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Identification of Interleukin 1-Responsive Genes in Human Chondrosarcoma SW1354 cells by cDNA Microarray Technology

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－Abstract－

Background : Accumulating evidence shows that interleukin(IL)-1 plays a critical role in inflammation and connective tissue destruction observed in both osteoarthritis and rheumatoid arthritis. IL-1 induces gene expression related to cytokines, chemokines and matrix metalloproteinases by activation of many different transcription factors.

Materials and Methods : The chondrosarcoma cell line, SW1353, is known to be a valuable in vitro system for investigating catabolic gene regulation by IL-1β in chondrocytic cells. To explore and analyze the changes in gene expression by IL-1 responsible for arthritis, SW1353 was treated with IL-1 for 1, 6 and 24 h and then total RNAs were purified for each time. The changes in gene expression were analyzed with 17k human cDNA microarrays and validated by semi-quantitative RT-PCR.

Results : Greater than a two-fold change was observed in 1,200 genes including metallothioneins, matrix metalloproteinases, extracellular matrix proteins, antioxidant proteins, cytoskeleton proteins, cell cycle regulatory proteins, proteins for cell growth and apoptosis, signaling proteins and transcription factors. These changes appeared to be correlate with the pathophysiological changes observed in early osteoarthritis.

Conclusion : cDNA microarray analysis revealed a marked variability in gene expression, and provided insight into the overall molecular changes. The result of this study provide initial information for further studies to identify therapeutic targets in osteoarthritis pathogenesis.

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Introduction

Osteoarthritis is primarily a disease affecting joint cartilage resulting in severe pain and is the most common debilitating condition in the elderly. The social, medical and financial aspects are rapidly increasing as society is aging. Previously, osteoarthritis had been considered part of the natural aging process but recently there have been studied concerning the metabolic alterations of the articular cartilage independent on the natural aging processes. It is widely accepted that osteoarthritis characteristically involves the processes of cartilage synthesis and degradation resulting in mechanical and biochemical instability with fibrotic changes marked by soft brownish discoloration and a rough surface. As the lesion progresses, subchondral bone becomes exposed with full thickness loss of the cartilage matrix. Especially in early stage osteoarthritis, the main pathologic changes are induced by an imbalance between synthesis and degradation of extracellular matrix rather than chondrocyte loss caused by apoptosis and necrosis.[1] Chondrocyte metabolic activity is controlled by various growth factors and cytokines. Insulin-like growth factor-1 and transforming growth factor-β enhance extracellular matrix formation, but interleukin-1 and tumor necrosis factor-α induce matrix metalloproteinase expression and result in degradation of the extracellular matrix.[2] IL-1, the prime proinflammatory cytokine, plays an important role in the pathogenesis of osteoarthritis. As has been shown in animal model, IL-1 antagonist can prevent inflammation and tissue loss.[3] IL-1 has 3 subtypes IL-1α, IL-1β, IL-1Ra. IL-1α and IL-1β are agonists for IL-1 receptor, but IL-1Ra is antagonistic for the IL-1 receptor.[4] IL-1α and IL-1β are synthesized from a 31kDa precursor protein and IL-1β becomes active if broken into 17kDa by an IL-1β cleavage enzyme (ICE, caspase-1). IL-1β is known to exist in cartilage, cartilage synovial membrane and cartilage synovial fluid.[5] Activated IL-1β signals the receptor IL-1R on the cell membrane, activates mitogen-activated protein kinase (MAPK) extracellular-signal regulated kinase (ERK), protein 38 (*p*38) and c-jun N-terminal kinase (JNK). The resultant increases in transcription factors such as NF-κB and AP-1 activate the signaling processes, thus leading to diverse gene expressions.[6] Golring et al. showed that IL-1β decreases the expression level of collagen II but increases the expression of immediate early genes such as egr-1, c-jun, c-fos, jun-B, collagenase, stromelysin and matrix metalloprotease.[7] Islam et al. reported the expression of protein tyrosin kinases

using cDNA microarrays, RT-PCR and Western blot technique in the human osteoarthritis chondrocyte.[8] Vincenti and Brinckerhoff reported changes in expression of translation factors, cytokines, growth factors, protease, extracellular matrix, cell adherent protein and signaling molecules, by analyzing genes with 1.2k cDNA microarrays after IL-1β treatment in human chondrosarcoma cells (SW1353).[9] Aigner et al. investigated genes expressed in the cartilage of normal, early, and late phase osteoarthritis employing 1.2k cDNA microarrays and identified the enhanced expression of collagen fiber type 2, 6, fibronectin, and MMPs.[10] Knorr et al. reported that the expressions of chitinase-3 like protein 2 and chitinase-3 like protein 1 were decreased in osteoarthritis as compared to normal cartilage.[11] Huh et al. investigated the genes controlled by TNF-α in rheumatoid arthritis.[12] Shi et al. analyzed IL-1 or TNF-α inducible genes in SW1353 cells using the Affimatrix oligonucleotide array, which contains approximately 12,600 probe sets interrogating about 8,700 annotated genes, and revealed that IL-1 and TNF-α each activate a distinct set of genes in SW1353 cells and chondrocyte. IL-1β might serve an important role in the pathogenesis osteoarthritis.[13] However, it is difficult to understand the overall mechanisms and comprehensive gene expressions involved in inflammation and to identify the novel genes involved in pathogenesis. This study was designed to elucidate more thoroughly the mechanisms induced by IL-1β using gene expression analysis in the human chondrosarcoma cell SW1353 by employing highly concentrated human 17k cDNA microarrays.

Materials and Methods

Materials. Human chondrosarcoma, SW1353 (HTB-94) was obtained from the Korea Tissue Cell Bank (Seoul, Korea). Human 17 k cDNA microarrays were purchased from GenomicTree Inc. (Daejeon, Korea). Dulbecco's modified Eagle's medium (DMEM), diapase, and a penicillin-streptomycin-fungizone antibiotic solution were from Life technologies, Inc. (Gaithersburg, MD) and MMLV reverse transcriptase, dNTP mix, nick translation system from Promega Corp. (Madison, WI), and interleukin-1β (IL-1β) from R&D Systems Inc., (Minneapolis, MN). Primers for IL-1β responsive genes were from Bioneer Inc. (Daejeon, Korea) (Table 1).

