Chondrogenic differentiation of canine myoblasts induced by cartilage-derived morphogenetic protein-2 and transforming growth factor-β1 *in vitro*

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Abstract. The meniscus has limited ability to repair itself following injury. However, tissue engineering provides new means of meniscus repair. Myoblasts, which possess the potential of multi-directional differentiation, may be ideal seed cells in meniscus tissue engineering. Myoblasts from different animals showed slight differences in morphology and in the potential to differentiate. In the present study, we isolated myoblasts from canines and induced chondrogenesis in order to establish a new experimental model of seed cells. Myoblasts were isolated and harvested from Beagle canines. To induce chondrogenesis, cartilage-derived morphogenetic protein-2 (CDMP-2) at different concentrations (10, 20, 50 and 100 ng/ml), transforming growth factor-β1 (TGF-β1; 10, 20, 30 and 50 ng/ml), and different concentrations of CDMP-2 (10, 20, 50 and 100 ng/ml) together with TGF-β1 (20 ng/ml) were added to the cultured pellets. After 21 days of *in vitro* culture, chondrogenic differentiation was evaluated by histological and immunohistochemical techniques. The degree of gene expression was measured by quantitative RT-PCR. Based on the histological staining of glycosaminoglycan, using the toluidine blue dye-binding method, we found that CDMP-2 initiated chondrogenic differentiation of myoblasts, as did TGF-β1. Furthermore, CDMP-2 conferred a stronger stimulatory effect than TGF-β1. The combination of CDMP-2 and TGF-β1 synergistically induced chondrogenesis of myoblasts. This synergistic chondrogenic effect of CDMP-2 together with TGF-β1 was further confirmed by quantification of glycosaminoglycan using dimethylmethylene blue dye-binding assay and immunohistochemical analysis of the expression of cartilage-specific proteins collagen I and II. Canine myoblasts can be induced into chondrocytes by CDMP-2 and TGF-β1 *in vitro*, suggesting that myoblasts are suitable as seed cells for meniscus tissue engineering.

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Introduction

Meniscus is a complex fibrocartilaginous tissue, which is essential to the knee joint for shock absorption, load distribution, maintenance of stability and protection of articular cartilage (1). For symptomatic meniscus injury, a partial or total meniscectomy is often performed as the intrinsic healing capacity of the meniscus is limited (2). This, however, often leads to osteoarthritis (3). Other therapeutic methods of preserving the function of the meniscus are limited, and the result is not always satisfactory (4).

In recent years, meniscal regeneration using tissue engineering techniques have been carried out based on the loading and culture of suitable cells into appropriate scaffolds (5). To date, several cell sources have been used for meniscus repair, including meniscus fibrochondrocytes, chondrocytes or bone marrow-derived mesenchymal progenitor cells (6). However, the use of these cell sources have prompted issues concerning cell selection, acquisition of seed cells and the quality of repaired tissues (7). Myoblasts are regarded as ideal donor cells due to their abundant source, obtainability, stem cell-like potential, extended survival ability and rapid proliferative activity *in vitro* (8). Myoblasts are considered superior in biopsy, manipulation and clinical application than other stem cells, such as chondrocytes or bone mesenchymal stem cells (9). Therefore, myoblasts may be suitable for providing autogenous cells for the treatment of meniscal injuries.

Chondrogenesis proceeds through the aggregation of cells into prechondrogenic condensation and then differentiation of cells into chondrocytes. Numerous growth factors in the transforming growth factor-β1 (TGF-β1) superfamily have been found to enhance or induce certain stem cells to become chondrocytes *in vitro* (10,11). Since cartilage-derived morphogenetic protein-2 (CDMP-2) belongs to the TGF-β superfamily, it is a key factor in the regulation of the development of the appendicular skeleton, particularly at the early stages of chondrogenesis of the limb (12). Expression of CDMP-2 is mainly

localized at the condensing mesenchyme of the limb as well as in future joint spaces, and is directly correlated with the location, number and timing of developing joints. Nevertheless, little is known about the effects of CDMP-2 on the differentiation of myoblasts *in vitro*. TGF-β1 was initially reported to be essential for the chondrogenesis of certain stem cells (13). However, TGF-β1 alone may not be sufficient for the induction of the differentiation of myoblasts into chondrocytes. We, therefore, tested the chondrogenic effect of CDMP-2 alone and together with TGF-β1 on the differentiation of myoblasts into chondrocytes using a high-density pellet culture system.

