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Interaction between synovium derived stem cells and chondrocytes in chondrogenesis and arthritis

연골재생과 관절염에서 활막줄기세포와 연골세포의 상호작용

2018 년 8 월

서울대학교 대학원

의학과 정형외과학 전공

김 태 우

A thesis of the Degree of Doctor of Philosophy

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Interaction between synovium derived stem cells and chondrocytes in chondrogenesis and arthritis

by

Tae Woo Kim , M.D.

A thesis submitted to the Department of Medicine in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Medicine (Orthopedic Surgery)
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Approved by Thesis Committee:

Professor _____Chairman

Professor _____Vice chairman

Professor _____

Professor _____

Professor _____

Abstract

Interaction between synovium derived stem cells and chondrocytes in chondrogenesis and arthritis

Tae Woo Kim

Medicine (Orthopedic Surgery)

The Graduate School

Seoul National University

Purpose: The purpose of this study was to evaluate interaction between chondrocytes and synovium-derived stem cells (SDSCs) in chondrogenesis and arthritis. The author evaluated whether direct coculture of human chondrocytes and SDSCs can enhance chondrogenesis. Also, we analyzed the inflammation-related secretory proteins between chondrocytes and SDSCs by secretome antibody microarray and immunoblotting .

Materials and Methods: To evaluate chondrogenic effect between human chondrocytes and SDSCs, cartilage and synovial tissues were harvested from patients undergoing total knee arthroplasty for osteoarthritis and expanded in monolayer. Passage 2 chondrocytes and SDSCs were directly cocultured with various mixed ratio (3:1, 1:1, or 1:3). GAG synthetic activity was accessed with GAG assay and Safranin−O staining. Expression of chondrogenesis-related genes (collagen types I, II, X, aggrecan, and SOX-9) were analyzed by RT-qPCR and immunohistochemistry staining.

To investigate inflammation-related secretory mediators between human chondrocytes and SDSCs, passage 2 chondrocytes and SDSCs were obtained

in the same way and cocultured indirectly. Secretomes obtained from monoculture or indirect coculture of each cells were analyzed by antibody microarray. Up or down regulated proteins more than 1.5 folds in coculture group compared with monoculture group were collected and extensive literature review was performed to select inflammation-related secretory proteins, as a candidate for inflammatory mediator between two cells. RT-PCR and immunoblotting (Western blot) was performed to confirm the expression of candidate mediators.

Results : In direct coculture study, GAG/DNA ratio in 1:1 and 1:3 coculture groups were significantly increased compared to those in chondrocyte and SDSC monoculture groups. Type II collagen and SOX-9 were significantly up-regulated in the 1:1 coculture group compared to those in chondrocyte and SDSC monoculture groups. On the other hand, osteogenic marker (type I collagen) and hypertrophic marker (type X collagen) were significantly down-regulated in the coculture groups compared to those in the SDSC monoculture group.

In secretome antibody microarray, 51 proteins were up-regulated and 36 proteins were down-regulated more the 1.5 folds in the coculture group compared with monoculture of each cell. Among these proteins, MMP-2, angiopoietin-1,Caveolin-1,ICAM-1,FABP4, Fibronectin, and VEGF-B were selected as up or down-regulated candidate for inflammatory mediators and subsequent RT-PCR and immublotting (Western blot) was performed. Finally, up-regulation of MMP-2 in cocultured chondrocyte and SDSCs, and down-regulation of FABP4 in cocultured SDSCs were consistently observed in all of microarray, RT-PCR, and immunoblotting.

Conclusion: Direct coculture of human chondrocytes and SDSCs significantly enhanced chondrogenic potential, especially at 1:1 ratio, compared to chondrocyte or SDSC monocultures. In secretome microarray and immunoblotting under indirect coculture of chondrocytes and SDSCs, up-regulated MMP-2 and down-regulated FABP4 could be selected as possible inflammatory mediators. However, further study is necessary to clarify the role of MMP-2 and FABP4 in the interaction between two cells.

Keywords: synovium derived stem cell, chondrocyte, coculture, chondrogenesis , secretome, microarray, inflammatory mediator

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Introduction

In osteoarthritis(OA) or cartilage injury cartilage has limited capacity for intrinsic healing due to its avascularity and low regeneration rate of chondrocyte. Despite various efforts to treat cartilage injury, repair of innate cartilage tissue remains a challenging issue. Cell based therapies have been developed to overcome the poor healing potential of cartilage. Autologous chondrocyte transplantation (ACT) composed of chondrocyte harvest, *in vitro* chondrocyte expansion, and implantation of cultivated chondrocytes has been introduced as a promising cell based treatment for cartilage repair (1, 2). However, dedifferentiation of chondrocytes during *in vitro* expansion decreases the chondrogenic phenotype, resulting in the production of repair tissue whose mechanical properties are inferior to those of hyaline cartilage (3, 4).

To overcome the limitation of the current ACT technique and improve clinical outcomes, various trials have been performed on cartilage tissue engineering. Coculture strategy has been developed to enhance chondrogenic phenotype of chondrocytes and mesenchymal stem cells(MSCs) in tissue engineering for cartilage repair. Coculture of these two cell types synergistically promotes the redifferentiation of chondrocyte and increases chondrogenic differentiation of MSCs during *in vitro* expansion, resulting in enhanced chondrogenesis (5-11). Numerous studies have been performed on cocultures involving various kinds of MSCs such as bone marrow, umbilical cord blood, adipose tissues, and synovium in the last decade. Among these tissues, synovium derived stem cells (SDSCs) is known to possess chondrogenic potential superior to MSCs derived from other tissues(12-14).

However, very few coculture study on chondrocytes and SDSCs has been reported. Wang DA et al. have shown that coculture of SDSCs and TGF-b3 gene transfected chondrocyte can improve chondrogenesis in mixed coculture as well as indirect coculture (15, 16). However, these studies were performed using animal SDSCs and chondrocyte such as rabbit or porcine. Kubosch EJ et al. have reported that indirect coculture of human SDSCs and chondrocytes can enhance chondrogenic phenotype of SDSCs through paracrine effect of cocultured chondrocyte (17). However, cell to cell interaction between human SDSCs and chondrocytes cannot be evaluated in this indirect coculture setting.

The first purpose of this study was to investigate whether direct mixed coculture of human chondrocytes and SDSCs could enhance chodrogenesis compared to monoculture of SDSC or chondrocyte. As we know, this is the first study that investigates direct coculture of human chondrocyte and SDSCs. Three different mixed ratio groups of cocultures were evaluated to determine the ideal mixed ratio of the two cell types.

Another strategy to treat osteoarthritic change of the joint including cartilage degradation is to understand pathophysiology of OA and control the key material in that pathway. While OA has been historically defined as “wear and tear” disease of articular cartilage, it is increasingly recognized that inflammatory reaction of the joint structures has important role in the progression of OA. With the development of molecular biology, detection of various mediators such as cytokines and prostaglandins that can increase the production of matrix metalloproteinases by chondrocytes derived the “inflammatory” theory of OA. Subchondral bone is also known to have important role in the OA progression as a source of inflammatory mediators related with pain perception and degradation of deep layer of cartilage. Thus,

OA is a very complex disease related with inflammatory mediators released from the cartilage, subchondral bone, synovium, and adipose tissues(18).

It is well known that synovitis and proiflammatory cytokines have important role in the pathogenesis of OA(19). Catabolic and proinflammatory mediators such as neuropeptides, nitric oxide, prostaglandin E2, and cytokines generated from the inflamed synovium break down the homeostasis of cartilage matrix degradation, resulting in the loss of cartilage (20). Catabolic effect of synovitis is also observed between the chondrocytes and synovial cells. Steinhagen et al. and Huch et al. reported that co-culture of synovial fibroblast and chondrocyte resulted in decrease of proteoglycan synthesis of chondrocyte(21, 22). Beekhuizen et al. also showed that osteoarthritic synovial tissue inhibited production of proteoglycan in osteoarthritic knee cartilage(23).

On the contrary, Koh et al. recently reported that coculture of SDSCs and articular chondrocyte can reduce inflammatory activity of chondrocyte(24). When the anti-inflammatory effect of SDSCs is proved, injection or implantation of SDSCs can be considered clinically to inhibit the inflammatory progression of OA. Also, SDSCs can exist in the normal or inflammed synovial tissues with fibroblast(25). If the inflammatory mediators that enhance or inhibit the inflammatory response between chondrocytes and SDSCs is detected, it can be a target for the new disease modifying drugs. However, to our knowledge, little is known about the inflammatory interaction between SDSC and chondrocyte, especially in inflammatory mediators between SDSC and chondrocyte.

The second purpose of this study was to investigate inflammatory mediators between human osteoarthritic SDSCs and chondrocytes using indirect

coculture system. Secretome microarray was performed to analyze up or down regulated inflammation-related secretory proteins in coculture compared with monoculture of each cell. Then candidate proteins were confirmed with immublotting method (Western blot).

Chapter I.

Assessment of chondrogenic effect in direct coculture of synovial stem cells and chondrocytes

I-1. Materials and Methods

Harvest of synovium and cartilage tissue

Synovium and cartilage tissues were obtained from six female osteoarthritis patients (age of 66 to 72 years) undergoing total knee arthroplasty(TKA). In all patients, Kellgren Lawrence grade was 4 and osteoarthritis progressed at medial side of knee. Therefore, study was performed using relatively intact cartilage from lateral femoral and tibial condyles. Synovium was harvested from the suprapatellar pouch. Ethical approval for this study was obtained from Seoul National University Boramae Medical Center Institutional Review Board (06-2012-25). Those who had inflammatory arthritis, prior knee joint infection, and intraarticular trauma were excluded.