Cell culture. SW1353 cells were cultivated in DMEM supplemented with 10% FBS and 1% antibiotic-antimycotic solution at 37℃ in a humidified atmosphere containing 5% CO2 and 95% air.

IL-1β treatment. Cells were cultured at 70% confluence in 150 mm culture plates. After discarding the media, cells were starved in DMEM for 24 h. Cells were washed 2 times with DMEM and treated with or without 10 ng/mL IL-1 for 1, 6, and 24 h at

Table 1. Primers for PCR

GenBank Acc. No.	Gene name	Symbol	Primer name	Sequence	DNA size (bp)
AA488084	superoxide dismutase 2	MnSOD	SOD2-126F	ACATCAACGCGCAGATCA	445
			SOD2-570R	TAAGCGTGCTCCCACACA	
AA148737	syndecan 4	SYND4	SDC4-1948F	CTGTTTGGGACCCAGCTG	460
			SDC4-2407R	AGCAAGGGAGGGTGGTTC	
AA470081	musculin (activated B-cell factor-1)	MSC	MSC-1338F	AAGAAGGGCGAGTGGCTT	414
			MSC-1752R	GTGGTGTTGGAGTTGGGG	
AA126958	RNA helicase	RIG-I	RIG-2455F	ACGCCTTCAGACATGGGA	258
			RIG-2712R	TGCTTTGGCTTGGGATGT	
T95113	cig5 mRNA, partial sequence htb1	vipirin	VIP-1712F	TGCCTTTATGCCATTGCA	373
			VIP-2084R	GGGCCAACCAGCTACTCA	
AI970057	secretory leukocyte protease inhibitor	SLPI	SLP1-224F	TGTGGCATCAAATGCCTG	313
			SLP1-537R	GCTGTGTGCCAAGCCTTT	
AA888172	butyrophilin, subfamily 3, member A2	BTN3A2	BTN-176F	TCCCTGGCTAATTGCCTG	377
			BTN-552R	TTGCTCTGCCTGGAAACC	
AI206156	Proto-oncogene c-CBL	CBL	CBL-2540F	TCCTCCTTTGGCTGGTTG	453
			CBL-2992R	TCCCACAGAGAGGGCAAG	
AA678971	regulator of G-protein signalling 9	RGS9	RGS9-1726F	AAGTGCCCTGCTGTGTCC	412
			RGS9-2137R	AGAAGGAGTTGGCCCCTG	
AA454609	forkhead box J1	FOXJ1	FOXJ1-1926F	TAACCCCCTGGAGGCTTC	402
			FOXJ1-2327R	GGTACCCCGCTTCTTGGT	
AA432143	Cbp/p300-interacting transactivator	CITED1	CIT-604F	GATGAGCTCCGTGGCCTA	427
			CIT-1030R	CAACCCCAGTTCCACCAC	
AA699782	transcription factor 21	TCF21	TCF21-2558F	ACAGACGCCTGAACGGAG	401
			TCF21-2958R	GGGACCAGCAGCAATGAC	
AA486533	early growth response 1	EGR-1	EGR1-2670F	CCTTTTGTGTGATGCCCC	401
			EGR1-3070R	TGGGCAATAAAGCGCATT	
AA857015	ephrin-A1	EFNA1	EFNA-997F	AAGGCACAGTGGGAGCTG	334
			EFNA-1330R	CATGGGCACTGCCCTTAC	
AA446027	early growth response 2	EGR-2	EGR2-2242F	GGGACTGATTTGGGGGAC	404
			EGR2-2645R	CATCACACAAGGCGACCA	
AA476272	tumor necrosis factor, alpha-induced protein	TNFA1P2	TNFAIP-3335F	TTGTTGGGGCATGAGCTT	432
			TNFAIP-3766R	CATCCCTGCTCCTTCCCT	
AA625806	ninjurin 1	NINJ1	NINJ-627F	GACCAGCCCTTGCTCTGA	432
			NINJ-1058R	CCAAGCCCAGGCACTTTA	
AA146773	2′,5′-oligoadenylate synthetase 1	OAS1	OAS1-783F	CACAGCCCAAGGATTTCG	446
			OAS1-1228R	TGGTGCAGGTCCAGTCCT	
AA283007	granzyme A	GZMA	GZMA-348F	CCATGCTATGACCCAGCC	404
			GZMA-751R	CGAGGGTCTCCGCATTTA	
N25945	phospholipid scramblase 1	PLSCR1	PLSCR-713F	TGCTGTGGGCCATCTAGAC	474
			PLSCR-1186	GCTGCCAGTGCTTTCAAAA	
AI953299	Interferon-induced protein with tetratricopeptide repeats 1	IFIT-1	IFIT-938F	GGGCTTTGCTACAAGGCA	518
			IFIT-1456R	CAGGGCCCGCTCATAGTA	
AA401441	B-factor, properdin	BF	BF-1993F	CTGCTCCCTGCACAGGAT	449
			BF-2441R	GTCCAGCAGGAAACCCCT	
AA458965	natural killer cell transcript 4	NK4	NK4-620F	CCTGTCCCGGATGTTGAG	461
			NK4-1080R	TTAAGATGCCAGGGCGAC	
AA286908	myxovirus (influenza) resistance 2	MX2	MX2-2300F	TAAGGGGAGTCGGTGCAG	436
			MX2-2735R	GCCTGGGCTTGAGCAATA	

AA863383	pim-2 oncogene	PIM2	PIM2-1611F PIM2-1926R	CTTACCTGCCTCAGCCCA GCTCAGGAGGAGGTTGCA	328
AA236164	cathepsin S	CTSS	CTSS-3614F CTSS-3941R	TCTGCCTGCTGTTCTCCC ACGAGGGGCTCCATAAGG	328
AA668821	chitinase 3-like 2	CHI3L2	CHI-727F CHI-1194R	ACAGAGGGCCAAGCTCCT TTCACAAGGAGCCAAGGC	468
AA708905	WAS protein family, member 2	WASF2	WAS-2974F WAS-3354R	GGTTTGGGCCTAATGGCT CTGAGGAGGCTTCGCAAG	381
AI659145	serum amyloid A2	SAA2	SAA2-70F SAA2-455R	TCAGCAGCCGAAGCTTCT ACTTTGAATCCCTGCCCC	386
AI921598	glycoprotein, synaptic 2	GPSN2	GPSN-267F GPSN-588R	CACGTGGCTCTTCCTGCT GGACAGGCGGGCTTTATT	322
AA455235	aldehyde dehydrogenase 1	ALDH1A3	ALDH-3012F ALDH-3385R	TGGCTTCCCTTCATCAGC AGGATCGGAATTCCCAGG	374

37℃ in 5% CO2 humidified air.