Materials and methods

Myoblast culture. Beagle canines from the Agricultural Institute of Shanghai Jiaotong University were used as the source of myoblasts. Myoblasts from the hind limb of adult Beagle canines were isolated and harvested by mechanical decomposition and two-step enzyme digestion (14). The cells were then purified by a method involving differential adherent velocity and flow cytometry. Myoblasts were resuspended in media containing Dulbecco's modified Eagle's medium/F12 (DMEM/F12; Gibco BRL) supplemented with 10% fetal bovine serum (FBS; Gibco BRL), 100 U/ml penicillin G, 100 μg/ml streptomycin and 0.25 μg/ml amphotericin B. The myoblast suspension was seeded on 100-mm dishes and cultured at 37˚C in a humidified atmosphere of 95% air and 5% CO_2. To remove the non-adherent cells, the medium was changed on the fifth day after seeding, and it was changed again every four days thereafter. Myoblasts were passaged four times prior to being collected for use.

Pellet culture. For chondrogenic differentiation, $3.0x10^5$ myoblasts in a 15-ml conical polypropylene tube were centrifuged at 500 g for 5 min at 20˚C (15) and cultured at 37˚C with 5% CO_2 in 0.5 ml DMED-high glucose (Sigma) medium containing 1% FBS, insulin (6.25 μg/ml; Sigma) and ascorbate 2-phosphate (50 μg/ml; Sigma). Human CDMP-2 at different concentrations (10, 20, 50 and 100 ng/ml; PeproTech EC), TGF-β1 (10, 20, 30 and 50 ng/ml; Sigma) and different concentrations of CDMP-2 (10, 20, 50 and 100 ng/ml) together with TGF-β1 (20 ng/ml) were added to the cultured pellets. The medium was changed every 3 days for 21 days. The concentration of CDMP-2 and TGF-β1 selected was based on a previous study (16).

Histology. Pellets were harvested following 21 days of culturing, fixed in 10% buffered formalin for 2 h, embedded in 2% agarose for easy manipulation and kept in 70% ethanol overnight. Samples were embedded in paraffin and 5-μm sections were cut. Sections were stained with toluidine blue (Sigma) to access pericellular sulfated glycosaminoglycan (GAG) deposition.

Immunohistochemistry. Collagen type I and II were detected using monoclonal antibodies (LSL Co.). Briefly, following deparaffinization, sections were predigested with trypsin at 37˚C for 30 min to facilitate antibody access, endogenous peroxidase was quenched by the treatment of 0.3% H_2O_2 in methanol at room temperature for 30 min, and non-specific antibody binding was blocked by incubation of the sections in 10% normal goat serum at 37˚C for 30 min. Mouse anti-canine collagen type I and II diluted 1:100 in 0.01 M PBS (pH 7.4) were applied as a primary antibody at 4˚C, overnight. Sections were then incubated with the secondary antibody, rabbit anti-mouse immunoglobulin (Dako) for 60 min followed by mouse PAP kit (Dako). Collagen type I and II were visualized by the reactions with 0.05% diaminobenzidine containing 0.01% H_2O_2.

RT-PCR. Pellets (n=20) cultured for 21 days were digested with collagenase I and II (3 mg/ml; LSL Co.), hyaluronidase (1 mg/ml; Sigma) and 0.25% trypsin (Sigma) for 3 h at 37˚C. RNA was extracted from the digested pellets using TRIzol (Invitrogen), then converted to cDNA and amplified by PCR in a PCR machine with 1 cycle at 94˚C (5 min), 35 cycles at 94˚C (30 sec), 57˚C (30 sec) and 72˚C (30 sec), followed by 1 cycle at 72˚C (5 min). The mRNAs analyzed were collagen I (681 bp) and collagen II (447 bp) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (211 bp). Prime sequences for GAPDH, collagen I and collagen II were as follows: GAPDH, sense 5'CCTCTATGCCAACACAGTGC3', antisense 5'GTACTCCTGCTTGCTGATCC3'; collagen I, sense 5'ATGCCCAAGACTACCAGTGG3', antisense 5'TCCTGGAAGCTCTTCTCAGT3'; collagen II, sense 5'TTTCCCAGGTCAAGATGGTC3', antisense 5'CTTCAG CACCTGTCTCACCA3'.

