility and abundance. Adipose tissue-derived mesenchymal stem cells (ATMSCs) have also been demonstrated to have multilineage potential [6].

Two important limitations are evident during chondrogenesis from MSCs, i.e., inadequate differentiation and hypertrophy [7,8]. Although MSCs are known to differentiate into any lineage of musculoskeletal tissue, individual cells have different clonal capacities in terms of becoming chondrocytes [9,10]. Chondrogenic growth factors promote chondrogenesis, but do not eradicate these undesired events [7]. In particular, hypertrophy poses a great challenge, because they represent the normal fate of chondrocytes during endochondral ossification in the developmental process [8,11]. Only chondrocytes of articular cartilage are absolved from these changes [11]. It is important to suppress hypertrophic changes because the purpose of cartilage tissue engineering is to produce neocartilage with the characteristics of hyaline articular cartilage.

The maturation and hypertrophic changes of growing cartilage are affected by the PTHrP-Indian hedgehog (IHH) axis [11,12]. PTHrP was first identified as a factor involved in humoral hypocalcaemia of malignancy [13]. PTHrP inhibits chondrocyte maturation, and plays a key role in regulating the rate of differentiation of chondrocytes in growth plates. PTHrP acts to maintain chondrogenic phenotypes and suppresses the expression of IHH. IHH promotes the proliferation and maturation of chondrocytes, which lead to hypertrophic changes [11,14]. Considering that many molecular events which occur during embryogenesis are recapitulated in the chondrogenesis from MSCs in

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three dimensional in vitro cultures, these molecules might enhance chondrogenesis and inhibit hypertrophic changes in engineered cartilage.

Thus, the purpose of this study was to test the hypothesis that chondrogenesis is enhanced and that unwanted hypertrophic changes are suppressed by PTHrP in bone-marrow-derived and adipose tissue-derived MSCs.

Materials and methods

Procurement of samples, cell isolation, and cultivation. The bone marrow samples used to isolate mesenchymal stem cells (BMMSCs) were obtained from three patients (mean age: 50 years, range: 37–64 years) undergoing total hip replacement due to osteoarthritis. The adipose tissue-derived mesenchymal stem cells (ATMSCs) were isolated from lipoaspirates generated during elective liposuction procedures on three patients (mean age: 37 years, range: 30–42 years). This study was approved by the institutional review board, and informed consent was obtained from all individuals included in the study. BMMSCs and ATMSCs were isolated from fresh bone marrow samples and lipoaspirates, and then expanded as described previously [7,15,16].

Induction of in vitro chondrogenesis. To induce chondrogenesis, in vitro pellet cultures were carried out using 2.5×10^5 MSCs at passage 3 in DMEM/F-12 supplemented with 1% ITS, 10^{-7} M dexamethasone, 50 μM ascorbate-2-phosphate, 50 μM L-proline, and 1 mM sodium pyruvate; 5 ng/ml of TGF-β_2 was also added for BMMSCs, and 5 ng/ml of TGF-β_2 plus 100 ng/ml of BMP-7 for ATMSCs. The starting concentrations used for the growth factors, i.e., 5 ng/ml of TGF-β_2 and 100 ng/ml for BMP-7, were on our previous findings, both published and unpublished [15,16]. From the 14th day of culture, subsets of pellets were additionally treated with PTHrP [0, 10 ng/ml, 100 ng/ml] (Sigma–Aldrich, St. Louis, MO), and after two further weeks of in vitro culture in their respective media, pellets were harvested for analysis. For pellet cultures, one milliliter of the cell suspension was aliquoted into 15 ml polypropylene centrifuge tubes, and spun in a benchtop centrifuge at 500*g* for 5 min. Tubes were incubated in 5% CO_2 atmosphere for up to 4 weeks. Caps of tubes were loosened in order to allow air exchange. The medium was changed every third day.

