Gene Expression Profile during Chondrogenesis in Human Bone Marrow derived Mesenchymal Stem Cells using a cDNA Microarray

Hyun Jung Yoo[1], Sung Soo Yoon[1], Seon Yang Park[1], Eun Young Lee[1], Eun Bong Lee[1], Ju Han Kim[2] and Yeong Wook Song[1]

[1]Department of Internal Medicine, Rheumatism Research Institute, [2]Graduate Course of Biomedical Informatics (SNUBI), Seoul National University College of Medicine, Seoul, Korea

Address for Correspondence:
Yeong Wook Song, MD
Department of Internal Medicine, Seoul National University Hospital, 103 Daehak-ro, Jongno-gu, Seoul 110-744, Korea
Tel: +82.2-2072-2234, Fax: +82.2-762-9662
E-mail: ysong@snu.ac.kr

Mesenchymal stem cells (MSCs) have the capacity to proliferate and differentiate into multiple connective tissue lineages, which include cartilage, bone, and fat. Cartilage differentiation and chondrocyte maturation are required for normal skeletal development, but the intracellular pathways regulating this process remain largely unclear. This study was designed to identify novel genes that might help clarify the molecular mechanisms of chondrogenesis. Chondrogenesis was induced by culturing human bone marrow (BM) derived MSCs in micromass pellets in the presence of defined medium for 3, 7, 14 or 21 days. Several genes regulated during chondrogenesis were then identified by reverse transcriptase-polymerase chain reaction (RT-PCR). Using an ABI microarray system, we determined the differential gene expression profiles of differentiated chondrocytes and BM-MSCs. Normalization of this data resulted in the identification of 1,486 differentially expressed genes. To verify gene expression profiles determined by microarray analysis, the expression levels of 10 genes with high fold changes were confirmed by RT-PCR. Gene expression patterns of 9 genes (*Hrad6B, annexinA2, BMP-7, contactin-1, peroxiredoxin-1, heat shock transcription factor-2, synaptotagmin IV, serotonin receptor-7, Axl*) in RT-PCR were similar to the microarray gene expression patterns. These findings provide novel information concerning genes involved in the chondrogenesis of human BM-MSCs.

Key Words: Chondrogenesis; Mesenchymal Stem Cells; cDNA Microarray

INTRODUCTION

Mesenchymal stem cells (MSCs) are present in a variety of tissues during human development, and in particular, are prevalent in adult bone marrow (1). MSCs isolated from bone marrow (BM) and expanded in vitro in their undifferentiated phenotype, retain an extensive capacity for multi-lineage differentiation into chondrocytes, adipocytes, osteoblasts, and tenocytes under appropriate environmental cues (2). The presence of specific, distinct antigens identified by the monoclonal antibodies SH2, SH3, and SH4, on the surfaces of marrow-derived MSCs, that are not present on osteocytes and osteoblasts, suggests that these epitopes are developmentally regulated. The antigen which bound to SH2 antibody was identified as endoglin (CD105), a receptor for TGF-β₃, which potentially plays a role in mediating the chondrogenic differentiation of MSCs and in their interactions with hematopoietic cells (3).

Chondrogenesis, the differentiation of MSCs into chondrocytes, is crucial required for skeletal development and maturation, since the cartilage anlage is the model for bone formation. Cartilage development thus includes the differentiation of MSCs into chondrocytes, followed by their maturation, and eventual their hypertrophy and death (4). Differential gene expression profiling has been widely performed to identify and characterize candidate genes that play potentially important roles in particular biological process (5).

Although the amount of information regarding the role of growth factors and cytokines as inducers and mediators of MSC differentiation continues to increase, little is known about the gene expression profiling of MSC chondrogenic differentiation. In this study, we employed ABI GeneChips (representing > 30,000 genes) to identify genes differentially expressed during BM-MSC chondrogenesis. ABI is introduced technology based on nylon-spotted 60 mer oligonucleotides, that uses on oligomers to detect each gene for most genes, chemiluminescence to measure gene expression levels, and fluorescence to grid to normalize and identify microarray features. The ABI gene list was compiled from information in public and Celera databases (6). This study was designed to identify differential gene expression profiles and novel genes that might be involved in BM-MSC chondrogenesis.

MATERIALS AND METHODS

Cell culture

Mononuclear cells from BM aspirates were isolated by density Ficoll-Paque gradient separation. BM was placed in a 50 mL syringe containing 5,000 units of preservative-free heparin, diluted 1:1 with phosphate buffered saline (PBS), resuspended in PBS to a final volume of 10 mL, and layered over an equal volume of Histopaque-1,077 (Sigma Chemical Co., St. Louis, MO, USA). After centrifugation at 2,000 rpm for 30 min, mononuclear cells were recovered from the gradient interface, rinsed twice in PBS, adjusted to a concentration of 1.5×10^7 cells/10 mL, and seeded onto 100-mm culture plates in Dulbecco's Modified Eagle's Medium-Low Glucose (DMEM-LG; 1 g/L glucose, JBI, Seoul, Korea) containing 1% penicillin-streptomycin (P/S; 10,000 units/mL, Gibco/BRL, New York, NY, USA) and 10% (v/v) heat-inactivated fetal bovine serum (FBS; Hyclone, Logan, UT, USA). Total numbers of nucleated and viable cells were determined using a hemocytometer and trypan blue (Gibco/BRL, Gaithersburg, MD, USA) staining. Cells were incubated at 37°C in a humidified 5% CO_2 atmosphere and allowed to adhere for 72 hr. Non-adherent cells were then removed. The medium was changed twice a week. When cells were 80%-90% confluent, adherent cells were trypsinized (0.05% trypsin, Gibco/BRL) at 37°C for 5 min and re-plated in 100-mm culture plates. After passage 3, a morphologically homogenous population of adherent cells was obtained. During this expansion, medium was changed every 4-5 days.