GAG measurement. Pellets were harvested following 21 days of induction for GAG quantitation, washed with PBS and digested with 200 μl papain solution (Sigma; 280 μg/ml in 50 mM sodium phosphate, pH 6.5, containing 5 mM N-acetyl cystein and 50 mM EDTA) for 24 h at 65˚C. GAG content was measured by the reaction with dimethylmethylene blue (DMMB; Sigma). Optical density was determined at 525 nm using shark chondroitin 4-sulfate (Sigma) as the standard.

Statistical analysis. The data are expressed as the means ± SD. Statistical significance for total GAG content (μg/pellet) [^{3}H] thymidine incorporation value between groups was determined using one-way analysis of variance (ANOVA).

Results

CDMP-2 promotes chondrogenesis of myoblasts. All pellets of the myoblasts showed a spherical shape after 21 days under different culture conditions. In the absence of factors or in the presence of TGF-β1 alone, the expression of collagen II in the pellets was not detected by immunohistochemistry (Fig. 1A). Expression of collagen II in the pellets was upregulated by either CDMP-2 (Fig. 1C) alone, or in combination with TGF-β1 (Fig. 1D). Higher cell density in the collagen II-positive region in the pellets indicated that more cartilage matrix was produced after myoblasts differentiated into chondrocytes. Only some cells incubated with TGF-β1 alone differentiated into chondrocytes (Fig. 1B). The expression of collagen II was observed all over the pellets when treated with CDMP-2 together with TGF-β1 (Fig. 1D). The results suggest a synergistic effect of CDMP-2 and TGF-β1 on chondrogenesis related to myoblasts.