DNA quantification. Cell pellets were digested overnight in papain buffer at 60 °C. DNA contents were determined using the DNA binding fluorochrome Hoechst 33258. Briefly, a 10 μl sample of each lysate was transferred to a 96-well polystyrene plate, to which 90 μl of TNE buffer (10 mM Tris, 2 M NaCl, at pH 7.4 and 1 mM EDTA) was added. Next, 100 μl of Hoechst 33258 (Sigma–Aldrich) solution (1 mg/ml) was added to a concentration of 20 μg/ml in TNE buffer per well in a 96-well polystyrene plate. Plates were then read using a Fluostar optima fluorescence plate reader (BMG Labtech, Offenburg, Germany) at 460 nm (emission) and 355 nm (excitation). DNA contents were derived using a standard curve of serial dilutions of calf thymus DNA.

Analysis of glycosaminoglycan (GAG) contents. Pellets were digested in papain buffer at 60 °C overnight, and then transferred to 1.5 ml microcentrifuge tubes. For 1,9-dimethylmethylene blue (DMMB) assays, 50 μl of each sample was added to a total volume of 100 μl with appropriate buffer. GAG production was determined using a Blyscan kit (Biocolor, Carrickfergus, Northern Ireland). This assay is based on the specific binding of the cationic dye 1, 9-DMMB to the sulfated GAG (s-GAG) chains of proteoglycans and protein-free s-GAG chains. The procedure was carried out according to the manufacturer's instructions. Briefly, standard solutions (0.1, 0.2, 0.3, 0.5, 1.0, 2.0, 3.0, and 5.0 μg of chondroitin-4-sulphate in 100 μl) and test samples (100 μl) were mixed with 1 ml of Blyscan dye reagent for 30 min at room temperature. S-GAG–dye complex was recovered by centrifugation and pellets were washed and resuspended in 1 ml of dissociation buffer. Absorbances were measured at 570 nm in a Spectra max plus 384 (Molecular Devices, Sunnyvale, CA). GAG contents were expressed in micrograms of GAG per microgram of DNA.

Reverse transcription and real-time PCR analysis. RNA was isolated using the standard guanidine isothiocyanate Tri-Reagent® (Sigma–Aldrich). Isolated RNA samples were converted to cDNA using reverse transcriptase (SuperScript III®; Invitrogen, Carlsbad, CA) and oligo (dT) primers. All PCR reactions were performed on the LightCycler 480 system® (Roche Diagnostics, Mannheim, Germany) in standard 20 μl reactions volumes containing 5 μl cDNA, 0.5 μl of 100 mM sense, and 0.5 μl of 100 mM antisense primer, 10 μl LightCycler 480 SYBR Green I Master mix (Roche), and 4 μl RNAse free water. The expressions of the following genes were examined: collagen type I (COL1A1), collagen type II (COL2A1), collagen type X (COL10A1), SOX-9, and Runx-2; glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a housekeeping gene. The primers and reaction conditions used for amplification are listed in Table 1. After polymerase activation (95 °C for 10 min), 45 amplification cycles were run (10 s denaturation at 95 °C, 10 s annealing at 65 °C and 10 s extension at 72 °C). Melt-curve analysis was performed immediately after the amplification protocol using the following conditions: 5 s at 95 °C (holding time on reaching temperature), 1 min at 65 °C, and 1 s at 97 °C. The temperature change rate used was 20 °C/s (except for the final step, during which the temperature change rate was 0.1 °C/s). The peak melting temperatures obtained were considered to be those of the specific amplified products. In order to guarantee the reliability of the results obtained, all samples were processed in triplicate. Each assay was performed using positive and negative controls. The threshold cycle (C_t) value of each gene was measured for each RT sample. The C_t value of GAPDH

Table 1
Primers used for real-time PCR

Gene	Sequence	Accession No.	Amplicon (bp)	Reference
Collagen type I	5′-CCGCCGCTTCACCTACAGC-3′ 5′-TTTTGTATTCAATCACTGTCTTGCC-3′	NM_000088	83	[26]
Collagen type II	5′-CCGAATAGCAGGTTCACGTACA-3′ 5′-CGATAACAGTCTTGCCCCACTT-3′	NM_001844	79	[26]
SOX-9	5′-CACACAGCTCACTCGACCTTG-3′ 5′-TTCGGTTATTTTTAGGATCATCTCG-3′	Z46629	76	[26]
Collagen type X	5′-AAAGGCCCACTACCCAACAC-3′ 5′-CTTCCGTAGCCTGGTTTTCC-3′	NM_000493	182	
Runx-2	5′-CCCAGCCACCTTTACCTACA-3′ 5′-TATGGAGTGCTGCTGGTCTG-3′	NM_001024630	128	
GAPDH	5′-ATGGGGAAGGTGAAGGTCG-3′ 5′-TAAAAGCAGCCCTGGTGACC-3′	NM_002046	70	[26]