RNA extraction. Total RNA in SW1353 cells treated with or without IL-1β was purified by acid-phenol-guanidium thiocyanate-chloroform extraction.[14] RNA concentrations were determined by measuring absorbance at 260 nm using a UV-spectrophotometer (Shimadzu, Japan) and stored at -70℃.

Northern blot analysis. Ten micrograms of total RNA were separated on a 1% formaldehyde agarose gel. The RNAs were then transferred to a nylon membrane by vacuum transfer and cross-linked with UV. The membranes were then prehybridized overnight at 42℃ with a hybridization buffer (0.1 M sodium phosphate pH 7.2, 0.25 M sodium chloride, 2.5 mM EDTA, 50% formamide, and 7% SDS) containing a 32P-labeled c-Jun and c-Fos probes. Membranes were then washed and exposed to X-ray films at 70℃. Equal RNA loadings were confirmed by hybridization with a 32P-labeled GAPDH probe.

cDNA microarray experiments and data analysis. cDNA microarray experiments were performed as described by Yang et al.[15] Briefly, total RNA (100 mg)was reverse transcribed in the presence of Cy3-dUTP or Cy5-dUTP (25 mM stock, NEN Life Science Products) at 42℃ for 2 h. The labeled cDNA was then hybridized with the cDNA microarray at 65℃ for 16 h. The hybridized slides were washed, scanned with an Axon 4000B scanner (Axon Instruments), and analyzed using GenePix Pro 4.0 (Axon Instruments). Raw data were normalized and analyzed using GeneSpring 6.0 (Silicon Genetics). Genes were filtered according to their intensities in the control channel.

If control channel values were below 80 in all of the samples, we considered them to be unreliable genes. Intensity-dependent normalization (LOWESS) was performed, where the ratio was reduced to the residual of the Lowess fit of the intensity versus ratio curve. Average normalized ratios were calculated by dividing the averaged normalized signal channel intensity by the averaged normalized control

channel intensity. Welch's ANOVA test was performed for p-values =0.1 or 0.05 to identify genes in differentially expressed samples. Correlation analysis was performed using Pearson correlation (-1 to 1). Spots showing changes of 2-fold or more were considered significant.

Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR). To validate the differential expressions of genes screened by cDNA microarray analysis, we used semi-quantitative RT-PCR.[16] The levels of amplified DNAs by RT-PCR were quantified using the UTHSCSA ImageTool program (developed at the University of Texas Health Science Center at San Antonio, Texas and available at http://ddsdx.uthscsa.edu/dig/ itdesc.html).

Results

Northern blot analysis to check responses of SW1353 cells to IL-1β treatment showed peak c-fos expression by 1 h and declined by 6 and 24 h (Fig. 1), indicating that the signaling processes of SW1353 were adequate for IL-1β stimulation. With these adequate responses, the RNAs controlled by IL-1β were analyzed using cDNA microarrays.

After treatment of SW1353 with IL-1β for 1, 6, and 24 h, cells were harvested, RNAs were purified and then analyzed by 17k cDNA microarray. For the RNAs from control cells without IL-1β treatment, cDNA probes were manufactured with cy3-dUTP of green fluorescence and for the RNAs of treated cells, cDNA probes with cy5-dUTP of red fluorescence. The mixture of same amounts of cDNA probes from control cells and IL-1β treated cells was applied to 17k cDNA microarrays. On microarray analysis, the genes suppressed by IL-1β treatment exhibited green fluorescence, while enhanced genes exhibited red fluorescence with yellow meaning no changes (Fig. 2). Among the genes showing fluorescence above 200 in green or red, there were 4,200 increasing

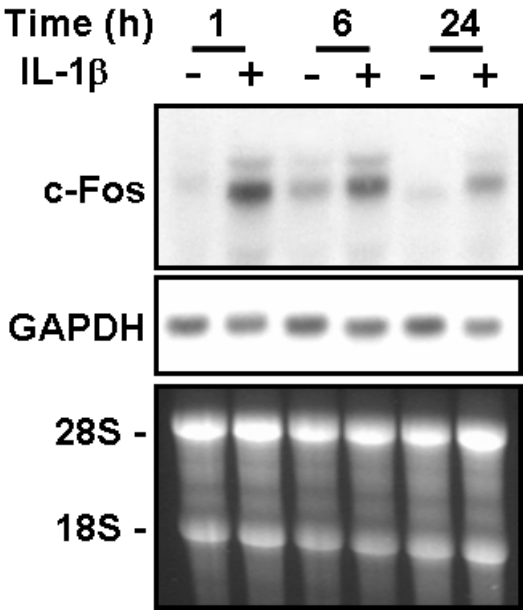

Fig. 1. Fos expression in IL-1β-stimulated cells. SW1353 cells were treated with or without 10 ng/mL IL-1β for the indicated times. The level of c-Fos expression was analyzed by Northern blotting. Equal loading of RNA was estimated with a GAPDH probe and by RNA staining with ethidium bromide in the agarose gel.