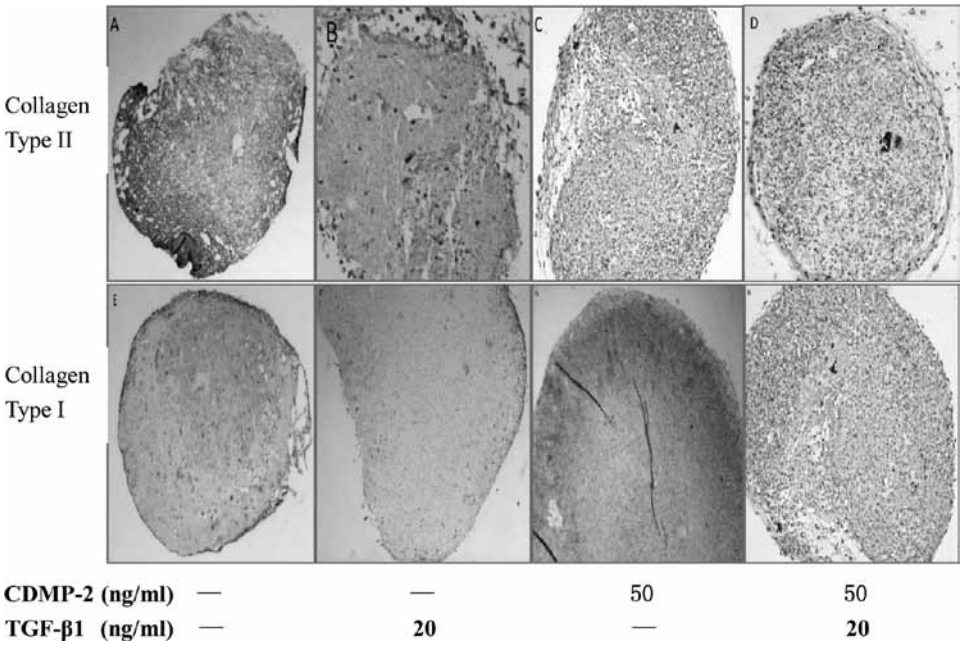

Figure 1. Effect of CDMP-2 and TGF-β1 on the differentiation of myoblasts into chondrocytes. Myoblast pellets were incubated: without CDMP-2 or TGF-β1 (control) (A and E), with 50 ng/ml CDMP-2 (C and G), with 20 ng/ml TGF-β1 (B and F) and with CDMP-2 (50 ng/ml) combined with TGF-β1 (20 ng/ml) (D and H), respectively, for 21 days. Immunohistochemical analysis of collagen II (A-D) and collagen I (E-H) expression in paraffin sections of pellets. The brown color is indicative of a positive region of collagen II or collagen I, and the blue color represents karyon counterstained with hematoxylin. Bar, 50 μm.

Collagen I, expressed by chondrocytes, was expressed under both induced situations of cell culture but was more strongly expressed in the sections that expressed collagen II (Fig. 1E-H). CDMP-2 in the presence of TGF-β1 strongly upregulated the expression of collagen I in the pellets. The results indicated that most cells treated with CDMP-2 and TGF-β1 had differentiated into chondrocytes.

Chondrogenesis of the pellets was further confirmed by RT-PCR (Fig. 2). Products of all PCR amplifications were sequenced to exclude the possibility of false-positive results. Results of the RT-PCR were in accordance with the immunohistochemical analysis. In all pellets, collagen I mRNA was uniformly expressed. Collagen II mRNA was expressed in both pellets cultured with CDMP-2 alone or in combination with TGF-β1 (Fig. 2, lanes 3 and 4), but it was not detected in pellets cultured without either growth factor or in the presence of TGF-β1 alone (Fig. 2, lanes 1 and 2).

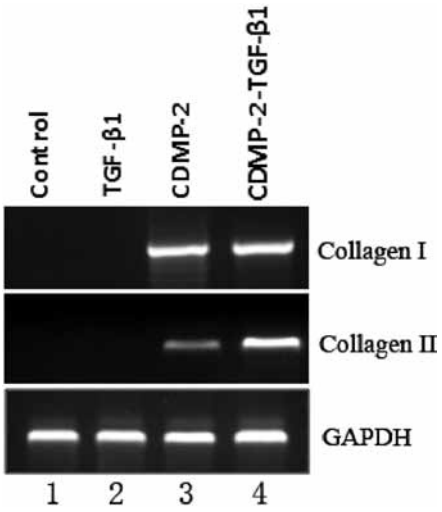

Figure 2. RT-PCR analysis of collagen I and collagen II expression of different pellets for 21 days cultured without CDMP-2 or TGF-β1 (control) (lane 1), with TGF-β1 (20 ng/ml) (lane 2), with CDMP-2 (50 ng/ml) (lane 3), and with CDMP-2 (50 ng/ml) combined with TGF-β1 (20 ng/ml) (lane 4), respectively.

Dose effect of CDMP-2 and TGF-β1 on myoblast chondrogenesis. To evaluate the dose effect of CDMP-2 and TGF-β1 on myoblast chondrogenesis, the pellets were cultured in medium containing different doses of CDMP-2 (10, 20, 50 and 100 ng/ml), TGF-β1 (10, 20, 30 and 50 ng/ml) and CDMP-2 (10, 20, 50 and 100 ng/ml) together with TGF-β1 (20 ng/ml), respectively, for 21 days. We assessed the GAG (a main matrix element of cartilage) expression in the pellets by histological staining using the toluidine blue dye-binding method. Metachromatic-staining of pellets represented GAG expression by differentiated chondrocytes. The metachromatic-staining region of the pellets grown in the presence of CDMP-2 combined with TGF-β1 (20 ng/ml) was correlated to the CDMP-2 concentration. An apparent effect was observed at 50 ng/ml (Fig. 3C); the maximal chondrogenic effect was noted at 100 ng/ml (Fig. 3D).