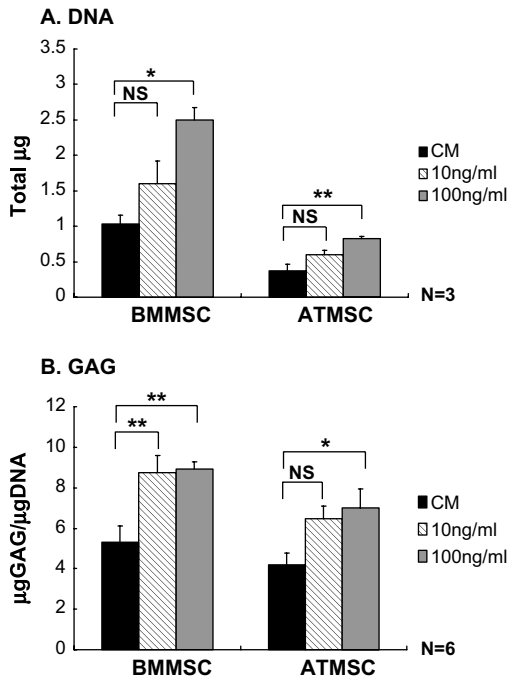

Fig. 1. Amount of DNA measured in cultured pellets after 4 weeks of culture (A). The bars represent means ± SEM ($n = 3$: $^{*}p < 0.05$, $^{**}p < 0.01$, NS: not significant). CM: MSCs treated with their respective chondrogenic medium. GAG normalized DNA amounts in pellets after 4 weeks of culture (B). The bars represent means ± SEM ($n = 3$: $^{*}p < 0.05$, $^{**}p < 0.01$, NS: not significant). CM: MSCs treated with their respective chondrogenic medium.

was used as an endogenous reference for normalization purposes (User bulletin #2 Applied Biosystems, Roche Molecular System, Alameda, CA). The values thus obtained were normalized versus the negative control, and are expressed as fold changes.

Histological analysis. After 4 weeks of culture, pellets were fixed in 4% paraformaldehyde solution for 3 h, dehydrated with 100% ethanol, washed with xylene, and embedded in paraffin. Four micrometer thick sections were cut from paraffin blocks and coated on glass slides. Safranin-O staining for proteoglycan and immunohistochemistry for collagen types I, II, X, and Runx-2 were then performed. For Safranin-O staining, sections were deparaffinized with xylene and ethanol, aqueous Safranin-O (0.1%) was applied for 30 min, and then sections were washed with distilled water. For immunohistochemistry, we used the Dakocytomation LSAB2 System HRP kits® (DAKO, Hamburg, Germany). Tissue sections were deparaffinized, treated with Pepsin Soluble® (Fine Life Science, Seoul) for 15 min and washed in a 1× wash buffer (DAKO). Sections were incubated for 5 min with a peroxidase-blocking solution, and reacted with the appropriate primary antibodies in goat serum overnight at 4 °C [mouse anti-chicken collagen type II monoclonal antibody (200 μg/ml: Chemicon, Temecula, CA, USA) diluted at 1:100, rabbit anti-human collagen type I antibody (50 μg/ml: AbD Serotec, Oxford, UK) diluted at 1:500, mouse monoclonal anti-human collagen type X antibody (1.0 mg/ml: Sigma–Aldich) diluted at 1:1000, mouse monoclonal anti-human antibody (1.0 mg/ml: Abcam, Cambridge, UK) diluted at 1:500]. After three washes in 1× wash buffer, sections were incubated with horseradish peroxidase (HRP)-labeled anti-mouse goat secondary antibodies (DAKO) for 30 min, extensively washed, reacted with substrate