Table 2. The groups of genes demonstrating similar patterns

GenBank Acc. No.	Gene name	Folds IL-1b treatment (h)			Gene Ontology	
		1	6	24	function	localization
Group A						
AA699697	tumor necrosis factor (TNF superfamily, member 2)	61.58	12.79	0.47	apoptosis	membrane
AA111969	CD83 antigen	8.71	2.17	0.40	immune response	membrane protein
Group B						
AA476272	tumor necrosis factor, alpha-induced protein 3	118.02	40.17	9.14	anti-apoptosis	cytoplasm
AA995402	colony stimulating factor 2 (granulocyte-macrophage)	117.21	82.03	8.07	immune response	extracellular
AI285199	chemokine (C-C motif) ligand 20	71.50	39.70	4.21	inflammation	extracellular
Group C						
AA190825	TNF-induced protein	1.92	6.29	1.48	anti-apoptosis	
AA447730	pim-1 oncogene	3.78	10.62	2.51	cell growth	cytoplasm
AI351740	lymphotoxin beta (TNF superfamily, member 3)	7.98	16.91	1.89	immune response	membrane
Group D						
T50675	caspase 7, apoptosis-related cysteine protease	0.86	4.23	1.23	apoptosis	cytoplasm
AA160783	FAT tumor suppressor homolog 1 (Drosophila)	0.91	4.79	1.20	cell adhesion	membrane
AA281936	zinc finger protein 147 (estrogen-responsive finger protein)	1.12	4.63	1.37	transcription factor	
Group E						
AA457114	tumor necrosis factor, alpha-induced protein 2	6.07	21.60	6.98	angiogenesis	extracellular
AA488084	superoxide dismutase 2, mitochondrial	9.99	35.55	25.68	antioxidant	mitochondrion
AI268937	chemokine (C-C motif) ligand 8	4.07	62.90	19.98	immune response	extracellular
AA040170	chemokine (C-C motif) ligand 7	3.75	23.13	9.09	inflammation	extracellular
Group F						
N63988	nterferon-induced protein with tetratricopeptide repeats 2	0.81	117.74	17.97	immune response	
AA489640	interferon-induced protein with tetratricopeptide repeats 1	0.87	48.31	22.02	immune response	cytoplasm
AA878880	chemokine (C-X-C motif) ligand 10	2.24	143.40	20.54	inflammation	extracellular
Group G						
AA485371	bone marrow stromal cell antigen 2	0.89	6.26	23.25	cell proliferation	membrane
AA448478	interferon, alpha-inducible protein (clone IFI-6-16)	0.95	7.86	26.82	immune response	membrane
AI659145	serum amyloid A2	2.15	05.00	>500.00	inflammation	extracellular
R41839	solute carrier family 35, member B1	0.93	1.41	2.49	transport(UDP-gal)	microsome
AA410188	chromosome 1 open reading frame 29	0.63	10.16	26.36		
Group H						
W92764	tumor necrosis factor, alpha-induced protein 6	3.36	88.40	98.22'	cell adhesion	extracellular
N69322	matrix metalloproteinase 13 (collagenase 3)	2.21	71.01	149.20	proteolysis(collagen)	extracellular
W51794	matrix metalloproteinase 3 (stromelysin 1, progelatinase)	1.79	101.16	318.93	proteolysis(collagen)	extracellular
AA143331	matrix metalloproteinase 1 (interstitial collagenase)	1.55	94.96	193.12	proteolysis(collagen)	extracellular
Group I						
AA171613	carbonic anhydrase XII	0.91	0.98	5.12	bone resorption	membrane
AI983645	deoxyribonuclease II, lysosomal	0.86	1.16	5.46	metabolism(DNA)	lysosome
R60343	5'-nucleotidase, ecto (CD73)	0.94	1.04	2.07	metabolism(DNA)	membrane
Group J						
AI380314	beaded filament structural protein 1, filensin	0.94	0.34	0.13	cytoskeleton	
AI634172	adenylate kinase 5	0.91	0.51	0.15	metabolism(energy)	
T50121	v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian)	0.75	0.95	0.16	transcription factor	Nucleus
H24316	aquaporin 1 (channel-forming integral protein, 28kDa)	0.87	0.48	0.12	transport(water)	membrane
Group K						
AA463225	bone morphogenetic protein 4	0.80	0.17	0.65	bone formation	extracellular

W93379	NIMA (never in mitosis gene a)-related kinase 2	0.64	0.27	0.88	cell cycle	nucleus
AI818293	D site of albumin promoter (albumin D-box) binding protein	0.89	0.02	1.44	transcription factor	
AA505136	PHD protein Jade-1	0.73	0.24	0.72		
Group L						
AA287316	histone 1, H4b	1.15	0.26	0.28	chromosome assembly	nucleus
AA868008	histone 1, H4f	1.28	0.27	0.32	chromosome assembly	nucleus
AA482119	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	1.10	0.18	0.33	transcription corepressor	nucleus
N74882	distal-less homeo box 5	0.47	0.15	0.31	transcription factor	nucleus
AA452909	nuclear receptor subfamily 2, group F, member 1	0.74	0.26	0.27	transcription factor	nucleus

fluorescence by more than 2 fold according to IL-1β treatment times. For those genes having a value above 700, there were 1,200 that doubled. Stratified analysis showed the changing patterns of these 1,200 genes (Fig. 3). Green means decreased expression, red; enhanced expression, black; no change according to IL-1β treatment times and the intensity of color is proportionate to change. The groups of genes demonstrating similar changing patterns were analyzed by k-means clustering and included 12 groups (group A to group L) (Fig. 4 and Table 2). The genes of

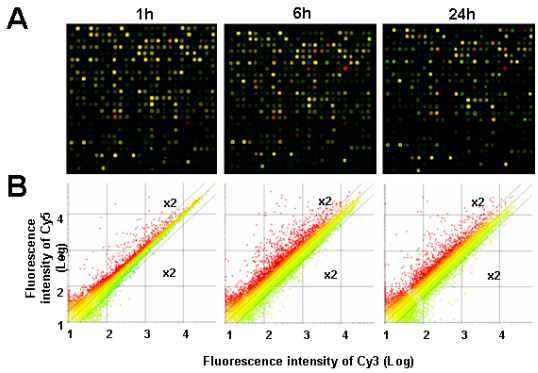

Fig. 2. cDNA microarray images (A) and scattered plots (B) for IL-1b responsive genes in SW1353 cells. Cy5-labeled cDNA (red, IL-1b-stimulated cells) and Cy3-labeled cDNA (green, IL-1b-unstimulated cells) were mixed and hybridized with 17k cDNA microarrays. The microarrays were scanned and analyzed. One Total The ratios of Cy5 to Cy3 fluorescent intensities of each genes were compared and plotted.