Immunofluorescence staining

MSCs that adhered to spot slide bottoms were fixed with -20°C methanol (100%) for 5 min. Cells were then rehydrated in PBS for 5 min at room temperature, washed three times with PBS, blocked with 3% bovine serum albumin in PBS, and incubated overnight at 4°C with SH2 (American Type Culture Collection [ATCC], Rockville, VA, USA) as a positive control. Primary antibody (SH2) was removed by washing three times with PBS, and cells were then incubated with fluorescein isothiocyanate (FITC)-labeled affinity-purified antibody to mouse IgG + IgM (H + L) (DiNonA Inc., Seoul, Korea) for 1 hr at room temperature. Secondary antibodies were removed by washing three times with PBS. Coverslips were mounted onto slides with a solution containing 50% PBS and 50% glycerol. Labeled cells were observed under an Axiovert 200 (Zeiss, Thornwood, NY, USA).

Flow cytometry

Flow cytometry was performed to determine MSCs positive for SH2. Cells were permeabilized with ice cold 75% methanol in PBS for 30 min at 4°C, and washed three times. A FITC-conjugated SH2 antibody (DiNonA), diluted 1:500 in PBS, was then added, and cells were incubated for 1 hr at 4°C. Cells were analyzed within 2 hr of staining using a flow cytometer (FACScali-

bur, Becton Dickinson, Bedford, MA, USA). A total of 1×10^6 cells were collected for each measurement. Negative control samples were stained with an isotype-matched irrelevant mAb.

In vitro chondrogenesis

To induce chondrogenic differentiation, 200,000 MSCs were placed in a 15-mL polypropylene tube and centrifuged at 1,000 rpm for 5 min. Pellets were then cultured at 37°C in 5% CO_2 and 300 μL of serum-free chondrogenic medium consisting of Dulbecco's modified Eagle medium-high glucose (DMEM-HG, JBI) supplemented with 10 ng/mL of transforming growth factor-β_3 (TGF-β_3, R&D Systems, Minneapolis, MN, USA), 100 nM dexamethasone (Sigma-Aldrich, St. Louis, MO, USA), 50 μg/mL ascorbate-2-phosphate, 100 μg/mL pyruvate, and 50 mg/mL ITS + Premix (Becton Dickinson Biosciences, Bedford, MA, USA; 6.25 μg/mL insulin, 6.25 μg/mL transferrin, 6.25 ng/mL selenious acid, 1.25 mg/mL bovine serum albumin [BSA], and 5.35 mg/mL linolenic acid); the medium was replaced every 2-3 days for 3, 7, 14, or 21 days.

RNA preparation

Total RNA was extracted from undifferentiated MSCs and from pellets after 3, 7, 14, or 21 days of differentiation using RNeasy kits (Qiagen, Valencia, CA, USA), according to the manufacturer's instructions. And 1,200 μL of RLT buffer supplemented with beta-mercaptoethanol (10 μL/mL) was added to the washed cells. RNA integrity was assessed by gel electrophoresis and RT-PCR and concentrations were determined by measuring absorbance at 260 nm.

Microarray

Total RNA was processed using the Genesys Applied Biosystem Facility (Genesys, Munster, Germany), according to manufacturer's recommendations. Each RNA pool (2 μg) was labeled with Digoxigenin-UTP using the ABI Chemiluminescent RT-IVT Labeling Kit v 1.0. Double-stranded cDNA was prepared from total RNA. UTP-digoxigenin-labeled complementary RNA (cRNA) was synthesized by in vitro transcription. Labeled cRNA (10 μg) was hybridized to ABI Human Genome Survey Microarray v 2.0, which was then incubated with alkaline phosphatase-linked digoxigenin antibody. Phosphatase activity was then initiated to produce the chemiluminescent signal. Chemiluminescent (cRNA) and fluorescent (spot background) signals of the cRNA and standard control spots were then scanned. Chemiluminescent detection and image acquisition was performed using an Applied Biosystems 1,700 (AB1,700) Chemiluminescent Microarrays Analyzer, according to the manufacturer's instructions. Using the software supplied with the AB1,700 apparatus, the spot chemiluminescent signal was normalized over the fluorescent signal of the same spot to obtain normalized signal value. For inter-array normalization, global median normalization was ap-

plied across all microarrays (7).