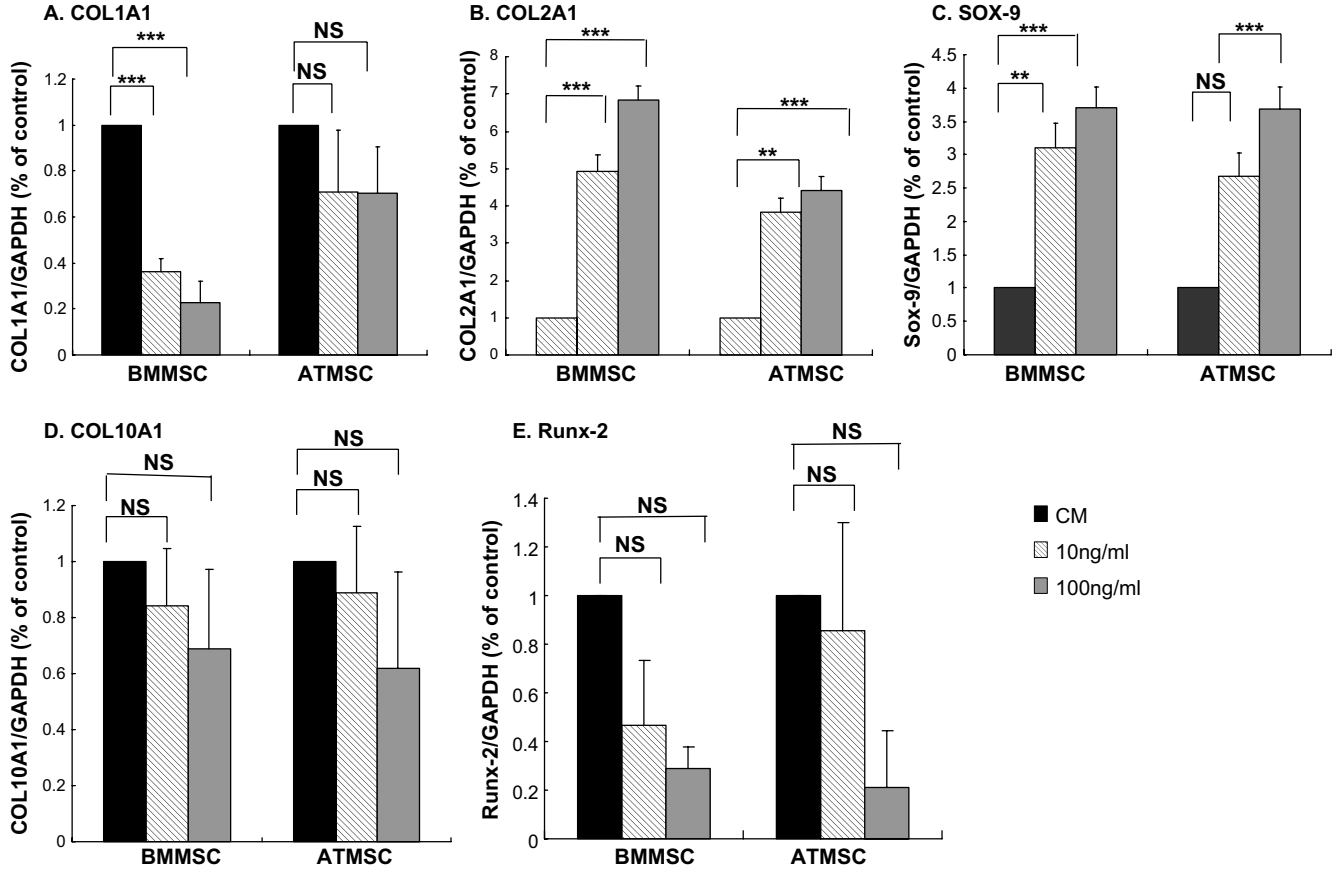

Fig. 2. Reverse transcription and real-time PCR of the cells after 4 weeks of culture. The mRNA expressions of COL1A1 (A), COL2A1 (B), SOX-9 (C), COL10A1 (D), and Runx-2 (E) were measured and normalized versus GAPDH. Values are expressed as fold of the control (CM: MSCs treated with their respective chondrogenic medium).The bars represent means ± SEM ($n = 3$: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, NS: not significant).

buffer and DAB (diaminobenzidine) chromogen (DAKO, 50:1) for 10 min, and then mounted.