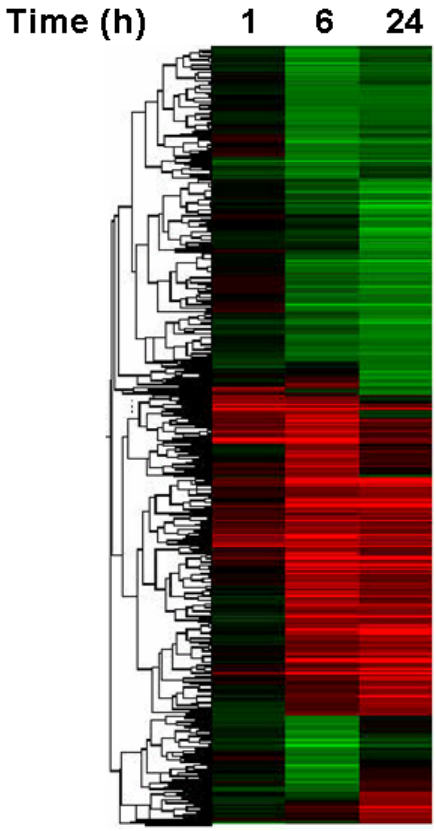

Fig. 3. Hierarchical clustering of IL-1b-responsive genes in SW1353 cells. Genes showing changes of more than 2 folds in its expression by IL-1b treatment were clustered using Genespring 6.1.

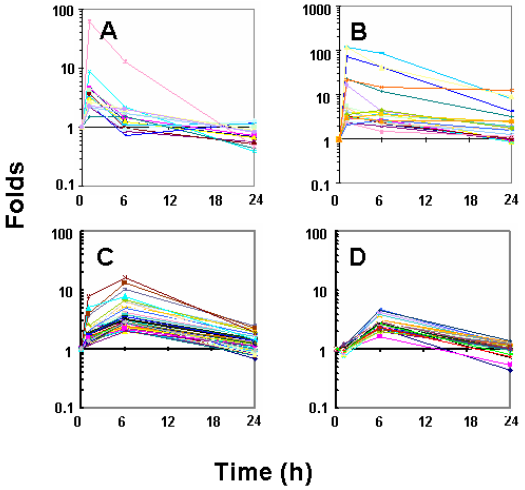

Fig. 4-1. K-mean clustering of IL-1b -responsive genes in SW1353 cells. Genes showing changes of more than 2 folds in its expression by IL-1b treatment were clustered using Genespring 6.1.

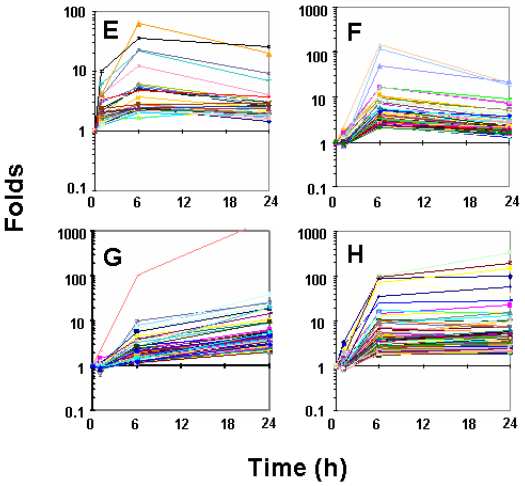

Fig. 4-2. K-mean clustering of IL-1b-responsive genes in SW1353 cells. Genes showing changes of more than 2 folds in its expression by IL-1b treatment were clustered using Genespring 6.1.

group A had a pattern that peaked at 1 h, and then decreased to pre-treatment levels

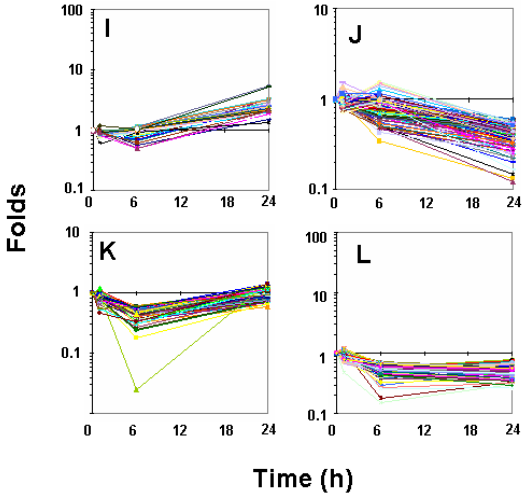

Fig. 4-3. K-mean clustering of IL-1b-responsive genes in SW1353 cells. Genes showing changes of more than 2 folds in its expression by IL-1b treatment were clustered using Genespring 6.1.

(Fig. 4-1-A). Many genes related to cell signal transmission, especially expression of TNF for cell death and CD83 antigen for immune response, were prominent in this group. Group B peaked at 1 h and decreased at 6 and 24 h, although remaining elevated above base line values (Fig. 4-1-B). This group consisted of colony stimulating factor 2, chemokine (CC motif) ligand 20, TNF-α induced protein 3 and others involved in inflammation and immune responses. The genes belonging to group C started to increase at 1 h, peaked at 6 h and then returned to the one hour reading by 24 h (Fig. 4-1-C). About 44 genes were in group C, lymphotoxin-β of TNF- family for cell death, pim-1 for cell growth and TNF-induced protein to inhibit cell death showed remarkable changes in