Data analysis

Data analysis and data normalization were performed using the method described by Quackenbush (8). For background correction, the mean intensities of areas surrounding spots were subtracted from spot intensities (local area background). Data sets were normalized by dividing the mean intensity value of every spot (in duplicate) by sum of all spot intensities within a sample to eliminate experimental or data acquisition variations. Normalized data were used to calculate the gene expression level ratios of different culture stages. A two-fold expression cut-off

Table 1. Selected genes and their RT-PCR primer sequences

Gene	Primer sequences
Hrad 6B	F: 5´- TCC AAA TAA ACC ACC AAC TG -3´
	R: 5´- GGA CTG TTA GGA TTC GGT TC -3´
Annexin A2	F: 5´- CAT TCT GAC TAA CCG CAG CA -3´
	R: 5´- CGG TTA ATC TCC TGC AGC TC -3´
BMP-7	F: 5´- CCT ACC CCT ACA AGG CCG TCT TC -3´
	R: 5´- TGC TCC CCG TGG ACC GGA TGC TG -3´
Contactin-1	F: 5´- CAA TAG TGC AGG GTG TGG AC -3´
	R: 5´- TGG CTA GGA GGT GCT TTC TT -3´
Peroxiredoxin-1	F: 5´- ATG TCT TCA GAG TGC AAA AAT TG -3´
	R: 5´- TCA CTT CTG TTA GCG AAA TAC TCT -3´
Heat shock transcription factor-2	F: 5´- GAA GAA CCT GTT TCA GCA CAT AGT C -3´
	R: 5´- GAT GTT ATG ATT CAA AAT GGA ATC CAT C -3´
Synaptotagmin IV	F: 5´- GCG GGA TCC GTG CAC GTG CTT AAA GGA G -3´
	R: 5´- GCG GAA TTC CTC TTG ATG ATC TCT CTG G -3´
Serotonin receptor-7	F: 5´- ATC GGC TCC ATC CTG ACG CTC AT -3´
	R: 5´- CCA AAG TCC TGG CTG ATC AAG CA -3´
Axl	F: 5´- GAG GAT GAA CAG GAT GAC TGG -3´
	R: 5´- ACG AAG GTC TGA TGT CCC AGA -3´
IL-15	F: 5´- CAC ATT TGA GAA GTA TTT CCA TCC AGT GC -3´
	R: 5´- CAT CCT AGC AAA CAA CAG TTT GTC TTC -3´

F, forward primer; R, reverse primer.

was applied. For hierarchical gene cluster analysis, expression ratios were calculated for all genes as described by Eisen et al. (9).

Reverse transcriptase-polymerase chain reaction (RT-PCR)

First strand cDNA was synthesized using reverse transcriptase (RT) and 1 μg of total RNA. Reactions were conducted in 20 μL of buffer containing; 0.5 μL oligo $(dT)_{12-18}$ primer (Gibco/BRL, Grand Island, NY, USA), 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 40 mM DTT, 0.5 mM deoxynucleotide triphosphate (dNTP) mixture (Invitrogen, Carlsbad, CA, USA), 10 unit RNase inhibitor (Gibco/BRL), and 200 units of MMLV reverse transcriptase (Invitrogen). After incubation at 37°C for 60 min, reactions were stopped by heating at 70°C for 15 min. To remove remaining RNA, 1 μL of *E. coli* RNase H (4 mg/mL) was added to reaction mixtures and incubated at 37°C for 30 min. cDNAs obtained were used as a template for PCR amplification using gene-specific primers for target genes and for *glyceraldehyde 3-phosphate dehydrogenase* (*GAPDH*). Primer sequences are listed in Table 1.

PCR amplification of cDNA was performed in an automated thermal cycler (GeneAmp PCR System 2,400, Applied Biosystems, Foster City, CA, USA), in a final volume of 25 μL containing; 4 μL of cDNA solution, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 0.2 mM dNTP mixture, 0.4 pM of each primer, and 5 units of *Taq* DNA polymerase (Promega, Madison, WI, USA). After PCR, amplified products were analyzed by electrophoresis in 1.5% agarose gel and visualized by ethidium bromide staining under UV light illumination.