Results

Assessments of cell number based on DNA contents, and of proteoglycan production by GAG analysis

After 2 weeks of in vitro culture in chondrogenic medium (CM) and an additional 2 weeks under CM plus PTHrP, BMMSCs and ATMSCs were harvested and analyzed. The addition of PTHrP at 10 ng/ml increased DNA contents by more than 50% and at 100 ng/ml by 150% in BMMSCs versus the PTHrP untreated controls ($p = 0.012$). ATMSCs had much lower DNA contents than BMMSCS. Nevertheless, they increased by 62% after treatment with 10 ng/ml PTHrP, and by 124% with 100 ng/ml PTHrP ($p = 0.019$). Cell number was observed to be dose-dependently increased by PTHrP in both BMMCSs and ATMSCs (Fig. 1A). GAG contents increased by 74% after adding 10 ng/ml PTHrP ($p = 0.021$), and by 78% after adding 100 ng/ml ($p = 0.027$). ATMSCs demonstrated considerably lower GAG contents than BMMSCs, but they still increased by 50% after treatment with 10 ng/ml PTHrP, and by 61% with 100 ng/ml PTHrP (Fig. 1B).

Gene expressions of COL1A1, SOX-9, COL2A1, COL10A1, Runx-2 by qRT-PCR

In BMMSCs, the expression of COL1A1 decreased by two-thirds ($p = 0.001$) and three-fourths ($p = 0.0001$) after treatment with 10 ng/ml or 100 ml PTHrP, respectively. This decrease was not evident in ATMSCs. In BMMSCs, SOX-9, the master gene of chondrogenesis [17], mRNA increased from 1 to 3.1 ($p = 0.005$) and 3.7-fold ($p = 0.001$) versus the untreated control after treatment with 10 ng/ml or 100 ml PTHrP, respectively, and corresponding levels were 2.7- and 3.7-fold ($p = 0.001$) greater than the control in ATMSCs. The expression of COL2A1 dramatically increased to five ($p = 0.0001$ with 10 ng/ml) and sevenfold ($p = 0.0001$ with 100 ng/ml) on increasing the PTHrP dose, but stayed at around fourfold in ATMSCs ($p = 0.002$ with 1 ng/ml, $p = 0.001$ with 100 ng/ml). COL10A1, the marker of hypertrophic chondrocytes [18], gradually decreased on increasing PTHrP dose, by a third in both cell types. Runx-2, the master transcription factor for osteogenesis and a marker of hypertrophic chondrocytes [19], dropped more sharply, by three fourths in BMMSCs and by four fifths in ATMSCs.

In summary, PTHrP was more effective at enhancing chondrogenesis in BMMSCs than in ATMSCs. The 1 ng/ml dose was found to be almost as effective as 10 ng/ml, except for COL2A1 in BMMSCs and SOX-9 in ATMSCs, which showed clear dose–response relationships (Fig. 2).

Histological findings

Histological findings of Safranin-O and immunohistochemistry for type I, II, and X collagens and Runx-2 generally mirrored changes detected by GAG assays and qRT-PCR with minor variations. Safranin-O staining showed an increase of metachromatic staining after PTHrP treatment in both BMMSC and in ATMSCs. Type II collagen expression markedly increased in both BMMSC and in ATMSCs while type I collagen staining decreased in both cell types. Type X collagen expression moderately decreased after PTHrP treatment in both cell types. Runx-2 protein expression decreased dramatically after PTHrP treatment in BMMSCs. Untreated Runx-2 had low expression in ATMSCs, which further decreased after PTHrP (Fig. 3).