expression and the proteins combining metal ions, metallothionein, were noticeable. Group D genes had maximum expression by hour 6 but were unchanged at 1 h and lower than base level at 24 h (Fig. 4-1-D) especially those participated in transcription factors such as FAT tumor suppressor homolog 1 for cell adherence, zinc finger protein 147, and caspase 7 were prominent in this group. Genes in group E were enhanced at 1 h, peaked at 6 h, and then tended to decrease. However, at 24 h, levels were still elevated above that of 1h measurements, in contrast to group C (Fig. 4-2-E). Chemokine (CCmotif) ligand 7 and 8 related to inflammation, and mitochondrial superoxide dismutase and TNF-α induced protein 2 for angiogenesis were prominent in group E. Group F, even though it was similar to group C and group E, showed increases at 1 and 6 h, and a slight decrease at 24 h (Fig. 4-2-F). The expression of chemokine (CC motif) ligand 10, and interferon-induced protein with tetratricopeptide repeats 1, 2 were prominent in addition to the characteristic genes of transcription factors, cell death and immune inflammatory responses. Group G included genes showing a steady increase at 1, 6 and 24 h. Serum amyloid A2 was the most prominent among them, and was expressed more than 100 fold at 6 h and 200 fold at 24 h (Fig. 4-2-G). Others such as bone marrow stromal cell antigen 2, interferon-induced protein for cell growth, solute carrier family 35 (zinc transporter) and chromosome 1 open reading frame 29 of unknown function were noticeable. In group H, similar to group G, genes expressed a steady level at 6 and 24 h (Fig. 4-2-H). Expression of MMP-3 (stromelysin 1), MMP-1 (interstitial collagenase), MMP-13 (collagenase 3), TNF-induced protein 6 for cell adherence, B-factor (properdin) for proteolysis were prominent. Genes of Group I had enhanced expression at 24 h but no changes at 1and 6 h (Fig. 4-3-I) and DNAse II, 5′-nucleotidase, carbonic anhydrase XII for ECF acidity and bone resorption were remarkable. Group J had characteristics of decreased expressions at 6 and 24 h compared to those at 1 h (Fig. 4-3-J). The decreases were remarkable with beaded filament structural protein 1 (filensin) related to cytoskeleton, aquaporin-1 for water transportation, adenylate kinase 5 for intracellular ATP control and translation factor v-maf musculoaponeurotic fibrosarcoma oncogene homolog B. There were also genes responsible for cell adherence, cytoskeleton, glycoprotein metabolism, proteolysis, ribosome proteins, signaling proteins and translation factors. Group K genes had decreased expression at 1 h, nadir at 6 h, and then recoverd to basal level at 24 h (Fig. 4-3-K). Prominent in this group were D site of albumin promoter (albumin D-box) binding protein, bone morphogenetic protein 4, PHD protein Jade-1 and NIMA-related kinase 2. There were also other genes for controlling cell cycle, cell growth, DNA

Table 3. Validation of cDNA microarray data by RT-PCR

GenBank Acc. No.	Gene name	Symbol	Folds IL-1b treatment (h)			Expression pattern in RT-PCR
			1	6	24	
AA488084	superoxide dismutase 2	MnSOD	10.5	37.1	26.5	similar
AA148737	syndecan 4 (amphiglycan, ryudocan)	SYND4	4.24	16.7	2.73	different
AA470081	musculin (activated B-cell factor-1)	MSC	4.27	14.1	2.76	similar
AA126958	RNA helicase	RIG-I	1.47	18.7	7.9	similar
T95113	cig5 mRNA, partial sequence htb1	vipirin	0.7	55.1	8.54	similar
AI970057	secretory leukocyte protease inhibitor	SLPI	0.24	7.04	30.4	similar
AA888172	butyrophilin, subfamily 3, member A2	BTN3A2	0.99	2.67	30	similar
AI206156	Proto-oncogene c-CBL	CBL	0.53	0.04	1	No band
AA678971	regulator of G-protein signalling 9	RGS9	0.77	0.19	0.27	similar
AA454609	forkhead box J1	FOXJ1	0.14	0.15	0.13	No band
AA432143	Cbp/p300-interacting transactivator	CITED1	0.57	0.27	0.11	similar
AA699782	transcription factor 21	TCF21	1.18	6.45	0.7	No band
AA486533	early growth response 1	EGR-1	11.1	0.39	0.36	similar
AA857015	ephrin-A1	EPLG1	2.04	0.88	1.09	similar
AA446027	early growth response 2	EGR-2	6.87	1.88	0.94	No band
AA476272	tumor necrosis factor, alpha-induced protein	TNFA1P2	132	49.1	9.83	similar
AA625806	ninjurin 1	NINJ1	2.41	7.22	2.15	similar
AA146773	2′,5′-oligoadenylate synthetase 1	OAS1	0.99	9.75	10.7	similar
AA283007	granzyme A	GZMA	1	0.24	1	No band
N25945	phospholipid scramblase 1	PLSCR1	0.8	10.7	8.34	similar
AI953299	Interferon-induced protein with tetratricopeptide repeats 1	IFIT-1	0.99	69.2	44.2	similar
AA401441	B-factor, properdin	BF	0.89	38.4	65.9	similar
AA458965	natural killer cell transcript 4	NK4	2.95	25.4	32.1	similar
AA286908	myxovirus (influenza) resistance 2	MX2	1.11	9.95	12.8	similar
AA863383	pim-2 oncogene	PIM2	1.28	2.15	0.14	similar
AA236164	cathepsin S	CTSS	1.22	4.59	10.9	No band
AA668821	chitinase 3-like 2	CHI3L2	0.88	9.43	16.3	similar
AA708905	WAS protein family, member 2	WASF2	1.08	1.43	0.15	different
AI659145	serum amyloid A2	SAA2	1	1	1	similar
AI921598	glycoprotein, synaptic 2	GPSN2	0.24	1.03	0.88	different
AA455235	aldehyde dehydrogenase 1	ALDH1A3	0.79	0.4	0.1	different

repair and metabolism and translation in this group. Group L, the largest group, consisted of genes whose expressions were suppressed from hour 6 to hour 24 inclusively (Fig. 4-3-L). Group L contained genes for cell cycle, cytoskeleton, mitochondria, energy, protein, lipid, carbohydrate metabolism and translation factors. Among the cited genes, distal-less homeo box 5, inhibitor of DNA binding 3, nuclear receptor subfamily 2, histone 1 gene for forming nucleosome binding DNA had marked changes.