Isolation of SDSCs

Synovial tissue was minced in phosphate-buffered saline(PBS) and digested with 0.02% collagenase (Sigma, St. Louis, MO) overnight. Cells were filtered from undigested tissue with 70μm sieves and centrifuged at 1,500 rpm for 5 minutes. Then, cells were cultured in low glucose Dulbecco's modified Eagle's medium(LG-DMEM; Gibco, Paisley, UK) with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin/amphotericin at 37℃ with 5% CO_2. Medium was changed after 48 hours and nonadherent cells were removed during this procedure. Cells were cultured until they reach 80-90% confluence after 10-14 days (passage 0, P0). In our previous study, we found that synovium derived cells obtained from these procedures expressed MSC specific surface makers such as CD44, CD73, CD90, CD 105, CD106, and

CD166 (26-28). Also, change of chondrogenic phenotype in SDSCs was not observed after passage1 period(29). Therefore, Passage 2 cells were used as SDSCs without other negative selection procedures in the pellet coculture.

Isolation of chondrocytes

Cartilage was digested at 37℃ with 0.2% pronase (Sigma) for 1 hour and with 0.2% collagenase (Sigma) overnight. Cells were filtered from undigested tissue with 70μm sieves and centrifuged at 1,500 rpm for 5 minutes. Subsequently collected chondrocytes were cultured in low glucose Dulbecco's modified Eagle's medium(LG-DMEM; Gibco, Paisley, UK) with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin/amphotericin at 37℃ with 5% CO_2 and expanded on culture dish at a density of 1×10^6/150ml. The initial number of chondrocytes from each patients ranged from 1.5 x 10^6 to $3x10^6$. Passage 2 cells were used in the pellet coculture (30).

Mixed coculture of chondrocytes and SDSCs

Five groups of passage 2 cell suspensions containing 5×10^5 cells of chondrocytes or SDSCs or a combination of chondrocytes and SDSCs with three different ratio (Table 1) were centrifuged at 1,500 rpm for 5 minutes to obtain cell pellets. Cell pellets were cultured in chondrogenic medium (LG-DMEM; Gibco) containing 0.1 mmol/L ascorbic acid 2-phosphate, 100 nmol dexamethasone, 40 g/mL proline, 100 U/mL penicillin, 100 g/mL streptomycin, and ITS Premix(BD Biosciences, Bedford, MA) supplemented with TGF- β1. Change of culture medium was performed every other day until day 21. Chondrogenesis of cell pellets were evaluated at days 7,14, and 21(31).

Histology and Immunohistochemistry

For histological evaluation of glycosaminoglycan synthesis, cell pellets of each group were stained with Safranin-O and fast green staining at day 7,14, and 21. Staining was performed as we described in the previous study (26). The staining was graded using Bern Score that was developed to evaluate Safranin-O staining in three different scoring categories(uniformity and darkness, distance between cells, and cell morphologies) (32). To evaluate production of type II and X collagen histologically, immunohistochemical staining was performed in each group at day 7,14, and 21.Immunohistochemistry was also examined as we described in the previous study (26). In the interpretation of immunohistochemical results, positive finding was density of brown color and background was blue-purple color.

Biochemical analysis

To access synthetic activity of glycosaminoglycan(GAG), total contents of GAG and DNA were measured. GAG levels were evaluated with dimethylmethylene blue(DMB) assay(33). Cell pellets of each group were collected in two different fractions (matrix and media) at day 21. Cell pellets were digested in papain buffer (5 mM L-cysteine , 200 μg/ml papain, 0.1 M sodium acetate, pH 3.0) for 18 hours at 65°C, and centrifuged for 5 minutes at 6,000 rpm. Subsequently, aggregated cells were placed on 96 well plate with DMB solution. GAG levels were determined by absorbances measured at 530 and 590 nm usingimmunoassay reader. Absorbance value was standardized by chondroitin-6-sulfate.DNA content of pellets were measured with Quant-iT

PicoGreen dsDNA Assay Kit (Invitrogen, Eugene, OR) (5). GAG synthetic activity was accessed by total GAG content normalized versus total DNA content.

Reverse transcription quantitative Polymerase Chain Reaction

At culture day 21, expression of chondrogenesis-related genes including aggrecan, Sry-type high-mobility-group box transcription factor-9 (Sox-9*),* type I collagen, type II collagen, and type X collagen was evaluated using reverse transcription quantitative polymer chain reaction (RT-qPCR). Total RNA was purified from cell pellets using TRIzol reagent(Invitrogen) and complementary DNA was prepared with RNA to cDNA EcoDry™ Premix (Oligo dT) and cDNA Synthesis Kit (Takara Bio). Primer Express software version 1.5 (Abingdon, UK) was used in analytic procedure during RT-qPCR and the level of Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was compared as an endogenous reference. Relative quantification of gene expression was perform using ABI Prism 7000 Sequence Detection System with relative standard curve method (27).

Statistical Analysis

Statistical analysis was performed using SPSS 18.0 software (SPSS, Chicago, IL). Kruskal–Wallis tests was used to compare GAG synthetic activity, bern score, and gene expression among 5 groups. Intergroup difference was assessed with Mann–Whitney test. Statistical significance was considered when p value was less than 0.05.

	CC	Co 1	Co 2	Co 3	SDSC
Ratio (CC: SDSC)	4:0	3:1	2:2	1:3	0:4
Cellcount (CC: SDSC)	5×10^5	3.75×10^5 :1.25×10^5	2.5×10^5 :2.5×10^5	1.25×10^5 :3.75×10^5	5×10^5

Table 1. Mixed ratio and cell count of five pellet culture groups

CC : chondrocyte, Co : coculture, SDSC : synovium derived stem cell

I-2. Results

Cellularity and GAG synthesis

Total cellular DNA and GAG depositions were measured at day 21. There was no significant difference in total DNA content among the five culture groups. However, GAG content was significantly increased in the 3:1,1:1, and 1:3 coculture groups compared to that in chondrocyte or SDSC monoculture group. The coculture group at 1:3 ratio showed the highest GAG activity among the three coculture groups. Regarding the GAG/DNA ratio, coculture groups at 1:1 and 1:3 ratio was significantly higher than that in the chondrocyte or the SDSC monoculture group.

Histological analysis

Presence of proteoglycans was evaluated with Safranin O-fast green staining on day 7, 14, and 21 for all five groups (Fig 1-B). On day 7, weak staining was observed in chondrocyte monoculture and the three coculture groups. However, staining was not observed in the SDSC monoculture group. On day 21, dense and even staining was observed in coculture groups at ratio of 1:1 and 1:3. Partial staining was observed in the 3:1coculture group and the chondrocyte monoculture group. Staining in the SDSC monoculture group was very weak. Safranin O-fast green staining was also evaluated with Bern Score known to be significantly correlated with GAG contents (32). On day 21, Bern scores of the chondrocyte monoculture group and the three coculture groups were significantly higher than those of the SDSC group (Fig. 1-C).

Overall, the histological findings matched well with the results of GAG/DNA assay.

Gene expression analysis using PCR

Chondrogenesis-related gene expression was quantified with qRT-PCR at day 21 (Fig 2). Type II collagen, aggrecan, and SOX-9 was evaluated as chondrogenic markers. Levels of type II collagen and SOX-9 in the 1:1 coculture group were significantly higher compared to those in the 1:3 and 3:1 coculture groups as well as the chondrocyte and SDSC monoculture groups. Expression levels of aggrecan in chondrocyte monoculture and 1:1 coculture group were significantly increased compared to those in the SDSC monoculture group. However, there was no statistical difference in the expression level of aggrecan among 1:3 coculture, 3:1 coculture, and the SDSC monoculture group.

To assess dedifferentiation of chondrocyte and osteogenic induction of SDSC, the levels of type I collagen were evaluated. Type I collagen levels in chondrocyte monoculture and the three coculture groups were significantly lower than those of the SDSC monoculture group during the 21-day culture period. However, the 1:1 coculture group showed significantly higher level of type I collagen compared to the chondrocyte monoculture group.To exclude hypertrophic change during chondrogenesis, type X collagen was evaluated as a hypertrophic marker. As expected, the levels of type X collagen in the three coculture groups were significantly lower than those of the SDSC monoculture group and higher than those of the chondrocyte monoculture group.

Immunohistochemical analysis

Immunohistochemistry was performed for type II and type X collagen, the representative chondrogenic and hypertrophic marker in chondrogenesis, respectively. Staining of type II collagen was similar among the three coculture groups on day 7 (Fig 3). However, most dense and homogeneous staining was observed in the 1:1 coculture group on day 21. On the other hand, staining of type X collagen was most prominent in the SDSC monoculture group (Fig 4). Slight staining was observed in the chondrocyte monoculture group and the three coculture groups on day 21. Immunohistochemistry staining for type II and X collagens were well correlated with gene expression results based on qRT-PCR.

A

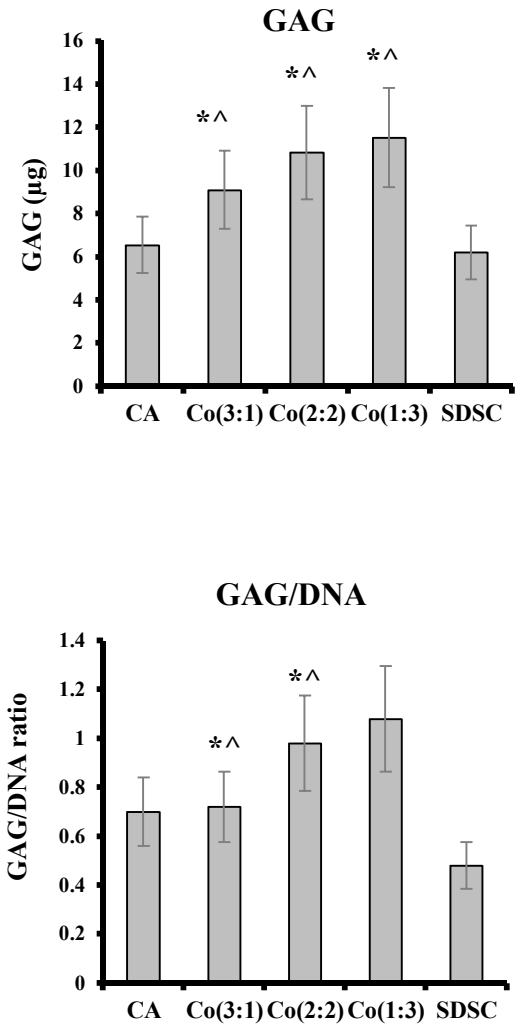

Figure 1-A. Evaluation of GAG synthetic activity (GAG content and GAG/DNA ratio).