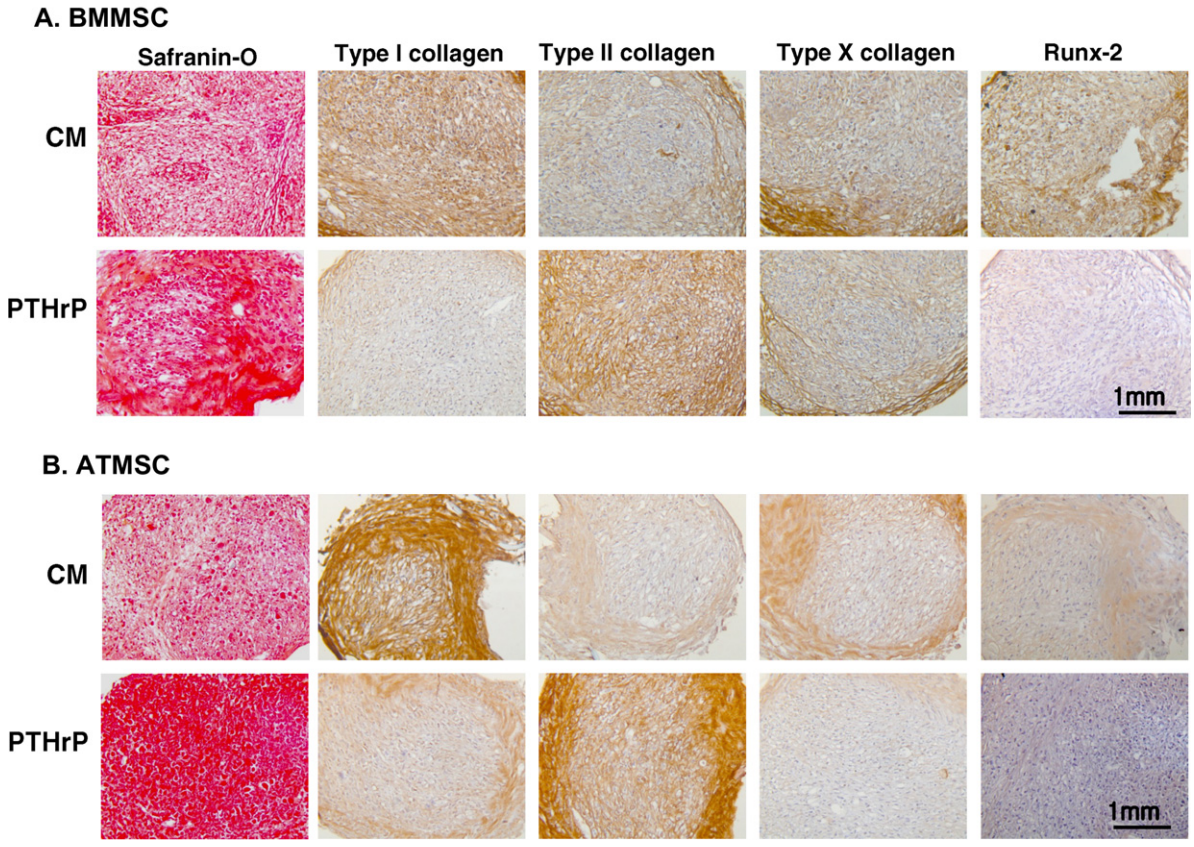

Fig. 3. Safranin-O staining (200×), immunohistochemistry (200×) for type I collagen , type II collagen, type X collagen, and Runx-2 of cells cultured in pellets of BMMSCs (A) and ATMSCs with (B). CM: MSCs treated with their respective chondrogenic medium.

Discussion

Cartilage tissue engineering offers a promising means of producing 3-dimensional neocartilages [3,20]. MSCs are an attractive option because of their abilities to proliferate and accessibilities [1,2]. However, the induction of chondrogenesis in MSCs is a challenging task. The qualities of neocartilage engineered from MSCs do not surpass those from chondrocytes [5]. Moreover, engineered cartilages undergo hypertrophic changes, which eventually lead to apoptosis or ossification [8]. However, the findings from the present study suggest a way of possibly circumventing these problems: PTHrP was found to promote chondrogenesis and to suppress hypertrophy, as evidenced by GAG analysis, gene expression studies, and histological findings.

The signaling mechanism by which PTHrP induces cellular phenotypes has been widely investigated. PTHrP signals through a G-protein-coupled receptor that activates proteins A and C [21–23]. Moreover, it has been shown that the phenotypic effects of PTHrP in chondrocytes are primarily facilitated downstream of protein kinase A [24]. PTHrP treatment increases cellular proliferation by suppressing the expression of the p57 gene, and this causes cell growth arrest in chondrocytes [25]. At the doses used in the present study, PTHrP increased the cell number of BMMSCs and ATMSCs. In the present study, levels of COL2A1 and SOX-9 mRNA were dose-dependently increased by PTHrP. Our results are different from those of Kafiensch et al., who found that type II collagen expression was unchanged and type I and X collagen expressions were suppressed after treating chondrogenic cultures of human MSCs with PTHrP [20]. We believe that their results were due to treating cells with PTHrP from the initiation of chondrogenic cultures while we started from the 14th day of culture.