Since cDNA microarray analysis revealed many false positives, it is necessary to repeat tests and statistical analyses or to validate the results with other methods of quantifying gene expression.[17] In this study RT-PCR was performed for 31 prominent genes in cDNA microarray analysis to verify the expression patterns. Most results were

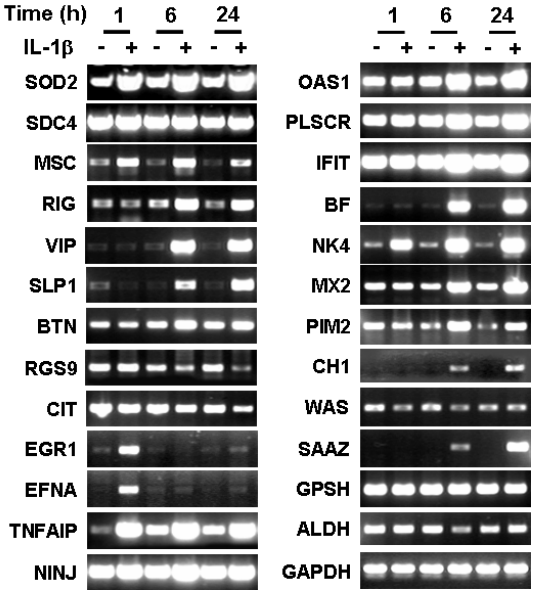

Fig. 5. Validation of cDNA microarray data with RT-PCR. RNAs were extracted from SW 1353 cells stimulated with or without IL-1b for the indicated times and cDNAs were prepare by reverse-transcription. DNAs for each genes were amplified by PCR, separated on agarose gels and visualized with ethidium bromide staining.

consistent with those with cDNA microarrays although 4 genes differed and 6 failed in amplification (Fig. 5, Table 3).

Discussion

In this study, we comprehensively measured the changes in genes related to the early inflammatory response to IL-1β treatment at 1, 6, and 24 h in a human chondrosarcoma cell line. Classified according to their functions, the genes were related to inflammatory responses, cell signalings, cell death and growth, cytoskeletons, transcription factors or metabolic functions. In early arthritis, proinflammatory cytokines such as TNF-α and IL-1β play important roles, as we verified in this study.

Gebauer et al. reported that chondrosarcoma cell line, SW1353 appear to be a valuable in vitro system for investigating catabolic gene regulation by IL-1β in chondrocytic cells and this implies that with appropriate methods some predictions from the SW1353 data can be made for the mechanism in primary human chondrocytes, despite the fact that the actual quantitative expression data within the two systems are quite diverse.[18] SW1353 cells treated with IL-1β showed diverse changes in the expression of inflammatory cytokines and chemokines participating in inflammation and immune response. Though the expression of those genes varied, the responses to IL-1 treatment were exponential in all, with proinflammatory cytokines; IL-1α, IL-1β, IL-6, IL-8, leukemia inhibitory factor (LIF) increasing, with anti-inflammatory cytokines; IL-13 decreasing. Zhang et al. reported that β_2-microglobulin was increased in synovial fluid and cartilage.[19] These results suggest that IL-1β induced proinflammatory cytokines and chemokines, as well as IL-1β, play important roles the early inflammatory changes of arthritis. It is well known that early changes of the cartilage caused by inflammation are characterized by the degradation of proteoglycan, an extracelluar matrix

protein, and by proteolytic proteins of MMPs.20) MMP-1 (interstitial collagenase), MMP-3 (stromelysin 1), MMP-13 (collagenase 3) are all known to be increased by IL-1β and were remarkable in expression among the MMPs. In addition, MMP-14, MMP-7 (matrilysin), and MMP-10 (stromelysin 2) were also rapidly elevated at 6 h, thereafter decreasing slowly by 24 h. A disintegrin like metalloprotease degrading aggrecan increased dramatically at 24 h, while some MMPs such as MMP-11, MMP-17, and MMP-12 were decreased, and proteolysis inhibitors such as serine (cysteine) proteinase inhibitor, protease inhibitor 3 were coexpressed. Though proteosome subunits, uniquitin conjugating enzyme, cathepsins, B-factor etc (group G, H) were enhanced and trypsin 2, ubiquitin specific protease 22, pronapsin etc (group J) were suppressed in expression. It is thought that not only changes of extracellular matrix but also the imbalance of intracellular proteins play a part in the pathogenesis of arthritis. Extracellular matrix consists of collagen fibers, elastin, proteoglycan, glycosaminoglycan, fibronectin and laminin. The joint cartilage is composed of 80% water, 20% organic substrates, while collagen fibers account for 50% of the dry weight. Among the collagen fibers, type 2 collagen is the most abundant with smaller amounts of type 5, 6, 9, 10 and 11.[21] Hypothetically, the changes of extracellular matrix in arthritis might result from of degradation by MMPs and impaired synthesis of extracellular matrix, so we analyzed the changes of gene expression related to collagen fibers. The results showed that type 4 α1,α6, type 5 α2, α3, type 7 α1, type 8 α1, type 11 α1, type 14 α1, type 22 α 1, lysine hydroxylase, prolyl-4-hydroxylase were increasing and type 3 α1, type 4 α4, type 16 α1 were decreasing. However, the gene for type 2 collagen was not included in these 17K cDNA microarrays. Lysine hydroxylase and prolyl-4-hydroxylase are involved in combining OH to proline residues and enhance the bindings between collagen fibers by increasing the hydrogen binding capacity.[22] Therefore, we have concluded that increased MMPs by IL-1β enhance lysis of intracellular matrix proteins. Thus the cells are activated to produce collagen fibers inducing lysine hydroxylase, prolyl-4-hydroxylase resulting in an increased binding between the collagen fibers. As to glycosaminoglycan synthesis, heparin sulfate 6-sulfotransferase for heparan sulfate synthesis, chondroitin 6-sulfotransferase, UDP-Gal: bGlcNAc β1,4-galactosyltransferases, UDP-N-acetylglucosa- mine transporter 3, UDP-galactose transporter for chondroitin sulfate synthesis and arylsulfatase B (N-acetylgalac-tosamine 4-sulfatase) for dermatan sulfate degradation were enhanced, but N-acetylg-lucosamine-6-sulphate sulphatase, chondroitin sulfate 2-sulfotransferase, chondroitin 6-sulfotransferase 1 for glycosaminoglycan degradation were decreased. As for proteoglycan,