B

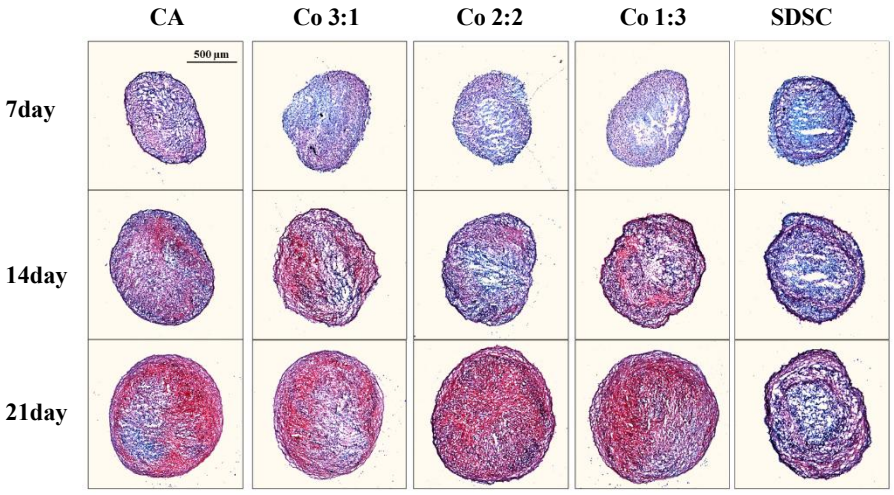

Figure 1-B. Histological evaluation of GAG production with Safranin-O staining.

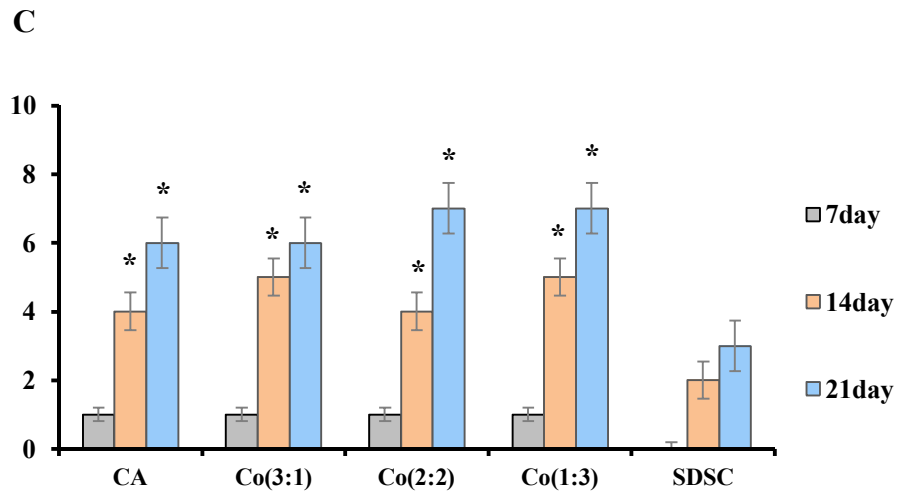

Figure 1-C. Histologic scoring (Bern Score). Results are presented as mean ± SD (n = 6). * and ^ indicate significant difference compared to SDSCgroup and chondrocyte group, respectively ($p < 0.05$). CA: chondrocyte; Co: coculture; SDSC: synovium derived mesenchymal stem cell

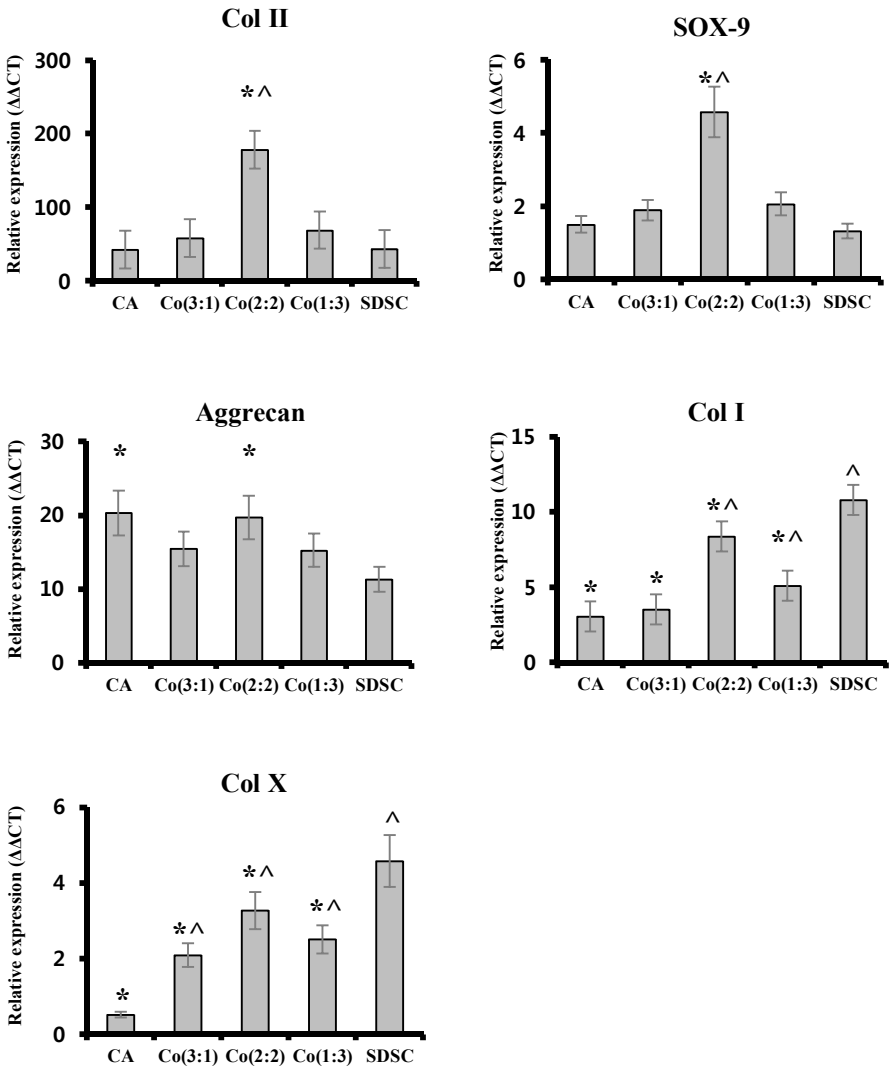

Figure 2. RT-PCR analysis for chondrogenesis-related gene expression after 21 days of culture period. Results are presented as mean ± SD (n = 6). * and

^ indicate significant difference compared to SDSC group and chondrocyte group, respectively ($p < 0.05$). CA: chondrocyte; Co: coculture; SDSC: synovium derived mesenchymal stem cell.

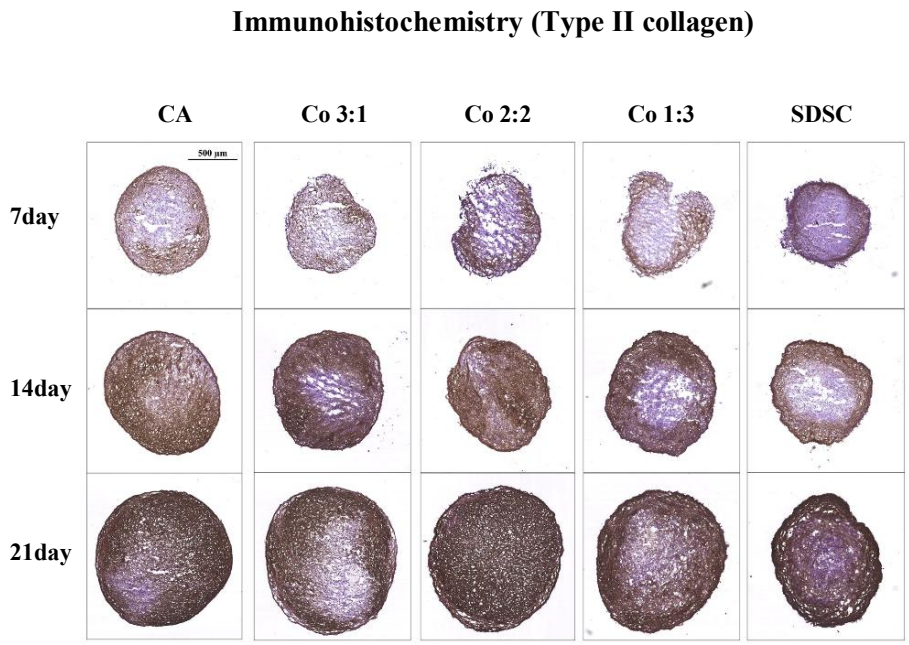

Figure 3. Immunohistochemistry for type II collagen chondrogenic marker. Staining on day 21 was the most prominent in the 2:2 ratio coculture group. CA: chondrocyte; SDSC: synovium derived mesenchymal stem cell.