To further evaluate the dose effect of CDMP-2 and TGF-β1 on myoblast chondrogenesis, we quantified GAG deposition of pellets using the DMMB-binding method (Fig. 4). Pellets cultured in the presence of growth factors showed a significant stimulation of GAG expression with CDMP-2 exhibiting a stronger stimulatory effect than TGF-β1. An increase in GAG expression was induced by TGF-β1 at 30 ng/ml ($P<0.01$) and by CDMP-2 at 50 ng/ml ($P<0.01$), but a significant stimulatory effect was detected at a lower dose of CDMP-2 (10 ng/ml) combined with TGF-β1 (20 ng/ml) ($P<0.01$).

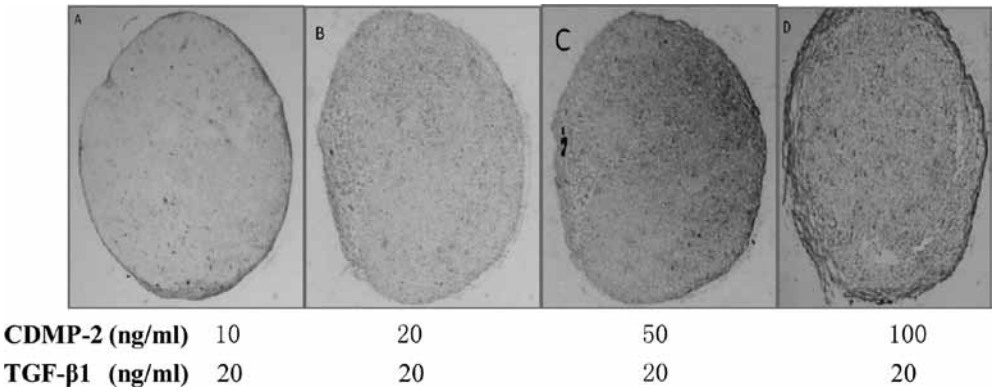

Figure 3. Dose effect of CDMP-2 and TGF-β1 on the chondrogenesis of myoblasts using pellet culture. Histochemical analysis of GAG expression of pellets cultured for 21 days in the medium containing CDMP-2 (10, 20, 50 and 100 ng/ml) together with TGF-β1 (20 ng/ml). The paraffin sections of pellets were stained with toluidine blue. The purple color is indicative of GAG and the blue color of background. Bar, 50 μm.