The COL1A1 downregulation and commensurate chondrogenic gene upregulation observed in our study is notable because previous studies and the authors' unpublished data show that type I collagen mRNA is persistent or upregulated after prolonged treatment with TGF-β [7,8,26]. This observed downregulation of COL1A1 would make another case for the merit of using PTHrP. Our in vitro data on the chondrogenesis of MSCs also shows that Runx-2 and COL10A1 are downregulated. PTHrP down regulates Runx-2 activation via various pathways that include PKC and Nkx3.2, which ultimately lead to the inhibition of chondrocyte maturation and suppress hypertrophic change in the growth plate of the chick embryo [24,27]. Our results suggest that this pathway is operational during the chondrogenesis of MSCs.

In conclusion, our study provides robust in vitro data which supports the benefit of using PTHrP for cartilage tissue engineering from MSCs. It demonstrates that PTHrP significantly enhances chondrogenesis and suppresses hypertrophy in both BMMSCs and ATMSCs. Further investigations are warranted to confirm the results of this study in different in vitro and in vivo models.

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References

[1] A.T. Caplan, Mesenchymal stem cells, J. Orthop. Res. 9 (1991) 641–650.
[2] 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.
[3] A.I. Caplan, Review: mesenchymal stem cells: cell-based reconstructive therapy in orthopedics, Tissue Eng. 11 (2005) 1198–1211.
[4] J.A. Buckwalter, H.J. Mankin, Articular cartilage: degeneration and osteoarthritis, repair, regeneration, and transplantation, Instr. Course Lect. 47 (1988) 487–504.
[5] 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.
[6] P.A. Zuk, M. Zhu, H. Mizuno, J. Huang, J.W. Futrell, A.J. Katz, P. Benhaim, H.P. Lorenz, M.H. Hedrick, Multilineage cells from human adipose tissue; implications for cell-based therapies, Tissue Eng. 7 (2001) 211–228.
[7] F. Barry, R.E. Boynton, B. Liu, J.M. Murphy, Chondrogenic differentiation of mesenchymal stem cells from bone marrow; differentiation-dependent gene expression of matrix components, Exp. Cell Res. 268 (2001) 189–200.
[8] K. Pelttari, A. Winter, E. Steck, K. Goetzke, T. Hennig, B.G. Ochs, T. Aigner, W. Richter, Premature induction of hypertrophy during in vitro chondrogenesis of human mesenchymal stem cells correlates with calcification and vascular invasion after ectopic transplantation in SCID mice, Arthritis Rheum. 54 (2006) 3254–3266.
[9] A. Muraglia, R. Cancedda, R. Quarto, Clonal mesenchymal progenitors from human bone marrow differentiate in vitro according to a hierarchical model, J. Cell Sci. 113 (2000) 1161–1166.
[10] K. Böhme, K.H. Winterhalter, P. Bruckner, Terminal differentiation of chondrocytes in culture is a spontaneous process and is arrested by transforming growth factor-beta 2 and basic fibroblast growth factor in synergy, Exp. Cell Res. 216 (1995) 191–198.
[11] H.M. Kronenberg, PTHrP and skeletal development, Ann. N.Y. Acad. Sci. 1068 (2006) 1–13.
[12] L.P. Lai, J. Mitchell, Indian hedgehog: its roles and regulation in endochondral bone development, J. Cell. Biochem. 96 (2005) 1163–1173.
[13] L.J. Suva, G.A. Winslow, R.E. Wettenhall, R.G. Hammonds, J.M. Moseley, H. Diefenbach-Jagger, C.P. Rodda, B.E. Kemp, H. Rodriguez, E.Y. Chen, et al., A parathyroid hormone-related protein implicated in malignant hypercalcemia:cloning and expression, Science 21 (1987) 893–896.