proteoglycan 1 gene expression was increased, however, chondroitin sulfate proteoglycan 5, chondroitin sulfate proteoglycan 6 (bamacan), dermatan sulfate proteoglycan 3, and keratin sulfate proteoglycan (lumican) genes were all decreased. Meanwhile, other extracellular matrix protein genes such as chitinase 3-like 1 (cartilage glycoprotein 36), fibronectin 1 gene expression were increased, luminin (β3, γ1, γ3 increased, α3, β1 decreased), integrin (β3, α5 increased, β5, α10, αE decreased) differed in expression according to their subtypes, and aquaporin 1 gene for the water channel of the cell membrane was decreased. Because the results of this study are consistent with those of Aigner et al.[10] that analyzed MMP, fibronectin and collagen fiber expressions with cDNA microarrays in osteoarthritis and because results from chondrocytes might be comparable with those of osteoarthritis, we concluded that in osteoarthritis the changes of extracellular matrix during the early inflammatory reaction involve the degradation of extracellular matrix protein, imbalance between extracelluar matrix proteins and glycosaminoglycan metabolism and the impairment of water transportation. Since the changes of molecules forcell and matrix adherence can also affect the functions of the cell during early inflammatory response, more sophisticated studies are needed. We observed that the expression of diverse genes related to cytoskeleton, cell movement such as cytoskeleton II, actin related protein 2, myosin X, myosin IB, myosin heavy polypeptide 11, tubulin β5, cytoskeleton-associated protein 4 increased and kinesin light chain 2, erythrocyte membrane protein band 4.1-like 1, calicin, myosin heavy polypeptide 1, 2, 8, VB, microtubule associated proteins were decreased. These changes could contribute to early pathogenesis in arthritis by affecting chondrocyte shape and inducing the change of the intracellular transportation system. Joint cartilages have no blood vessels, and thus use anaerobic metabolism if the oxygen supplied by diffusion is inadequate.[23] With IL-1β treatment, hexokinae 2, enolase 1, aldolase B gene expression for glycolysis and transketolase-like 1, ribulose-5-phosphate 3-epimerase expression for pentose phosphate pathway were enhanced, but glycogen phosphorylase for glycogenolysis was decreased. In respect to lipid metabolism, fatty acid Co-A ligase was increased, but dehydrogenase/reductase (SDR family) member 3, acyl-CoA dehydrogenase, and L-3-hydroxyacyl-CoA dehydrogenase were decreased. As for amino acid metabolism, branched chain aminotransferase 1, and serine dehydratasecitrate synthase were increased. Even though the expression of citrate synthase in mitochondrial TCA cycle was enhanced, genes related to electron transportation were distinctly decreased. These results suggest that anaerobic metabolism is brisk, with aerobic metabolism suppressed in the first response of the cartilage, which might

increase the ECF acidity and contribute to inflammatory reactions. This proves that active oxygen and nitrogen metabolites play important roles in arthritis pathogenesis[24, 25] and that IL-1β treated cells overproduce such metabolites.[26] In this study, metallothinein, mitochondrial superoxide dismutase and other known antioxidants were increased briskly, but glutathione-S-transferase, selenoprotein P, glutamate-cysteine ligase, thiredoxin reductase 3 and others were decreased. This might be due to the result of oxidative stress induced by IL-1β treatment disturbing redox conditions in chondrocytes.

In conclusion, this study was designed to identify the comprehensive mechanisms and potential molecules for early responses in arthritis with IL-1β treated SW1353, human chondrosarcoma cell lines using cDNA microarray analysis. We can verify the marked variety in expressions of not only the genes of cytokines, chemokines, extracellular matrix lysis proteins but also those related to extracellular matrix formation, degradation, energy metabolism, controlling intracellular red-ox condition, cell signaling and translation. We therefore conclude that IL-1β plays a diverse role in cell signaling and expression of various translation factors related to inflammatory reactions with the alteration of cellular metabolisms in arthritis.

요 약

배경 : 골관절염은 단순 노화로 인한 질병이 아니라 연골대사의 이상으로 인한 기계적 그리고 생화학적 불안정성이 나타나는 질환이다. Transforming growth factor-β와 같이 연골세포의 기능을 향상시키는 촉진인자가 있는 반면 Interleukin(IL)-1이나 Tumor necrosis factor-α는 염증반응을 증가시킨다. 이중 IL-1은 골관절염의 병인에서 가장 중요한 염증 유발 인자로 알려져 있으며 이에 대한 자료도 축적되고 있다. 이 논문의 목적은 IL-1β에 대한 human chondrosarcoma cell (SW1353)의 유전자 발현 양상을 파악하여 골관절염 병인의 이해를 증대시키는데 있다.

재료 및 방법 : Chondrosarcoma cell line (SW 1353)은 연골세포의 IL-1β를 통한 세포노화에 대한 유전자 조절을 실험실에서 연구하는데 유용한 것으로 알려져 있다. 골관절염에서 IL-1에 의한 전체적인 유전자 발현의 변화를 연구하고 분석하기 위해 SW1353을 각각 1시간, 6시간, 24시간동안 IL-1에 노출시킨후 각각 총 RNA를 정제하였다. 유전자 발현의 변화는 17k human cDNA microarray로 분석하였고 semi-quantitative RT-PCR로 확인하였다.

결과 : Metallothioneins, matrix metalloproteinases, extracellular proteins, antioxidant protein, cytoskeletal proteins, cell cycle regulatory proteins, 세포성장과 세포 자멸사에 대한 단백질, signal protein, transcriptional factor를 포함한 1,200개 유전자에서 2배 이상의 변화가 관찰되었다. 이러한 변화는 초기 관절염에서 보이는 병리생리학적 변화와 상관관계가 있는 것으로 생각된다.

결론 : cDNA microarray 분석으로 유전자 발현의 의미있는 변화를 관찰하였으며 골관절

염 발병기전에서 분자생물학적 변화에 대한 인식과 치료 목표를 정립하는데 대한 새로운 자료로서 가치가 있을 것으로 생각된다.

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