RESULTS

Cultures of human MSCs and their phenotypes

The in vitro growth pattern of MSC is shown in Fig. 1. Human bone marrow-derived MSCs were cultured and expanded. Dur-
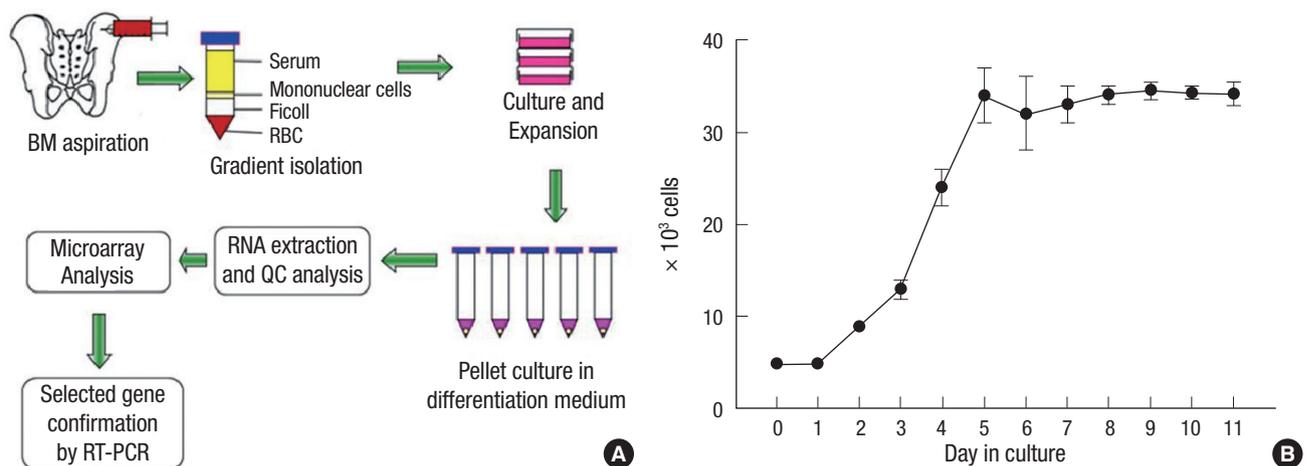
Fig. 1. Schematic representation of the experimental procedure (**A**) and growth curve of bone marrow derived mesenchymal stem cells (BM-MSCs) in culture (**B**). Increases in the numbers of viable adherent cells during incubation were measured by counting trypan blue negative cells. Cultures were started at 5,000 cells per well, harvested daily for 10 days, and numbers of adherent cells were counted. Data represent mean cell numbers ± SD of three experiments performed in duplicate. BM, bone marrow; RBC, red blood cell; QC, quality control.
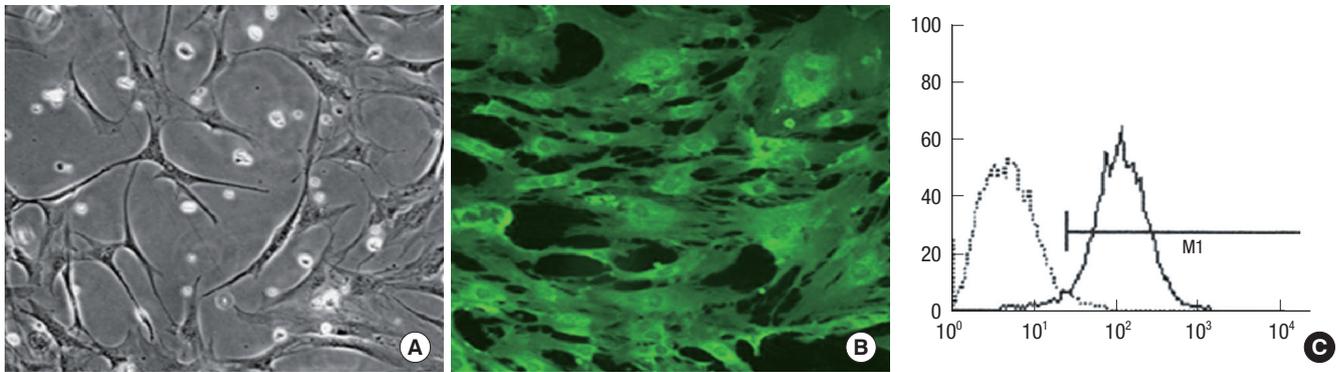
Fig. 2. Human bone marrow derived mesenchymal stem cells (BM-MSCs) confirmation by morphology, immunofluorescence staining, and flow cytometry. (**A**) BM-MSCs show a spindle-shaped fibroblastic morphology after culture expansion under the phase contrast microscope. Original magnification × 100. (**B**) Cultured BM-MSCs are positive for SH2 by immunofluorescence staining. (**C**) Flow cytometric analysis of BM-MSCs for the expression of SH2.
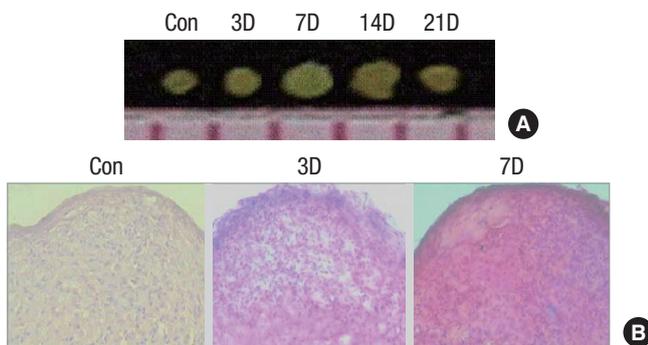
Fig. 3. Histochemical analyses of undifferentiated and differentiated human mesenchymal stem cells (MSCs). (**A**) Time course of MSC chondrogenesis. Macro picture of a differentiating MSC pellet on days 0, 3, 7, 14, and 21. A 1-mm rule is included. (**B**) Safranin O staining of undifferentiated (Con) and differentiated MSCs (after 3 and 7 days of differentiation).

ing the log phase of growth, cells proliferated with a population doubling time of 30 hr, and this growth period was followed by a confluent growth-arrested phase.

Colonies were examined approximately 7 days after initial plating. A morphologically homogeneous population of 90% confluent fibroblast-like cells was obtained after 2 weeks. The cells were replated into culture dishes and cultured for 2 weeks. The replated cells were used for subsequent experiments. The cultured MSCs were positive for SH2 by flow cytometry (Fig. 2).

Time course of BM-MSC chondrogenesis

MSCs were pelleted into micromasses and differentiated in serum-free medium in the presence of TGF-β₃ and dexamethasone. Immediately after centrifugation, the cells appeared as flattened pellets at the bottom of tubes. One day later, pellets had a thickened lip, and between days 2 and 3, pellet became spherical without any increase in size. Pellets then grew in size and pellet diameters increased to about 2-fold on days 7 and 14 (Fig. 3A).