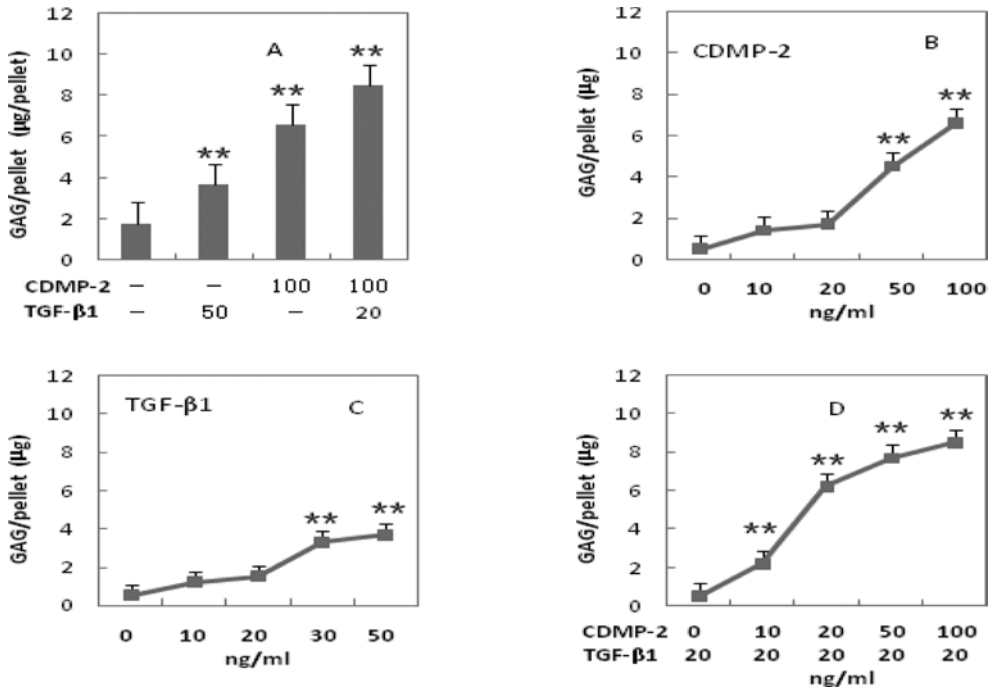

Figure 4. Quantification of the GAG content of pellets incubated with different concentrations of CDMP-2 and TGF-β1 using the DMMB dye-binding method. (A) Pellets were grown in the presence of CDMP-2 (100 ng/ml), TGF-β1 (50 ng/ml) or CDMP-2 (100 ng/ml) and TGF-β1 (20 ng/ml) for 21 days. Parallel cultures without the addition of CDMP-2 and TGF-β1 served as unstimulated control. (B-D) The chondrogenic effect of the four doses of CDMP-2 (10, 20, 50 and 100 ng/ml), TGF-β1 (10, 20, 30 and 50 ng/ml) or CDMP-2 (10, 20, 50 and 100 ng/ml) combined with TGF-β1 (20 ng/ml) on myoblasts were observed. Data are presented as the means ± SD (n=4). **P<0.01 vs. control.

Discussion

Meniscal injury is one of the most common injuries to the knee. The meniscus is essential for normal knee function, and loss of the meniscus increases the risk of subsequent development of degenerative alterations in the knee. Tissue engineering may offer new methods for the regeneration of meniscus lesions or for the complete replacement of a degenerated (part of total) meniscus by the production of newly synthesizing meniscal tissue. Tissue engineering is based on a smart and unique combination of exogenous cells, specific stimuli and matrix scaffold in an *in vitro* or *in vivo* environment (17). In terms of cell sources, several cell sources have been used for meniscus repair, including meniscus fibrochondrocytes, chondrocytes and bone marrow-derived mesenchymal progenitor cells (18).

Myoblasts are satellite cells that proliferate near the muscle fibers. Myoblasts have been considered a candidate cell source for tissue engineering and reparative medicine by virtue of their potential to differentiate into chondrocytes, fibroblasts, osteoblasts and other tissues of mesenchymal origin (19,20). Compared to the above-mentioned cell sources, myoblasts represent a more promising source for cartilage engineering, as they are relatively abundant and easily accessible with minimal donor site morbidity (21). At the same time, myoblasts further promote the development of tissue engineering, as they have higher cell yield and a more rapid proliferative ability during *in vitro* expansion (22).

In the present study, we demonstrated that expanded canine myoblasts seeded within conical polypropylene tubes can be efficiently induced to undergo chondrogenic differentiation when treated with CDMP-2. The *in vitro* chondrogenic differentiation of myoblasts requires the complex involvement of growth factors and cell-cell and cell-matrix interactions, similar to developmental chondrogenesis *in vivo*. Expression of members of the TGF-β superfamily of growth factors has been localized to sites of bone repair as well as to sites of embryonic bone and cartilage formation *in vivo* (23). Furthermore, the chondroinductive effects of the TGF-β superfamily members, particularly the bone morphogenetic proteins (BMPs) and the TGF-βs, have been well established in embryonic and adult mesenchymal cells (24).

Recent reports have demonstrated the critical role of intracellular signaling cascades activated by the TGF-β family members in promoting cartilage-specific gene expression, including the mitogen-activated protein (MAP) kinases, whose major subtypes include p38, extracellular signal-regulated kinase-1 (ERK-1) and c-Jun N-terminal kinase (JNK or stress-activated protein kinase). To date, several growth factors have been demonstrated to enhance or induce various stem cells to become chondrocytes *in vitro*. However, BMPs play an important role in the development of embryonic skeleton and growth and retension of adult skeleton (25). CDMP-2 is a member of the BMP family. CDMP-2 is essential for chondrogenesis. CDMP-2 initiates limb mesenchymal cells, periosteum-derived cells and ATDC5 (a mouse clonal teratocarcinoma chondrogenic cell line) to differentiate into chondrocytes *in vitro* and even cartilage-like structures *in vivo*. Moreover it enhances GAG production of chondrocytes (26).