[14] T. Kobayashi, D.W. Soegiarto, Y. Yang, B. Lanske, E. Schipani, A.P. McMahon, H.M. Kronenberg, Indian hedgehog stimulates periarticular chondrocyte differentiation to regulate growth plate length independently of PTHrP, J. Clin. Invest. 115 (2005) 1734–1742.
[15] G.I. Im, Y.W. Shin, K.B. Lee, Do adipose tissue-derived mesenchymal stem cells have the same osteogenic and chondrogenic potential as bone marrow-derived cells?, Osteoarthr. Cartilage 13 (2005) 845–853.
[16] G.I. Im, N.H. Jung, S.K. Tae, Chondrogenic differentiation of mesenchymal stem cells isolated from patients in late adulthood: the optimal conditions of growth factors, Tissue Eng. 12 (2006) 527–536.
[17] V. Lefebvre, W. Huang, V.R. Harley, P.N. Goodfellow, B. de. Crombrugghe, SOX-9 is a potent activator of the chondrocyte-specific enhancer of the pro-alpha1 (II) collagen gene, Mol. Cell. Biol. 17 (1997) 2336–2346.
[18] P. Castagnola, G. Moro, F. Descalzi-Cancedda, R. Cancedda, Type X collagen synthesis during in vitro development of chick embryo tibial chondrocytes, J. Cell Biol. 102 (1986) 2310–2317.
[19] H. Enomoto, M. Enomoto-Iwamoto, M. Iwamoto, S. Nomura, M. Himeno, Y. Kitamura, T. Kishimoto, T. Komori, Cbfa1 is a positive regulatory factor in chondrocyte maturation, J. Biol. Chem. 275 (2000) 8695–8702.
[20] W. Kafienah, S. Mistry, S.C. Dickinson, T.J. Sims, I. Learmonth, A.P. Hollander, Three-dimensional cartilage tissue engineering using adult stem cells from osteoarthritis patients, Arthritis Rheum. 56 (2007) 177–187.
[21] A. Iida-Klein, J. Guo, M.T. Drake, H.M. Kronenberg, A.B. Abou-Samra, F.R. Bringhurst, G.V. Segre, Structural requirements of parathyroid hormone/parathyroid hormone-related peptide receptors for phospholipase C activation and regulation of phosphate uptake, Miner. Electrol. Metab. 21 (1995) 177–179.
[22] F.R. Bringhurst, H. Juppner, J. Guo, P. Urena, J.T. Potts Jr., H.M. Kronenberg, A.B. Abou-Samra, G.V. Segre, Cloned, stably expressed parathyroid hormone (PTH)/PTH-related peptide receptors activate multiple messenger signals and biological responses in LLC-PK1 kidney cells, Endocrinology 132 (1993) 2090–2098.
[23] H. Jüppner, A.B. Abou-Samra, M. Freeman, X.F. Kong, E. Schipani, J. Richards, L.F. Kolakowski Jr., J. Hock, J.T. Potts Jr., H.M. Kronenberg, et al., A G protein-linked receptor for parathyroid hormone and parathyroid hormone-related peptide, Science 254 (1991) 1024–1026.
[24] T.F. Li, Y. Dong, A.M. Ionescu, R.N. Rosier, M.J. Zuscik, E.M. Schwarz, R.J. O'Keefe, H. Drissi, Parathyroid hormone-related peptide (PTHrP) inhibits Runx2 expression through the PKA signaling pathway, Exp. Cell Res. 299 (2004) 128–136.
[25] H.E. MacLean, J. Guo, M.C. Knight, P. Zhang, D. Cobrinik, H.M. Kronenberg, The cyclin-dependent kinase inhibitor p57 (Kip2) mediates proliferative actions of PTHrP in chondrocytes, J. Clin. Invest. 113 (2004) 1334–1343.
[26] N. Indrawattana, G. Chen, M. Tadokoro, L.H. Shann, H. Ohgushi, T. Tateishi, J. Tanaka, A. Bunyaratvej, Growth factor combination for chondrogenic induction from human mesenchymal stem cell, Biochem. Biophys. Res. Commun. 320 (2004) 914–919.
[27] S. Provot, H. Kempf, L.C. Murtaugh, U.I. Chung, D.W. Kim, J. Chyung, H.M. Kronenberg, A.B. Lassar, Nkx3.2/Bapx1 acts as a negative regulator of chondrocyte maturation, Development 133 (2006) 651–662.