Microarray data

Using normalized microarray data, we identified 1,486 differen-
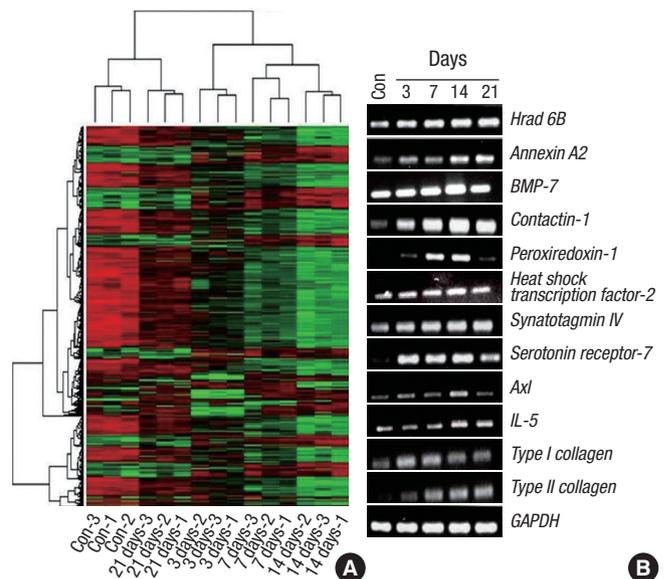
Fig. 4. Gene expressions of microarray and reverse transcriptase-polymerase chain reaction (RT-PCR). (**A**) Two-way hierarchical clustering of 1,486 genes expressed by undifferentiated (con) and differentiating (at 3, 7, 14, and 21 days) bone marrow derived mesenchymal stem cells. Expression profiles were clustered by average linkage hierarchical clustering. The color scale of standardized signal intensities in the microarray extends from bright green (down-regulation) to bright red (up-regulation). (**B**) RT-PCR analysis of 10 selected genes in undifferentiated (con) and differentiating stem cells (at 3, 7, 14, and 21 days). *Glyceraldehyde 3-phosphate dehydrogenase* (*GAPDH*) was used as an internal control.

tially expressed genes (Fig. 4A), which included 1,303, 121, 40, 20, and 2 genes exhibiting minimum 2 to < 5, 5 to < 10, 10 to < 20, 20 to < 70 and > 70-fold changes, respectively. To verify gene expression profiles determined by microarray analysis, the expression levels of 10 genes with high fold changes (2-48 fold changes, Table 2) were confirmed by RT-PCR.

Confirmation of gene expression by RT-PCR

The expression levels of the 10 genes selected (*Hrad 6B, annexin A2, BMP-7, contactin-1, peroxiredoxin-1, heat shock transcription factor-2, synaptotagmin IV, serotonin receptor-7, Axl,* and *IL-15*) were analyzed by RT-PCR, by using total RNAs obtained

Table 2. Identification of genes differentially expressed during chondrogenesis

Reporter ID	Reporter name	Fold
114958	5-hydroxytryptamine (serotonin) receptor 7 (adenylate cyclase-coupled)	48.93291
171467	nudix (nucleoside diphosphate linked moiety X)-type motif 8	40.41234
166535	heat shock transcription factor 2	39.17662
143528	tubulin, beta 1	37.39376
162651	hypothetical protein DT1P1A10	22.14788
195022	tumor suppressor candidate 3	21.78559
148565	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 8, 19 kDa	20.83195
186773	FLJ45224 protein; prostaglandin D2 synthase 21kDa (brain)	19.24891
202455	peroxiredoxin 1	18.53505
173923	fibronectin type 3 and ankyrin repeat domains 1	18.25457
160161	chromosome 6 open reading frame 210	17.19068
183606	FLJ22624 protein	16.76164
112057	ubiquitin-conjugating enzyme E2B (RAD6 homolog)	6.96857
144159	RYK receptor-like tyrosine kinase	6.29185
143111	glutamic pyruvate transaminase (alanine aminotransferase) 2	4.28248
142438	polymerase (DNA-directed), delta interacting protein 2	3.97053
209540	annexin A2	3.29699
177636	bone morphogenetic protein 7 (osteogenic protein 1)	3.27800
148029	NAD(P) dependent steroid dehydrogenase-like	3.26781
131216	BH3 interacting domain death agonist	2.72827
123506	gamma-aminobutyric acid (GABA) A receptor, beta 1	2.51372
139212	synaptotagmin IV	2.49410
136701	mannan-binding lectin serine protease 2	2.42219
136002	signal transducer and activator of transcription 1, 91 kDa	2.02504

This listed genes were selected from the most highly fold changed genes (2-48 fold).

from 5 samples (Fig. 4B). The expression levels of 9 genes (*Hrad 6B, annexin A2, BMP-7, contactin-1, peroxiredoxin-1, heat shock transcription factor-2, synaptotagmin IV, serotonin receptor-7, Axl*) were low in undifferentiated cells and increased in differentiated cells by RT-PCR and microarray, but the expression pattern of IL-15 was different. Expression level of IL-15 tended to be decreased in microarray, but increased in RT-PCR (Fig. 5).

DISCUSSION

In this study, we determined gene expression profiles in differentiated chondrocytes and BM-MSCs. The microarray technology used did not allow quantitative comparisons between the expressional levels of different genes, but did allow us to compare fold changes with time and quantify differences in the expressions of multiple genes. Our results show the sequences for gene expressional changes during BM-MSC chondrogenesis. Microarray data showed that *Axl, synaptotagmin IV, Hrad6B, peroxiredoxin-1, BMP-7, heat shock transcription factor-2, annexin A2, contactin-1 and serotonin receptor-7* expressions were maintained in differentiating BM-MSCs until day 14.