In the present study, we evaluated the effects of CDMP-2 alone and in combination with TGF-β1 on the chondrogenesis of myoblasts using a high-density pellet culture system *in vitro*. The results demonstrated that high concentrations of CDMP-2 (50 ng/ml) alone stimulated the chondrogenesis of myoblasts, as did CDMP-2 together with TGF-β1. The chondrogenic effect on myoblasts was the strongest in the pellets cultured with CDMP-2 (50 ng/ml) and TGF-β1 (20 ng/ml).

TGF-β1 is a key factor in the regulation of cellular differentiation in cartilage formation. TGF-β1 was found to be highly expressed in embryonic cartilage. It has been reported that TGF-β1 stimulated collagen II and GAG expression of periosteum-derived cells in a dose-dependent manner (27). Repairing of partial thickness articular defects *in vivo* was enhanced by TGF-β1 treatment that induced mesenchymal cell recruitment from the synovium (28). Our results showed that low doses of TGF-β1 alone were not sufficient to promote myoblast chondrogenesis. When tested at doses of TGF-β1 (10, 20, 30 and 50 ng/ml), we observed that higher doses (30 and 50 ng/ml) sufficiently initiated myoblast differentiation into chondrocytes. However, the chondrogenic effects of 50 ng/ml TGF-β1 showed a significant difference when compared to combined CDMP-2 (50 ng/ml) and TGF-β1 (20 ng/ml). These data indicate that TGF-β1 has weaker chondrogenic effects on myoblasts than CDMP-2. We provide evidence that CDMP-2 in combination with TGF-β1 synergistically promotes chondrogenesis of myoblasts.

In conclusion, in the present study we demonstrated that higher doses of CDMP-2 or TGF-β1 stimulated myoblast differentiation into chondrocytes. Low doses of CDMP-2 with TGF-β1 showed a marked chondrogenic effect on myoblasts. Therefore, combination of CDMP-2 and TGF-β1 may be beneficial for the treatment of cartilage lesions using myoblasts.

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References

1. Ahmed AM, Burke DL and Yu A: In-vitro measurement of static pressure distribution in synovial joints-part II: Retropatellar surface. J Biomech Eng 105: 226-236, 1983.
2. Hennerbichler A, Moutos FT, Hennerbichler D, *et al*: Repair response of the inner and outer regions of the porcine meniscus in vitro. Am J Sports Med 35: 754-762, 2007.
3. Hart R, Janecek M, Siska V, *et al*: Correlation of long-term clinical and radiological results after meniscectomies. Acta Chir Orthop Traumatol Cech 72: 304-307, 2005 (In Czech).
4. Van der Wal RJ, Thomassen BJ and van Arkel ER: Long-term clinical outcome of open meniscal allograft transplantation. Am J Sports Med 37: 2134-2139, 2009.
5. Ishida K, Kuroda R, Miwa M, *et al*: The regenerative effects of platelet-rich plasma on meniscal cells in vitro and its in vivo application with biodegradable gelatin hydrogel. Tissue Eng 13: 1103-1112, 2007.
6. Kang SW, Son SM, Lee JS, *et al*: Regeneration of whole meniscus using meniscal cells and polymer scaffolds in a rabbit total meniscectomy model. J Biomed Mater Res A 78: 659-671, 2006.
7. El Tamer MK and Reis RL: Progenitor and stem cells for bone and cartilage regeneration. J Tissue Eng Regen Med 3: 327-337, 2009.
8. Lu SH, Yang AH, Wei CF, *et al*: Multi-potent differentiation of human purified muscle-derived cells: potential for tissue regeneration. BJU Int 105: 1174-1180, 2009.
9. Moisset PA, Gagnon Y, Karpati G and Tremblay JP: Expression of human dystrophin following the transplantation of genetically modified mdx myoblasts. Gene Ther 5: 1340-1346, 1998.
10. Stewart K, Pabbruwe M, Dickinson S, *et al*: The effect of growth factor treatment on meniscal chondrocyte proliferation and differentiation on polyglycolic acid scaffolds. Tissue Eng 13: 271-280, 2007.
11. Diekman BO, Rowland CR, Lennon DP, *et al*: Chondrogenesis of adult stem cells from adipose tissue and bone marrow: induction by growth factors and cartilage-derived matrix. Tissue Eng Part A 16: 523-533, 2010.
12. Steinert AF, Ghivizzani SC, Rethwilm A, *et al*: Major biological obstacles for persistent cell-based regeneration of articular cartilage. Arthritis Res Ther 9: 213, 2007.
13. Steinert AF, Palmer GD, Capito R, *et al*: Genetically enhanced engineering of meniscus tissue using ex vivo delivery of transforming growth factor-beta 1 complementary deoxyribonucleic acid. Tissue Eng 13: 2227-2237, 2007.
14. Zhu W, Wang Y, Qiu G and Chen B: Characterization of the purification and primary culture of adult canine myoblasts in vitro. Mol Med Rep 3: 463-468, 2010.
15. Johnstone B, Hering TM, Caplan AI, *et al*: In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells. Exp Cell Res 238: 265-272, 1998.
16. Tian H, Yang S, Xu L, *et al*: Chondrogenic differentiation of mouse bone marrow mesenchymal stem cells induced by cartilage-derived morphogenetic protein-2 in vitro. J Huazhong Univ Sci Technolog Med Sci 27: 429-432, 2007.
17. Baker BM, Nathan AS, Huffman GR and Mauck RL: Tissue engineering with meniscus cells derived from surgical debris. Osteoarthritis Cartilage 17: 336-345, 2009.
18. Yamasaki T, Deie M, Shinomiya R, *et al*: Transplantation of meniscus regenerated by tissue engineering with a scaffold derived from a rat meniscus and mesenchymal stromal cells derived from rat bone marrow. Artif Organs 32: 519-524, 2008.