Immunohistochemistry (Type X collagen)

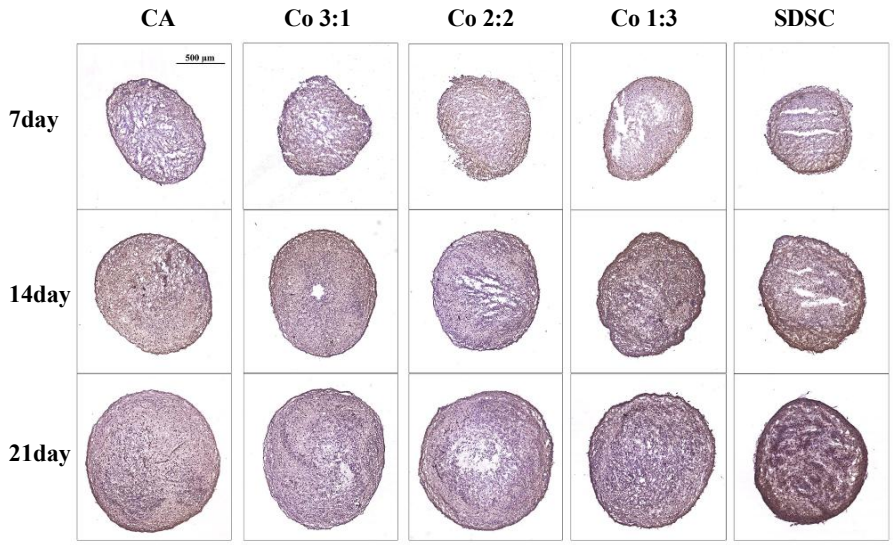

Figure 4. Immunohistochemistry for type X collagen hypertrophic marker. Staining of SDSC group was prominent compared to that in the three coculture groups on day 21. CA: chondrocyte; SDSC: synovium derived mesenchymal stem cell.

I-3. Discussion

Coculture strategy of chondrocytes and MSCs has been presented as a solution to improve autologous chondrocyte transplantation because the chondrogenic phenotype of chondrocytes can be maintained during *in vitro* expansion. In addition, required amount of cartilage for *in-vitro* culture can be reduced in proportion to mixed amount of MSCs, resulting in decrease of donor site morbidity. SDSC has been reported to possess superior chondrogenic potential to other MSCs and known to be tissue-specific for cartilage engineering (12-14, 34). Also, synovium can be obtained arthroscopically with minimum invasiveness during cartilage harvest procedure (13). Therefore, additional procedure for tissue harvest is unnecessary and complications such as pain and hematoma during BMSCs harvest can be avoided (35). However, whether direct coculture of human chondrocyte and SDSC can enhance chondrogenesis with reduced hypertrophy has not been clearly proved.

In the present study, direct coculture of human chondrocytes and SDSCs enhanced chondrogenesis compared to monoculture of chondrocyte or SDSC. Coculture groups were evaluated at three different mixed ratio of chondrocytes and SDSCs (3:1, 1:1, and 1:3) to find the optimal ratio for chondrogenesis. Results of GAG assay revealed that the GAG synthetic activities in the 1:1 and 1:3 coculture groups were significantly higher

compared to those of chondrocyte and SDSC monoculture groups. The 1:3 coculture group showed the highest GAG synthetic activity among the three coculture groups. These findings were very similar to the results of a coculutre study of Lai et al. using human chondrocytes from the patients undergoing TKA and adipose derived stem cells (36). Their results revealed that the coculture groups showed superior GAG synthetic activities to SDSC or chondrocyte groups, especially at 1:1 and 1:3 ratios. On the other hand, GAG activity of the chondrocyte group was comparable to that of coculture groups in the study of Meretoja et al. using bovine primary chondrocytes and BM-MSCs (5). We assum that chondrogenic potential of chondrocyte or coculture groups can be affected by donor age or cell passage of chondrocytes and MSCs.

Gene expression analysis revealed that the levels of type II collagen and SOX-9 were significantly increased in the 1:1 coculture group compared to those in chondrocyte and SDSC monoculture groups. However, the expression levels of aggrecan were similar to each other between the chondrocyte monoculture group and the 1:1 coculture group. Low level of type II collagen in chondrocyte group can be interpretated in the same way with low GAG activity in chondrocyte group as above. Overall, the levels of chondrogenesis-related genes were upregulated in the 1:1 coculture group compared to those in other groups. On the other hand, the level of type I collagen in the SDSC monoculture group was significantly increased compared to that of 1:3 and 3:1 coculture groups. Relatively higher level of type I collagen in 1:1 coculture group can be related with highly expressed type II collagen. However, exact cellular mechanism of this finding is not clear and further study including change of fibroblast following coculture

seem to be necessary. The expression of collagen type I and type II and aggrecan in this study were similar to those in the coculture study of Lai et al. except that the 1:3 ratio group also showed comparable chondrogenic potential to the 1:1 ratio group in their study (36). The difference between adipose derived MSC and synovium derived MSC in the two studies might have affected the optimal coculture ratio.

In previous direct coculture studies with various mixed ratio, the optimal ratio of chondrocytes to bone marrow-derived MSCs or adipose-derived MSCs ranged from 25% to 50% in the majority of these studies (5, 36, 37). However, coculture study of chondrocytes with SDSCs at different ratios has not been performed yet. In this study, optimal mixed ratio for coculture of chondrocytes and SDSCs was found to be from 25% to 50% of chondrocyte, similar to those of coculture studies using bone marrow-derived MSCs or adipose-derived MSCs.

Another remarkable finding of this study was the decrease of type X collagen, a hypertrophic marker, in the coculture groups compared to that in the SDSC monoculture group. MSCs can express hypertrophic phenotype under chondrogenic induction, resulting in calcification of extracellular matrix (38). These results can limit the clinical application of MSCs for the treatment of cartilage injury. Some authors suggest that type X collagen cannot be ideal hypertrophic marker for MSCs because it can increase before MSCs differentiate into chongrogenic cells(39). However, early expression of type X collagen was not proved in coculture study and various coculture studies have evaluated MSCs hypertrophy using type X collagen. Cooke et al. and Glovannini et al. have reportedthat coculture of chondrocyte and bone marrow derived MSCs can reduce the expression of type X collagen (9, 10).

Decreased hypertrophy of adipose derived MSC has also been observed in the coculture study of Lee JS et al. (40). However, whether coculture of SDSC and chondrocyte can reduce hypertrophy of SDSC has not been investigated yet. The results of the present study can be used as a basis for clinical use of SDSC in terms of hypertrophy prevention.

Regarding the enhanced chondrogenesis in direct mixed coculture, the exact cellular mechanism remains unclear. Some studies have suggested that MSC differentiation is essential to the chondrogenic mechanism following direct coculture (8, 40). On the other hand, Wu et al. have reported that MSCs can stimulate cartilage formation due to a trophic effect on chondrocytes rather than differentiating into chondrocytes incoculture pellets (37). In the present study,chondrogenic phenotype was expressed in both of chondrocyte and SDSC monocultures. Therefore, we can suggest that chondrogenesis of direct coculture can be achieved by mutual synergism of chondrocyte redifferentiation and chondrogenic differentiation of SDSC. Although exact contribution of each cell cannot be determined, it is clear that a combination of human chondrocyte and SDSC can enhance chondrogenesis and this combination can be a proper cell source to overcome the limitation of current ACT treatment.

There is a limitation in this study. The human chondrocytes and SDSCs investigated in this study were obtained from old female patients undergoing total knee arthroplasty. It has been reported that the proliferation and chondrogenic potential of chondrocyte can be influenced by donor age(41). Considering that autologous chondrocyte transplantation is recommended for patients under 45-50 years old, chondrocytes from TKA might be less ideal cell source. However, it is not easy to obtain healthy cartilage from young

donor for ethical reasons. This may be the reason why several coculture studies have also obtained human chondrocytes from arthroplasty surgery (17, 42, 43). On the other hand, Kubosch et al's recent study showed that expression level of type II collagen in SDSCs was not affected by age and arthritis of donor (44). Although donor age might be a limitation factor, this study demonstrated meaningful comparison of chondrogenic potential among coculture groups and monoculture groups of each cell type.

Chapter II.

Investigation on the inflammatory mediators between synovium derived stem cells and chondrocytes in indirect coculture

II-1. Materials and Methods

Isolation and culture of SDSCs and chondrocyte

Synovium and cartilage tissues were obtained from two female osteoarthritis patients (age of 68 and 71 years) undergoing total knee arthroplasty(TKA). Study was performed using relatively intact cartilage from lateral femoral and tibial condyles. Synovium was harvested from the suprapatellar pouch. Ethical approval for this study was obtained from Seoul National University Boramae Medical Center Institutional Review Board (06-2012-25). Those who had inflammatory arthritis, prior knee joint infection, and intraarticular trauma were excluded. Isolation and culture of SDSCs and chondrocyte was performed in the same way with direct coculture study as above. Passage 2 SDSCs and chondrocytes were used in all experiments.

Indirect co-culture with SDSCs and chondrocyte in transwell systems

SDSCs were seeded at cell density of 5×10^5 cells in monolayer on the bottom of Transwell plates. Chondrocytes were seeded at cell density of 5×10^5 cells onto the inserts (0.4-μm pore size; Corning) of Transwell using complete medium and appropriate culture environment. The control group was established separately with chondrocyte and SDSCs in culture plates. Cells

were then treated with IL-1β 10ng/ml (Peprotech, London, UK) in Opti-MEM (Gibco BRL, Grand Island, NY) containing 1% antibiotic–antimycotic solution (100U=mL penicillin, 100 mg=mL streptomycin, and 0.25 mg=mL amphotericin B) to reproduce osteoarthritic environment. Concentration of IL-1β was determined based on the previous studies that evaluated coculture of articular chondrocytes and MSCs in osteoarthritic model(45, 46). The medium was changed every 2–3 days and stored in deep freezing state for secretome analysis. Samples of cells and culture supernatant were taken on 7 days for gene and protein expression analysis.

Antibody array

Eight secretome samples (4 cocultured, 4 monocultured) from two different donors were tested and soluble form of proteins were analyzed. The protein was extracted by using protein extraction buffer (Fullmoon biosystems, Sunnyvale, CA) containing 1% protease inhibitor cocktail (Sigma, St. Louis, Mo) and 1% phosphotase inhibitor cocktail (Sigma, St. Louis, Mo) and lysis beads (Fullmoon biosystems, Sunnyvale, CA). After extraction, the protein solution was purified using gel matrix column that was included in antibody array assay kit (Fullmoon biosystems, Sunnyvale, CA). The column was vortex-mixed at 5 seconds and hydration-treated at 60 minutes on room temperature. After hydration, the column was centrifuged at 750 x g for 2 minutes. After centrifuge, the column was placed into a collect tube and the 100 ul of protein sample was transferred into column. The column was

centrifuged at 750 x g for 2 minutes. The concentration of purified sample was measured with BCA protein assay kit

(Pierce, Rockford, Ill) using NanoPhotometer^{TM} (Implen, UK). And the purity of purified sample was confirmed on UV spectrum.