Axl is overexpressed in a number of tumors (10), and *IL-15* is known to mediate the transactivation and upregulation of *Axl* with subsequent activation of PI3K/Akt and upregulations of Bcl-2 and Bcl-XL (11). On the other hand, *synaptotagmin IV* is required for the maturation of secretory granules in PC12 cells (12). Human homologues of yeast Rad 6 (*Hrad6B*) encode ubiq-

uitin-conjugating enzymes, and is highly expressed in lung cancer cell. It has been reported that DNA repair and UV mutagenesis are defective in *Saccharomyces cerevisiae* rad6 mutant (13). *Peroxiredoxin-1* is the most ubiquitously expressed member of the peroxiredoxin family, and is found in the cytoplasm, nucleus, mitochondria, and peroxisomes of many cell types (14). Furthermore, recent studies have reported high levels of *peroxiredoxin-1* expression in the bovine bladder, seminal vesicles, testes, adrenal gland (15), and in the rat liver, skin, lungs and nervous system (14). The role of *peroxiredoxin-1* in cell differentiation and proliferation suggests that it has a possible role in growth and development.

Recent studies have confirmed that BMP-7 is a strong chemotactic component in cartilage cells produced by mesenchymal stem cells, and it can promote cartilage cells to secrete specific extracellular matrix (proteoglycans and collagen type II). And *BMP-7* can induce the differentiation of BM-MSCs into cartilage cells, and that it offers a greater efficiency in repairing cartilage and subchondral bone defects (16).

Heat shock transcription factor-2 (*HSF-2*) has been shown to be a transcriptional regulator of heat shock protein gene expression during the differentiation and development of eukaryotic cells in a tissue dependent manner (17). *HSF-2* plays an important role in FGF-2 stimulated osteoclast formation, and HSF-2 deficiency was found to modulate gene expression in stromal/preosteoblast cells and affect osteoclastogenesis in the bone microenvironment (18). *Annexins* bind to negatively charged phos-
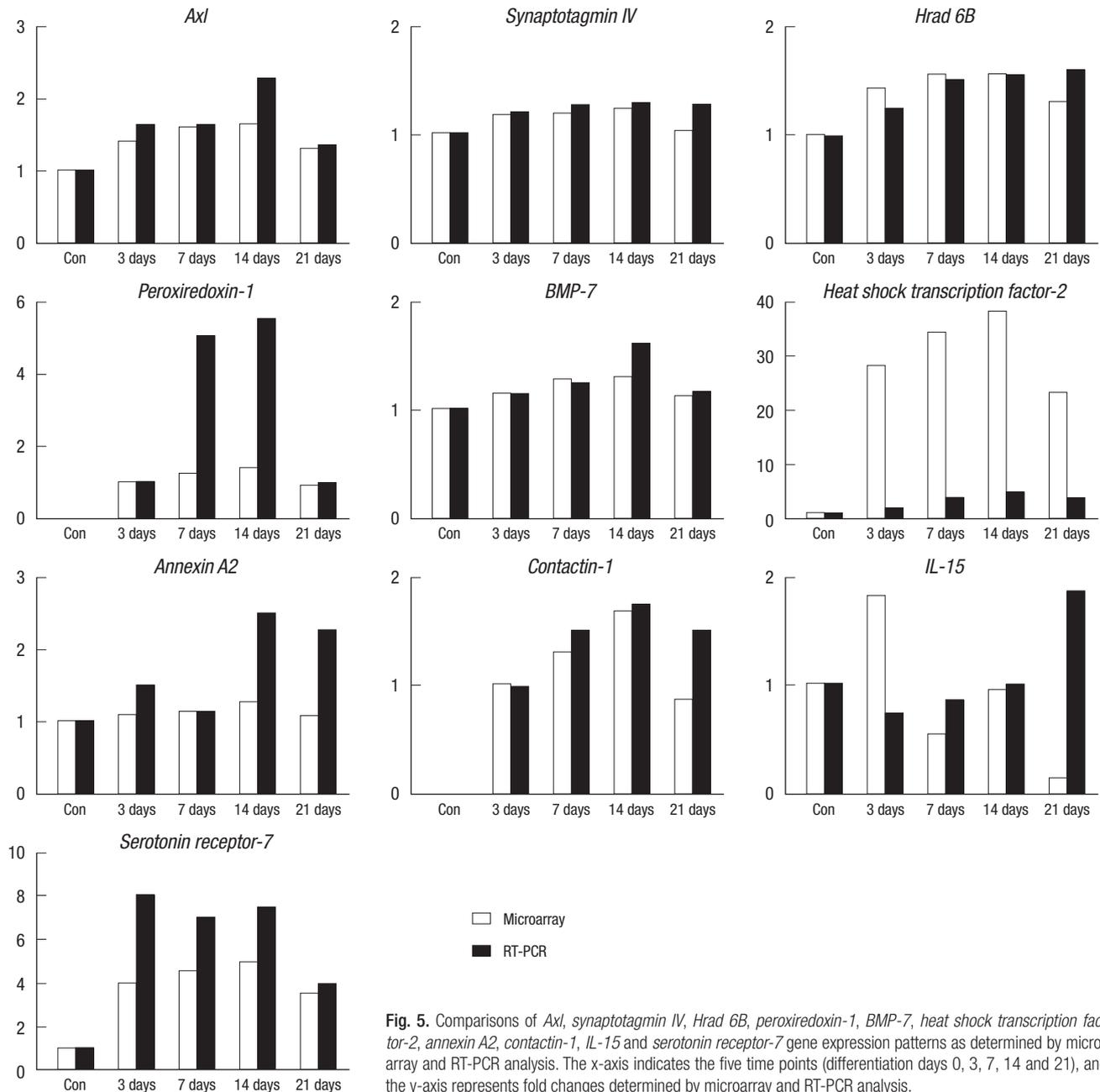
Fig. 5. Comparisons of *Axl*, *synaptotagmin IV*, *Hrad 6B*, *peroxiredoxin-1*, *BMP-7*, *heat shock transcription factor-2*, *annexin A2*, *contactin-1*, *IL-15* and *serotonin receptor-7* gene expression patterns as determined by microarray and RT-PCR analysis. The x-axis indicates the five time points (differentiation days 0, 3, 7, 14 and 21), and the y-axis represents fold changes determined by microarray and RT-PCR analysis.