19. Koning M, Harmsen MC, van Luyn MJ and Werker PM: Current opportunities and challenges in skeletal muscle tissue engineering. J Tissue Eng Regen Med 3: 407-415, 2009.
20. Singh D, Nayak V and Kumar A: Proliferation of myoblast skeletal cells on three-dimensional supermacroporous cryogels. Int J Biol Sci 6: 371-381, 2010.
21. Périé S, Mamchaoui K, Mouly V, *et al*: Premature proliferative arrest of cricopharyngeal myoblasts in oculo-pharyngeal muscular dystrophy: Therapeutic perspectives of autologous myoblast transplantation. Neuromuscul Disord 16: 770-781, 2006.
22. Stern-Straeter J, Bran G, Riedel F, *et al*: Characterization of human myoblast cultures for tissue engineering. Int J Mol Med 21: 49-56, 2008.
23. Horner A, Kemp P, Summers C, *et al*: Expression and distribution of transforming growth factor-beta isoforms and their signaling receptors in growing human bone. Bone 23: 95-102, 1998.
24. Tuli R, Seghatoleslami MR, Tuli S, *et al*: p38 MAP kinase regulation of AP-2 binding in TGF-beta1-stimulated chondrogenesis of human trabecular bone-derived cells. Ann NY Acad Sci 961: 172-177, 2002.
25. Bobick BE, Chen FH, Le AM and Tuan RS: Regulation of the chondrogenic phenotype in culture. Birth Defects Res C Embryo Today 87: 351-371, 2009.
26. Bobacz K, Gruber R, Soleiman A, *et al*: Cartilage-derived morphogenetic protein-1 and-2 are endogenously expressed in healthy and osteoarthritic human articular chondrocytes and stimulate matrix synthesis. Osteoarthritis Cartilage 10: 394-401, 2002.
27. Bai X, Xiao Z, Pan Y, *et al*: Cartilage-derived morphogenetic protein-1 promotes the differentiation of mesenchymal stem cells into chondrocytes. Biochem Biophys Res Commun 325: 453-460, 2004.
28. Hunziker EB and Rosenberg LC: Repair of partial-thickness defects in articular cartilage: cell recruitment from the synovial membrane. J Bone Joint Surg Am 78: 721-733, 1996.