The 50 ug of protein sample was filled up 75 ul with labeling buffer and treated 3 ul of the 10 ug/ul biotin/DMF solution. The sample was incubated at room temperature for 90 min with mixing. After incubation, the sample was treated 35 ul of stop reagent and incubated at room temperature for 30 min with mixing. The antibody microarray slide (Fullmoon biosystems, Sunnyvale, CA) was treated 30 ml of blocking solution in a petri dish and incubated on shaker at 60 rpm for 30 min at room temperature and washed with distilled water. This step was replicated three times. After blocking, the slide was rinsed with Milli-Q grade water.

The labeled sample was mixed in 6 ml of coupling solution. The blocked array slide was incubated with coupling mixture on shaker at 60 rpm for 2 hours at room temperature into coupling dish. After coupling, the slide was washed 6 times with 30 ml of washing solution into petri dish on shaker at 60 rpm for 5 minutes. And the slide rinsed with Milli-Q grade. The 30 ul of 0.5 mg/ml Cy3-streptavidin (GE Healthcare, Chalfont St. Giles, UK) was mixed in 30 ml of detection buffer. The coupled array slide was treated with detection mixture into petri dish on shaker at 60 rpm for 20 minutes at room temperature. After detecting, the slide was washed 6 times with 30 ml of

washing solution into petri dish on shaker at 60 rpm for 5 minutes. And the slide rinsed with Milli-Q grade water.

The slide scanning was performed using GenePix 4100A scanner (Axon Instrument, USA). The slides were absolutely dried before the scanning and scanned within 24-48 hours. The slides were scanned at 10 um resolution, optimal laser power and PMT. After got the scan image, they were grided and quantified with GenePix 7.0 Software (Axon Instrument, USA). The numeric data were analyzed using Genowiz 4.0^{TM} (Ocimum Biosolutions, India). After analyzing, the data about protein information was annotated using UniProt DB.

mRNA Expression in quantitative reverse transcriptase–polymerase chain reaction (RT-PCR)

After 7 days of culture , total RNA was extracted from SDSCs and chondrocyte using TRIzol reagent (Invitrogen). Complementary DNA was synthesize using RNA to cDNA synthesis kits (Clontech, CA). Briefly, 1 mg of RNA, oligo-dT hexamer primer, 5_reaction buffer, RNase inhibitor, and10mMdNTP mix were mixed with SMART MMLV reverse transcriptase. Real-time quantitative PCR assay was performed using Primer Express software version 1.5 AppliedBiosystems, Abingdon, UK) to overlay a junction between two exons to avoid hybridization to genomic DNA. mRNA levels were quantified

by SYBR premix Ex tag (Takara Bio Inc., Otsu,Japan) and ABI Prism 7000 (Applied Biosystems). Each sample (each reaction, 100 ng cDNA; total volume, 25 ml) was run in triplicate. The cycling parameters were 95℃ for 10 min, followed by 40 cycles of 95℃ for 15 s and 60℃ for 1 min and 72℃ for 30 sec. The expression of Fibronectin, MMP-2, Caveoline-1, ANGPT-1, FABP4, ICAM-1 and VEGFB was analyzed. *GAPDH* was used as housekeeping gene. Gene expressions were calculated using the relative expression levels were calculated using the $2^{-\Delta\Delta CT}$ method (ABI Prism 7000, User Bulletin 2). To investigate the effect of inflammatory environment on the results, RT-PCR was performed twice with or without IL-1beta treatment. The primers used for PCR are described in Table2.

Immunoblotting (Western blotting)

Immunoblotting was performed with cocultured chondrocytes and SDSCs. Total protein were isolated using RIPA buffer (Thermo Scientific) with 1% protease inhibitor complete (Roche, Manheim, Germany). Protein concentrations were determined by the bicinchoninic acid (BCA) protein assay (Pierce Chemical, Rockford, IL, USA). For each sample, 10ug of total protein fraction was separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels and transferred on polyvinylidene fluoride (PVDF) membranes by Western blotting. Membranes were blocked for 1 h in 0.5X Tris buffered saline-tween20 (TBS-T) containing 5% bovine serum

albumin, washed three times in 0.5X TBS-T. All of primary antibodies for Western blot analysis were purchased from R&D systems (MMP-2, ANGPT-1, FABP4, Fibronectin) and were diluted 1:1000 with 0.5X Tris-Buffered Saline-Tween-20 (TBS-T) and 5% bovine serum albumin. The membranes were further incubated with 1:3000 diluted horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. Developing was performed using ECL Western blotting substrate (Thermo Scientific). As a standard protein for protein quantization, β-actin(Sigma, Saint Louis, MO) was detected from same membrane. The blots were visualized by means of Amersham Imager 600 imagers system (AI600, GE Healthcare, Chalfont St. Giles, UK) using manufacturer's instruction. Image analysis and quantification were performed using the National Institute of Health Image J freeware (release 1.44X; http://rsb.info.nih.gov/ij/). Western blotting was also performed twice with or without IL-1beta treatment.

Statistics

Statistical analysis was performed using SPSS 18.0 software (SPSS, Chicago, IL). Mann–Whitney test was used to compare gene expressiong and protein intensity ratio. Statistical significance was considered when *p*-value was less than 0.05.

Table 2. Probe sequence of each gene used for RT-PCR

Gene	Primer
Fibronectin	Fw: AAACCAATTCTTGGAGCAGG
	Rv: CCATAAAGGGCAACCAAGAG
MMP2	Fw: TGGCAAGTACGGCTTCTGTC
	Rv: TTCTTGTCGCGGTCGTAGTC
Caveolin-1	Fw: CTGGGGGCAAATACGTAGA
	Rv: CTTGACCACGTCATCGTTG
ICAM-1	Fw: GGCCGGCCAGCTTATACAC
	Rv: TAGACACTTGAGCTCGGGCA
ANGPT1	Fw: AGCAACTGGAGCTGATGGAC
	Rv: TCTTCCTCTCTTTTTCCTCCCT
FABP4	Fw: GGAATGCGTCATGAAAGGCG
	Rv: GCGAACTTCAGTCCAGGTCA
VEGFB	Fw: GATCCGGTACCCGAGCAGTCAG
	Rv: CACCTGCAGGTGTCTGGGTTGA

GAPDH	Fw:	ATTGTTGCCATCAATGACCC
	Rv:	AGTAGAGGCAGGGATGATGTT

II-2. Results

Differential protein expression identified by AbM profiling

Differentially expressed proteins between coculture and monoculture secretomes were selected based on the fold change criteria (>1.5 -fold or <0.66 -fold). Compared with monoculture of SDSCs, 28 proteins were up-regulated and 32 proteins were down-regulated with statistical significance in the coculture secretome. Also, compared with monoculture of chondrocyte, 23 proteins were up-regulated and 54 proteins were down-regulated in the coculture secretome (Table 3). After an extensive literature review focused on the association of the expressed proteins with inflammatory and secretory properties, MMP-2, angiopoietin-1, and Caveolin-1 were selected as up-regulated candidate and ICAM-1,FABP4, Fibronectin, and VEGF-B were selected as down-regulated candidate for inflammatory mediator between chondrocytes and SDSCs

Evaluation of candidate protein expression in cell lines

-Validation of up-regulated proteins via quantitative RT-PCR and immunoblotting

To validate proteomic findings, the gene expression of up-regulated proteins (MMP-2, antiopoietin-1, and Caveolin-1) were examined by RT-PCR. The expression level of MMP-2 in cocultured chondrocytes and SDSCs were significantly increased compared with that of monocultures. Increased level of MMP-2 in cocultured chondrocytes and SDSCs also detected in the immunoblotting (western blot). Also, the expression level of ANGPT1 in chondrocyte cocultured with synovial cell was increased compared with monoculture in RT-PCR. However, increase of ANGPT1 in cocultured chondrocyte was not observed in the immunoblotting. On the contrary, the expression level of Caveolin-1 in synovial cells cocultured with chondrocytes were reduced compared with that of monoculture, and the result of RT-PCR was not coincide with the result of antibody array. Therefore, subsequent immunoblotting was not performed on Caveolin-1. On the basis of microarray, RT-PCR, and Western blot results, MMP-2 could be selected as up-regulated inflammatory mediator between chondrocyte and SDSCs.

- Validation of down-regulated proteins by quantitative RT-PCR and immunoblotting

To validate proteomic findings, the gene expression of down-regulated proteins (FABP4, Fibronectin, ICAM-1, and VEGF-B) were examined by RT-PCR.. The expression level of FABP4 in SDSCs cocultured with chondrocyte was significantly decreased compared with that of synovial cell moculture, and RT-PCR result was correlated well with antibody microarray results.

Decreased level of FABP4 in SDSCs cocultured with chondrocyte also detected in western blot. The expression of Fibronectin was also decreased in synovial cell cocultured with chondrocyte compared with SDSCs monoculture in RT-PCR. However, in the western blot, Fibronectin was increased in cocultured SDSCs compared with moculture. On the contrary, the expression level of ICAM-1 and VEGFB was increased in cocultured chondrocyte and the result of RT-PCR was not coincide with the result of antibody array. Therefore, subsequent immunoblotting was not performed on these two proteins. On the basis of microarray, RT-PCR, and Western blot results, FABP4 could be selected as down-regulated inflammatory mediator between chondrocyte and SDSCs.