pholipids in a Ca^{2+}-dependent manner and have a conserved structure. The human *annexin*, *annexin A2* (alternative names: *annexin II*, *p36*, and *lipocortin II*) is expressed abundantly in various human organs, including the placenta, lungs, heart, and liver (19). At the cellular level, *annexin A2* is expressed on endothelial cell surfaces and acts as a co-receptor for plasminogen and tissue plasminogen activator (20). Furthermore, *annexins* are commonly dysregulated in cancer (21) and *annexin A2* is upregulated in a variety of tumors and cancer cell lines (22, 23). *Contactin-1* is a cell surface adhesion molecule, which is normally expressed by neurons, oligodendrocytes, and human astrocytic gliomas (24, 25). Previous studies have reported that MSCs express *IL-15*, essential hematopoietic growth factor (26, 27) and

IL-15 is also a potent apoptosis inhibitor and has many immunomodulatory activities (28). The *Serotonin receptor 7* is the most recently identified member of the serotonin receptor family and is found in brain, mainly in the hypothalamus, thalamus, hippocampus, and cortex (29).

In the present study, we performed microarray analysis during BM-MSC chondrogenesis in vitro. We found that over 1,486 genes were expressed by BM-MSCs during chondrogenesis, and we identified genes that were differentially expressed. These data may provide novel information of the genes involved in chondrogenesis of human BM-MSCs.

REFERENCES

1. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. *Multilineage potential of adult human mesenchymal stem cells. Science 1999; 284: 143-7.*

2. Ho YC, Chung YC, Hwang SM, Wang KC, Hu YC. *Transgene expression and differentiation of baculovirus-transduced human mesenchymal stem cells. J Gene Med 2005; 7: 860-8.*

3. Haynesworth SE, Baber MA, Caplan AI. *Cell surface antigens on human marrow-derived mesenchymal cells are detected by monoclonal antibodies. Bone 1992; 13: 69-80.*

4. Izzo MW, Pucci B, Tuan RS, Hall DJ. *Gene expression profiling following BMP-2 induction of mesenchymal chondrogenesis in vitro. Osteoarthritis Cartilage 2002; 10: 23-33.*

5. Diatchenko L, Lau YF, Campbell AP, Chenchik A, Moqudam F, Huang B, Lukyanov S, Lukyanov K, Gurskaya N, Sverdlov ED, Siebert PD. *Suppression subtractive hybridization: a method for generating differentially regulated or tissue-specific cDNA probes and libraries. Proc Natl Acad Sci U S A 1996; 93: 6025-30.*

6. Bosotti R, Locatelli G, Healy S, Scacheri E, Sartori L, Mercurio C, Calogero R, Isacchi A. *Cross platform microarray analysis for robust identification of differentially expressed genes. BMC Bioinformatics 2007; 8 Suppl 1: S5.*

7. Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, Ellis B, Gautier L, Ge Y, Gentry J, Hornik K, Hothorn T, Huber W, Iacus S, Irizarry R, Leisch F, Li C, Maechler M, Rossini AJ, Sawitzki G, Smith C, Smyth G, Tierney L, Yang JY, Zhang J. *Bioconductor: open software development for computational biology and bioinformatics. Genome Biol 2004; 5: R80.*

8. Quackenbush J. *Microarray data normalization and transformation. Nat Genet 2002; 32 Suppl: 496-501.*

9. Eisen MB, Spellman PT, Brown PO, Botstein D. *Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci U S A 1998; 95: 14863-8.*

10. Goruppi S, Chiaruttini C, Ruaro ME, Varnum B, Schneider C. *Gas6 induces growth, beta-catenin stabilization, and T-cell factor transcriptional activation in contact-inhibited C57 mammary cells. Mol Cell Biol 2001; 21: 902-15.*

11. Budagian V, Bulanova E, Orinska Z, Thon L, Mamat U, Bellosta P, Adam D, Paus R, Bulfone-Paus S. *A promiscuous liaison between IL-15 receptor and Axl receptor tyrosine kinase in cell death control. EMBO J 2005; 24: 4260-70.*

12. Ahras M, Otto GP, Tooze SA. *Synaptotagmin IV is necessary for the maturation of secretory granules in PC12 cells. J Cell Biol 2006; 173: 241-51.*

13. Sasaki H, Moriyama S, Nakashima Y, Yukiue H, Fukai I, Fuji Y. *Decreased Hrad6B expression in lung cancer. Acta Oncol 2004; 43: 585-9.*

14. Immenschuh S, Baugmart-Vogt E, Tan M, Iwahara SI, Ramadori G, Fahimi HD. *Differential cellular and subcellular localization of heme-binding protein 23/peroxiredoxin I and heme oxygenase-1 in rat liver. J Histochem Cytochem 2003; 51: 1621-31.*

15. Leyens G, Donnay I, Knoops B. *Cloning of bovine peroxiredoxins-gene expression in bovine tissues and amino acid sequence comparison with rat, mouse and primate peroxiredoxins. Comp Biochem Physiol B Biochem Mol Biol 2003; 136: 943-55.*

16. Bai X, Li G, Zhao C, Duan H, Qu F. *BMP7 induces the differentiation of bone marrow-derived mesenchymal cells into chondrocytes. Med Biol Eng Comput 2011; 49: 687-92.*

17. Eriksson M, Jokinen E, Sistonen L, Leppä S. *Heat shock factor 2 is activated during mouse heart development. Int J Dev Biol 2000; 44: 471-7.*

18. Kajiya H, Ito M, Ohshima H, Kenmotsu S, Ries WL, Benjamin IJ, Reddy SV. *RANK ligand expression in heat shock factor-2 deficient mouse bone marrow stromal/preosteoblast cells. J Cell Biochem 2006; 97: 1362-9.*

19. Dreier R, Schmid KW, Greke V, Riehemann K. *Differential expression of annexins I, II and IV in human tissues: an immunohistochemical study. Histochem Cell Biol 1998; 110: 137-48.*

20. Diaz VM, Hurtado M, Thomson TM, Reventós J, Paciucci R. *Specific interaction of tissue-type plasminogen activator (t-PA) with annexin II on the membrane of pancreatic cancer cells activates plasminogen and promotes invasion in vitro. Gut 2004; 53: 993-1000.*

21. Fang YT, Lin CF, Liao PC, Kuo YM, Wang S, Yeh TM, Shieh CC, Su IJ, Lei HY, Lin YS. *Annexin A2 on lung epithelial cell surface is recognized by severe acute respiratory syndrome-associated coronavirus spike domain 2 antibodies. Mol Immunol 2010; 47: 1000-9.*

22. Esposito I, Penzel R, Chaib-Harrireche M, Barcena U, Bergmann F, Riedl S, Kayed H, Giese N, Kleeff J, Freiss H, Schirmacher P. *Tenascin C and annexin II expression in the process of pancreatic carcinogenesis. J Pathol 2006; 208: 673-85.*

23. Tanaka T, Akatsuka S, Ozeki M, Shirase T, Hiai H, Toyokuni S. *Redox regulation of annexin 2 and its implication for oxidative stress-induced renal carcinogenesis and metastasis. Oncogene 2004; 23: 3980-9.*

24. Falk J, Bonnon C, Girault JA, Faivre-Sarrailh C. *F3/contactin, a neuronal cell adhesion molecule implicated in axogenesis and myelination. Biol Cell 2002; 94: 327-34.*

25. Eckerich C, Zapf S, Ulbricht U, Müller S, Fillbrandt R, Westphal M, Lamszus K. *Contactin is expressed in human astrocytic gliomas and mediates repulsive effects. Glia 2006; 53: 1-12.*

26. Majumdar MK, Thiede MA, Mosca JD, Moorman M, Gerson SL. *Phenotypic and functional comparison of cultures of marrow-derived mesenchymal stem cells (MSCs) and stromal cells. J Cell Physiol 1998; 176: 57-66.*

27. Kim DH, Yoo KH, Choi KS, Choi J, Choi SY, Yang SE, Yang YS, Im YS, Im HJ, Kim KH, Jung HL, Sung KW, Koo HH. *Gene expression profile of cytokine and growth factor during differentiation of bone marrow-derived mesenchymal stem cell. Cytokine 2005; 31: 119-26.*

28. Bulfone-Paus S, Bulanova E, Pohl T, Budagian V, Durkop H, Ruckert R, Kunzendorf U, Paus R, Krause H. *Death deflected: IL-15 inhibits TNF-alpha-mediated apoptosis in fibroblasts by TRAF2 recruitment to the IL-15R alpha chain. FASEB J 1999; 13: 1575-85.*

29. Hedlund PB, Sutcliffe JG. *Functional, molecular and pharmacological advances in 5-HT7 receptor research. Trends Pharmacol Sci 2004; 25: 481-6.*

AUTHOR SUMMARY

Gene Expression Profile during Chondrogenesis in Human Bone Marrow derived Mesenchymal Stem Cells using a cDNA Microarray

Hyun Jung Yoo, Sung Soo Yoon, Seon Yang Park, Eun Young Lee, Eun Bong Lee, Ju Han Kim and Yeong Wook Song

Mesenchymal stem cells (MSCs) have the capacity to proliferate and differentiate into multiple connective tissue lineages such as cartilage and bone. In this study, using an ABI microarray system, the authors determined the differential gene expression profiles of differentiated chondrocytes and bone marrow (BM)-MSCs. Normalization of this data resulted in the identification of 1,486 differentially expressed genes. To verify gene expression profiles of microarray, RT-PCR was also performed. Gene expression patterns of 9 genes in RT-PCR were similar to the microarray results. These findings provide novel information concerning genes involved in the chondrogenesis of human BM-MSCs.