Table 3. Up or Down- regulated protein lists and fold change values in antibody microarraty profiling

Up-regulated Proteins	fold	Down-regulated Proteins	fold
Actin-alpha-1	4.723	NKX2.5	0.111
MMP-2*	2.971	C-Kit	0.293
KCNJ2	2.734	HSP90A	0.347
beta-2-Microglobulin	2.376	Fibronectin*	0.387
Dynamin-1	2.282	SOD1	0.392
GPR151	2.223	Tubulin alpha	0.397
Claudin 5	2.179	p42 MAPK	0.433
Pax-5	2.145	FABP4*	0.480
Lamin A	1.999	MUM1	0.515
Caveolin-1*	1.864	Ferritin	0.517
Tubulin alpha	1.838	VEGFB*	0.519
Angiopoietin-1*	1.811	Ferritin	0.520
MAPK 11	1.801	Claudin 3	0.530
ARSI	1.778	ARSI	0.533
Lck	1.742	ARC	0.535
ATP2C1	1.727	Ku70/80	0.541
GPR132	1.633	ICAM1*	0.546
Cullin 1	1.611	AFP	0.564
Desmin	1.580	FAK	0.566
DP-1	1.570	GSK3 beta	0.566

"*" indicates seven selected inflammation-related secretory proteins

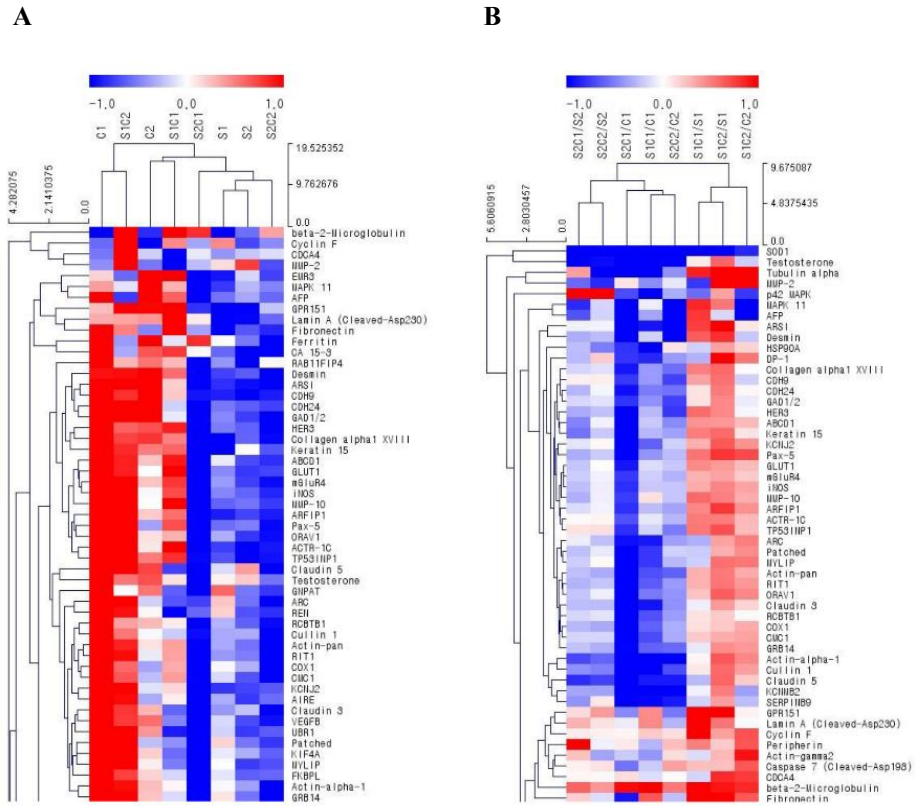

Figure 5. Antibody microarray profiling. **A.** Differential protein expression between indirect coculture of chondrocytes and SDSCs and monoculture of each cell. Red indicate higher expression and blue indicate lower expression of proteins. **B.** Expression of proteins up or down-regulated more than 1.5 folds in coculture groups. Red indicate up-regulation in coculture groups and blue indicat down-regulation in coculture group.

A

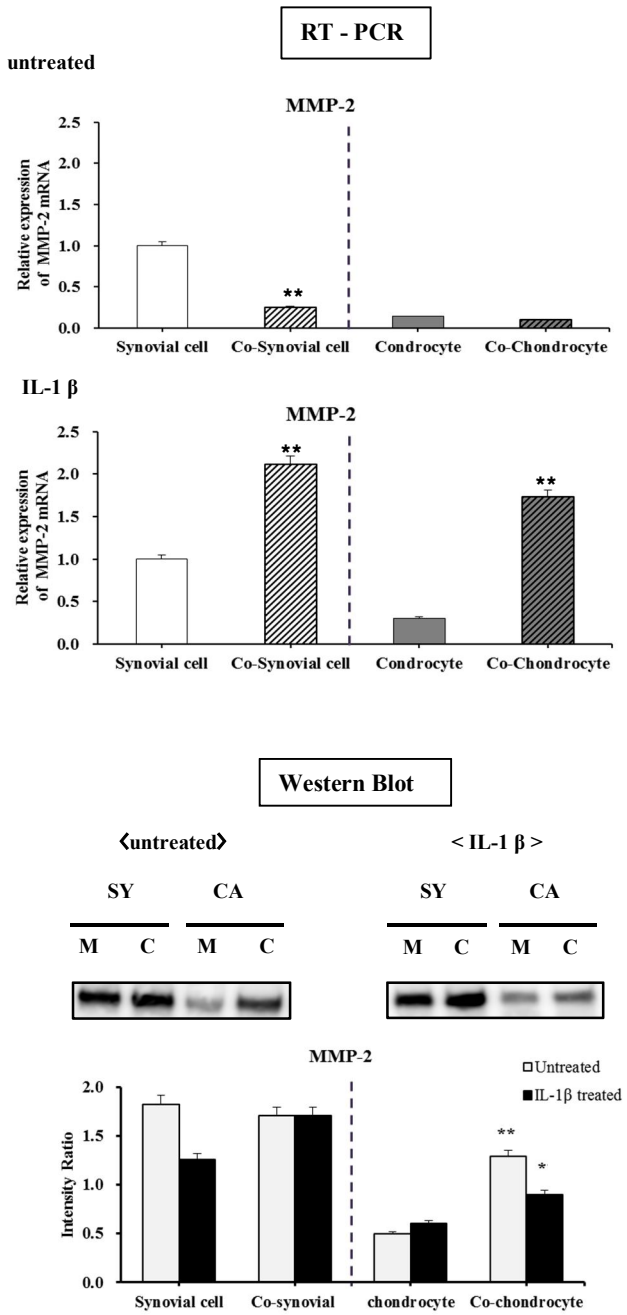

Figure 6-A. Up-regulation of MMP-2 in cocultured chondrocyte was observed consistently in microarray, RT-PCR, and western blot. Data in the bar graph represent the means±SD. **P*<0.05; ***P*<0.01

B

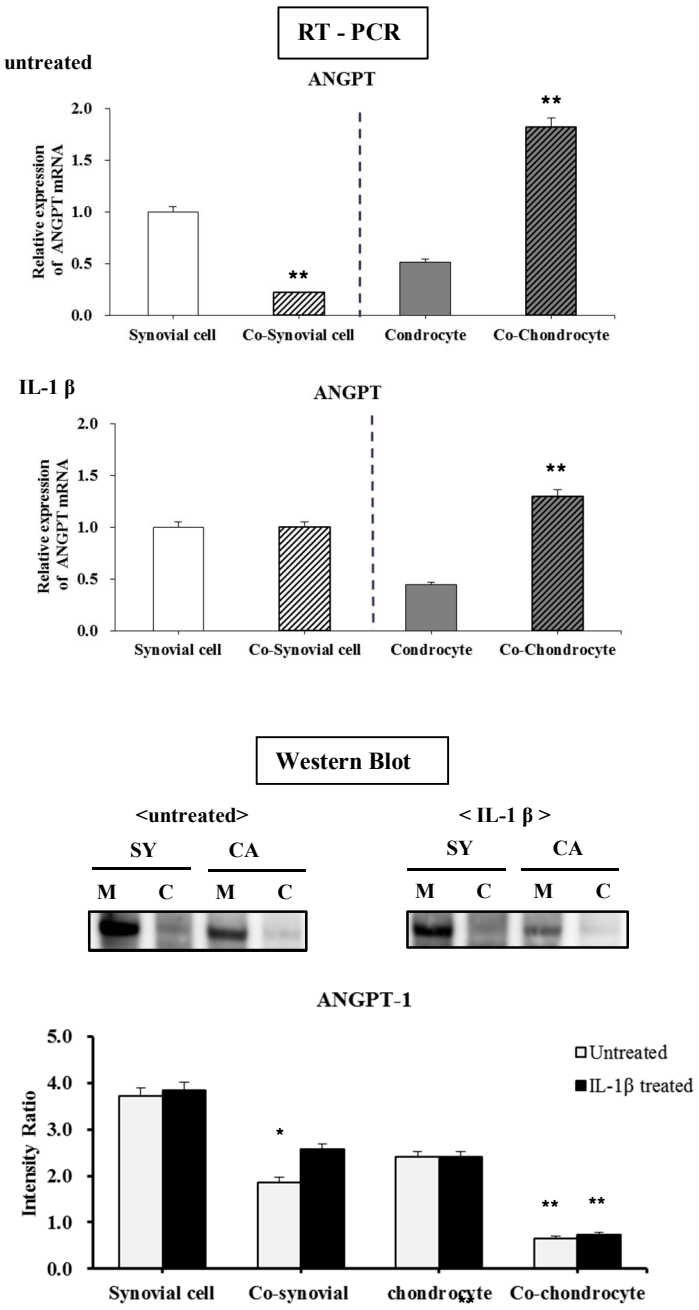

Figure 6-B. Up-regulation of ANGPT in cocultured chondrocyte was observed in microarray and RT-PCR. However, ANGPT was down-regulated in the western blot. Data in the bar graph represent the means±SD. **P*<0.05; ***P*<0.01

C

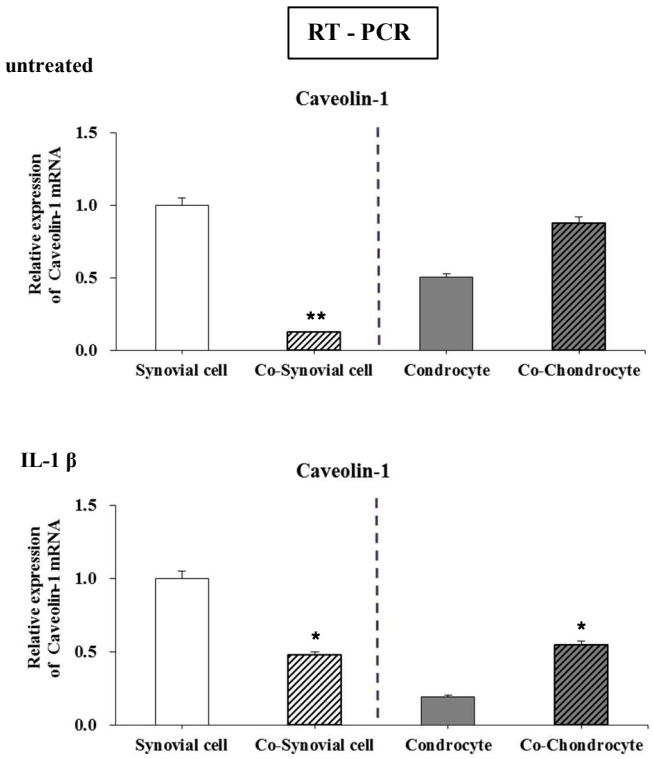

Figure 6-C. Up-regulation of Caveolin-1 in cocultured synovial cell was observed only in microarray. However, Caveolin-1 was down-regulated in RT-PCR. Therefore, western blot was not performed. Data in the bar graph represent the means±SD. **P*<0.05; ***P*<0.01

A

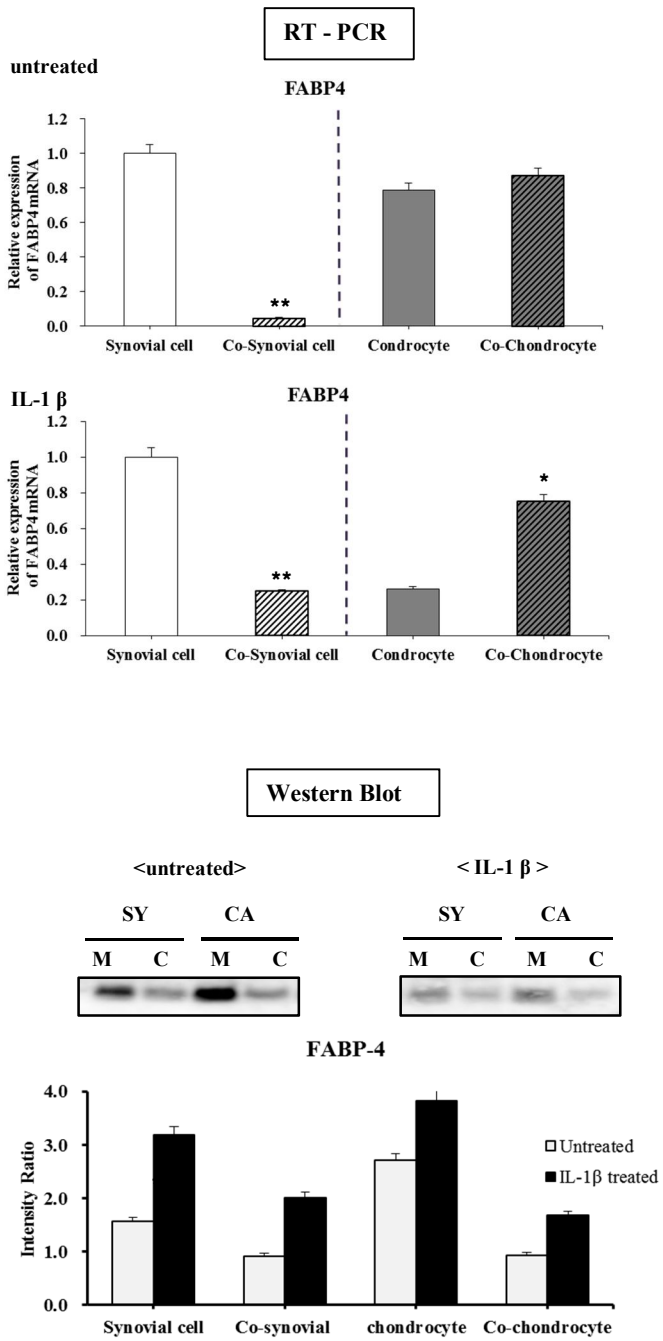

Figure 7-A. Down-regulation of FABP4 in cocultured SDSCs was observed consistently in microarray, RT-PCR, and western blot. Data in the bar graph represent the means±SD. **P*<0.05; ***P*<0.01

B

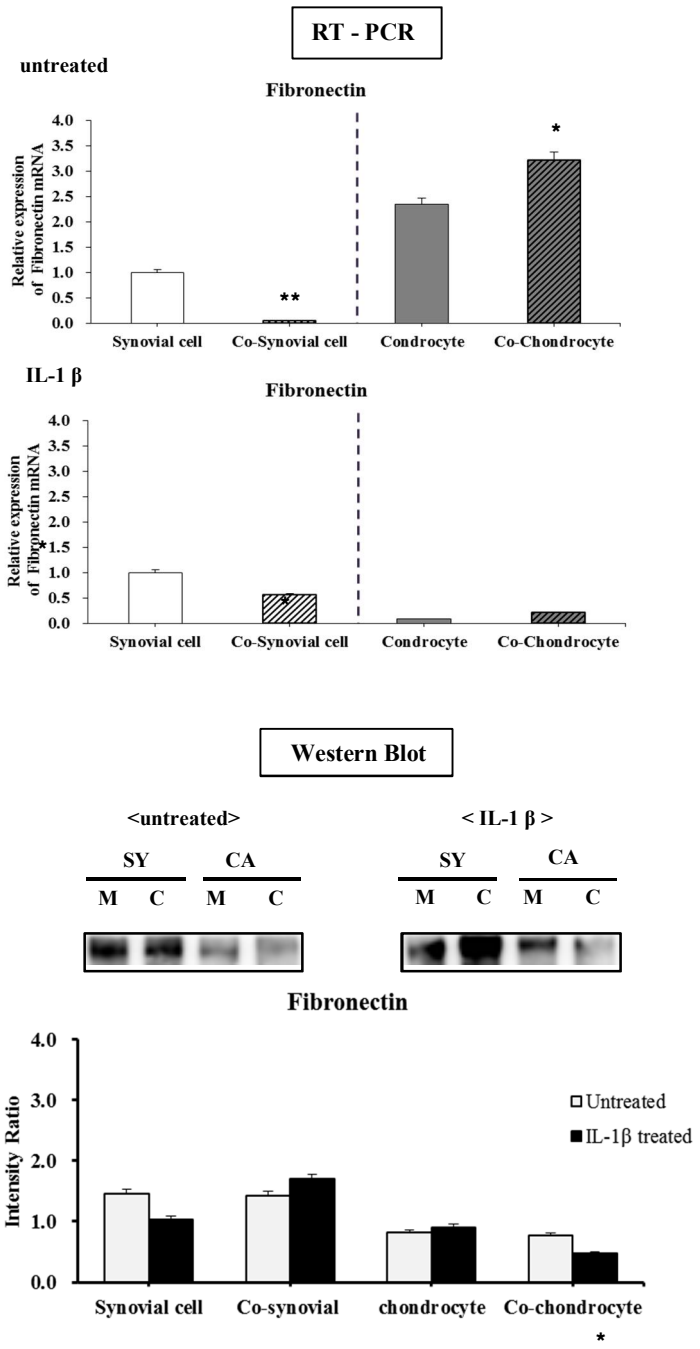

Figure 7-B. Down-regulation of Fibronectin in cocultured SDSCs was observed in microarray and RT-PCR. However, Fibronectin was up-regulated in the western blot. Data in the bar graph represent the means±SD. **P*<0.05; ***P*<0.01

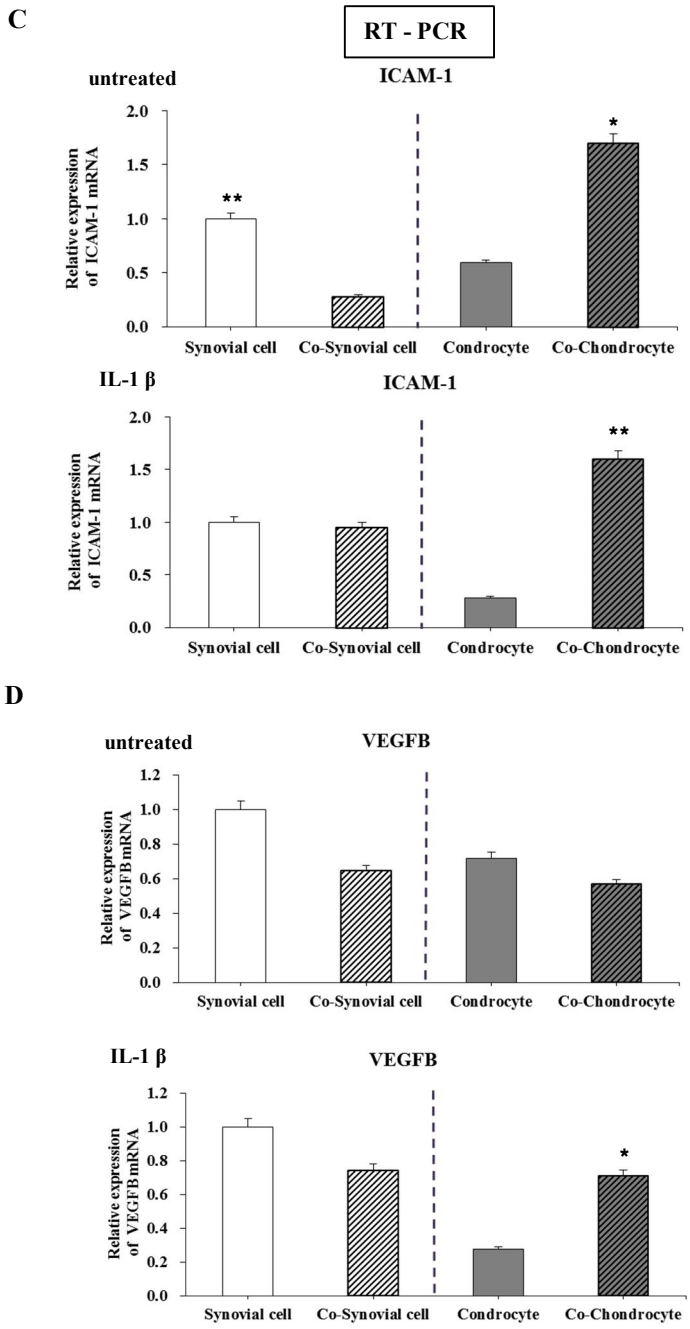

Figure 7-C,D. ICAM-1 and VEGFB was down-regulated in microarray. However, up-regulation of ICAM-1 and VEGFB was observed in RT-PCR. Therefore, western blot was not performed. Data in the bar graph represent the means±SD. **P*<0.05; ***P*<0.01

II-3. Discussion

Synovium is a specialized connective tissue that lines inner surface of synovial joints and it contains various type of synovial cells such as synovial fibroblast, macrophage, and even synovium derived stem cell(SDSC)(25). Inflammation of synovium and pro-inflammatory cytokines released from synovium have important role in the progression of osteoarthritis(OA) and degradation of cartilage(19). Interaction between synovial fibroblast and articular chondrocyte can inhibit the synthesis of proteoglycan and chondrogenesis(21-23).

On the contrary, Koh et. al recently reported that coculture of SDSCs and chondorocytes can reduce inflammatory activity of chondrocyte(24). Also, anti-inflammatory effect of other mesenchymal stem cells (MSCs) such as bone marrow derived MSCs and adipose tissue derived MSCs have been reported in several studies(47). However, little is known about the inflammatory interaction between SDSCs and chondrocytes. Also, we could not find any published data that analyzed the secretome between the two cells using protein microarray. Therefore, in this study, we intended to investigate inflammation related secretory proteins between SDSCs and chondrocytes using indirect coculture system and secretome antibody microarray.

In comparative proteomic analysis, MMP-2, angiopoietin-1, and Caveolin-1 were selected as up-regulated inflammation-related secretory proteins between SDSCs and chondrocytes. MMP-2 is type IV collagenase and regulate vascularization and inflammation in OA(48). Angiopoietin-1 has important role in vascular development and angiogenesis. It also, expressed in

the synovium of rheumatoid arthritis patients and related with inflammation in angiogenesis(49). Caveolin-1 is plasma membrane protein and can exist as secretory protein. It is related with regulation of synovial inflammation(50).
On the other hand, FABP4, Fibronectin, ICAM-1,and VEGF-B were selected as down-regulated inflammation-related secretory proteins between SDSCs and chondrocytes. FABP4 is expressed mainly in adipose tissue and reported to be increased in joint fluid in rheumatoid arthritis(51). Fibronectin and ICAM-1 are glycoprotein that regulate cell migration and related with rheumatoid arthritis(52, 53). VEGF-B is known to be related with inflammation-associated synovial angiogenesis(54).

Among these candidates, only up-regulated MMP-2 in cocultured cells and down-regulated FABP4 in cocultured SDSCs were consistently confirmed in both of RT-PCR and western blot. Although role of MMP-2 in controlling and regulating extracelluar matrix of cartilage has been studied extensively, limited numbers of studies that investigated the effects of MMP-2 on MSCs have been reported(48). Marquets et al. reported that MMP-2 is related with proliferation and migration of cord-derived MSCs(55). Also, Feng et al.'s study showed that MMP-2 regulated MSCs chondrogenesis(56). However, little is known about MMP-2 effect on SDSCs. FABP4 has been considered to be closely related with fat metabolism and related disease. However, Chaofan et al. reported that FABP4 is elevated in serum and joint fluid of OA patients and FABP4 can be biomarker for knee OA in recent study(57). Although the mechanism of FABP4 elevation in knee OA is not clear, down regulated FABP4 in cocultured SDSCs can be interpreted as beneficial change for osteoarthritis. To clarify the role of MMP-2 and FABP4 in the interaction of chondrocytes and SDSCs, further studies that investigate the effect of MMP-2

and FABP4 on chondrogenic, proliferative, and inflammation-related phenotypes of each cell (chondrocytes and SDSCs) is necessary.

This study has some limitations. First, secretome antibody microarray was performed one time and sample size was relatively small in this study. However, to compromise this limitation, chondrocytes and SDSCs from two different patients were combined each other and 4 monoculture secretomes and 4 coculture secretomes were evaluated in antibody microarray. Also, all kinds of proteins that were up or down regulated more than 1.5folds in any one of specimen were thoroughly evaluated. Second, in this study, western blot was performed with chondrocytes and SDSCs itself instead of secretomes. Evaluation of intracellular proteins can provide the information about the contribution of each cell to the up or down regulation of the inflammation-related proteins. However, the amount of secreted proteins can be changed with this method compared with secretome analysis. For more defined result of the study, further evaluation with secretomes should be considered.

Conclusion

Direct coculture of human chondrocytes and SDSCs significantly enhanced chondrogenic potential, especially at 1:1 ratio, compared to chondrocyte or SDSC monocultures. Chondrogenesis-related genes (Type II collagen, SOX-9, and Aggrecan) were highly expressed and GAG synthesis was increased in coculture groups. On the contrary, hypertrophic marker (Type X collagen) was decreased in the coculture group. In secretome antibody microarray based

on the indirect coculture of chondrocytes and SDSCs, up-regulated MMP-2 and down-regulated FABP4 was observed consistently in all of secretome microarray, RT-PCR, and immunoblotting. Therefore, MMP-2 and FABP4 can be selected as possible inflammatory mediators between chondrocytes and SDSCs. However, further study is necessary to clarify the role of MMP-2 and FABP4 in the interaction between the two cells.

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국문 초록

목 적: 본 연구에서는 인체활막줄기세포와 연골세포간의 직접공동배양이 각 세포의 단독배양보다 연골형성능을 향상시킬 수 있는지 조사하고자 하였다. 또한 분비단백질의 항체 유전자미세배열분석을 통해 활막줄기세포와 연골세포간의 염증성 매개 분비 물질을 규명하고자 하였다.

대상 및 방법: 인체활막줄기세포와 연골세포간의 직접공동배양이 연골형성능을 향상시킬 수 있는지를 평가하기 위해서 먼저, 연골세포와 활막줄기세포를 3:1, 2:2, 1:3의 서로 다른 비율로 혼합한 세 개의 공동배양군과 연골세포나 활막줄기세포의 단독배양군등 총 5개의 배양군을 대상으로 21일까지 pellet 배양을 시행하였다. 배양 7,14,21일에 각 군의 GAG, DNA, GAG/DNA ratio, 연골형성에 관련된 유전자(type I,II,X collagen,SOX-9,aggrecan)의 발현양상, 조직학적 염색소견등을 PT-PCR, 측정하여 각 군간의 비료평가를 시행하였다.

인체활막줄기세포와 연골세포간의 염증성 매개물질을 규명하기 위해서,두 세포의 간접공동배양과 단독배양에서 얻어진 단백질 분비물을 이용하여 항체 유전자미세배열분석을 시행하였다. 유전자미세배열분석결과,간접공동배양에서 단독배양보다 1.5배 이상 증가하거나 감소한 단백질을 정리하고 이 중에서 염증성 분비가 가능한 단백질들을 선별하였다. 이어서 유전자 분석과 면역블로팅법을 이용하여 선별된 단백질의 증감을 확인하였다.

결 과: 활막줄기세포와 연골세포를 공동배양한 세 개의 군에서 배양

21일째에 연골세포 또는 활막줄기세포 단독배양군보다 연골형성능이 유의하게 증가된 소견이 GAG/DNA assay와 gene expression study에서 관찰되었으며 이러한 양상은 2:2 비율의 공동배양군에서가장 뚜렷하게 나타났다. 이러한 결과는 GAG contents를 평가하기위한 safranin-O 염색과, typeII collagen에 대한 면연조직염색에서도 일치하는 소견을보였다. 또한 활막줄기세포의 배양시 발생할 수 있는 부작용인 과발현이나 type I collagen형성의 증가소견은 공동배양군에서 활막유래줄기세포 단독배양군보다 유의하게 감소한 결과를 보였다.

활막줄기세포와 연골세포의 간접공동배양에서 얻어진 분비단백질의 항체 유전자미세배열분석결과, 28개의 단백질이 단독배양군보다 공동배양군에서 1.5배이상 증가하였고 32개의 단백질이 단독배양군보다 공동배양군에서 1.5배이상 감소하였다. 이 중에서 염증성 분비가 가능한 7개의 단백질 (MMP-2, angiopoietin-1,Caveolin-1,ICAM-1,FABP4, Fibronectin, and VEGF-B)을 선별하여 유전자 분석과 면역블로팅을 시행하였다. 그 결과 공동배양된 연골세포와 활막줄기세포에서 MMP-2가 증가되고, 공동배양된 활막줄기세포에서 FABP4가 감소된 소견이 유전자미세배열분석, 유전자분석, 면역블포팅 세가지 검사에서 모두 동일하게 관찰되었다.

결 론: 인체연골세포와 활막줄기세포의 직접공동배양은, 특히 1:1 비율의 공동배양에서 각 세포의 단독배양보다 유의하게 향상된 연골형성능력을 보여주었다. 활막줄기세포와 연골세포의 간접공동배양에서 얻어진 분비단백질의 항체 유전자미세배열분석과 면역블로팅 검사상 MMP-2의

증가소견과 FABP4의 감소소견이 관찰되었다. 그러나 두세포간에서 MMP-2와 FABP4의 역할을 규명하기 위해서는 이에 대한 추가연구가 필요하겠다.

색인 단어: 활막줄기세포, 연골세포, 공동배양, 연골형성능, 분비단백질 유전자미세배열분석, 염증성 매개물질

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