

Comparison of molecular mechanisms of rheumatoid arthritis and osteoarthritis using gene microarrays

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Abstract. The present study aimed to compare the molecular mechanisms of rheumatoid arthritis (RA) and osteoarthritis (OA). The microarray dataset no. GSE29746 was downloaded from Gene Expression Omnibus. After data pre-processing, differential expression analysis between the RA group and the control, as well as between the OA group and the control was performed using the LIMMA package in R and differentially expressed transcripts (DETs) with \log_2 fold change (FC) > 1 and $P < 0.01$ were identified. DETs screened from each disease group were then subjected to functional annotation using DAVID. Next, DETs from each group were used to construct individual interaction networks using the BIND database, followed by sub-network mining using clusterONE. Significant functions of nodes in each sub-network were also investigated. In total, 19 and 281 DETs were screened from the RA and OA groups, respectively, with only six common DETs. DETs from the RA and OA groups were enriched in 8 and 130 gene ontology (GO) terms, respectively, with four common GO terms, of which two were associated with phospholipase C (PLC) activity. In addition, DETs screened from the OA group were enriched in immune response-associated GO terms, and those screened from the RA group were largely associated with biological processes linked with the cell cycle and chromosomes. Genes involved in PLC activity and its regulation were indicated to be altered in RA as well as in OA. Alterations in the expression of cell cycle-associated genes were indicated to be linked with the occurrence of OA, while genes participating in the immune response were involved in the occurrence of RA.

Introduction

Arthritis comprises a variety of diseases with joint pain as a common feature and includes osteoarthritis (OA), rheumatoid arthritis (RA) and psoriatic arthritis, while their underlying mechanisms are divergent (1). OA is a degenerative disease commonly manifesting with mechanical abnormalities of weight-bearing joints and hands, including knees and hips. It is characterized by loss of matrix proteoglycans, fibrillation of cartilage surface and eventual loss of collagenous matrix. Substantial studies have proven that synovial membrane inflammation, abnormal articular chondrocyte differentiation and bone remodeling contribute to the progression of OA (2-4). RA is a systemic autoimmune disease, in which the immune system targets body cells of the same organism. It features chronic inflammation of the synovium and subsequent cartilage destruction as well as bone erosion. The complement system is known to be involved in the induction and progression of inflammatory reactions in RA (5,6).

Synovial fibroblasts (SFs), the most abundant resident cell type in human synovial tissue, are thought to have an important role in the pathogenesis of chronic arthritis (7) and display marked hyperplasia in OA and RA (8). Furthermore, alterations in the expression of various genes have also been observed to be associated with the phenotypic changes in OASFs and RASFs (9,10). Previous microarray studies have confirmed a relatively high heterogeneity of the RASF phenotype (11,12). By comparison between healthy SFs, RASFs and OASFs, Del Rey *et al* (13) found that OASFs possessed a more homogeneous phenotype compared to RASFs. The present study subjected the microarray data from Del Rey *et al* (13) to a bioinformatics analysis to identify common and differential molecular mechanisms underlying the two arthritis sub-types. The transcriptional expression profiles of the OASF and RASF samples were compared with those of SFs from healthy controls and key genes were identified.

Materials and methods

Microarray data. Gene microarray dataset GSE29746 was downloaded from Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo/>) database (13). The data had been collected from SF cultures obtained from nine patients with RA, 11 age- and gender-matched patients with OA, and 11 age- and

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Key words: rheumatoid arthritis, osteoarthritis, differentially expressed transcripts, functional annotation, sub-network analysis

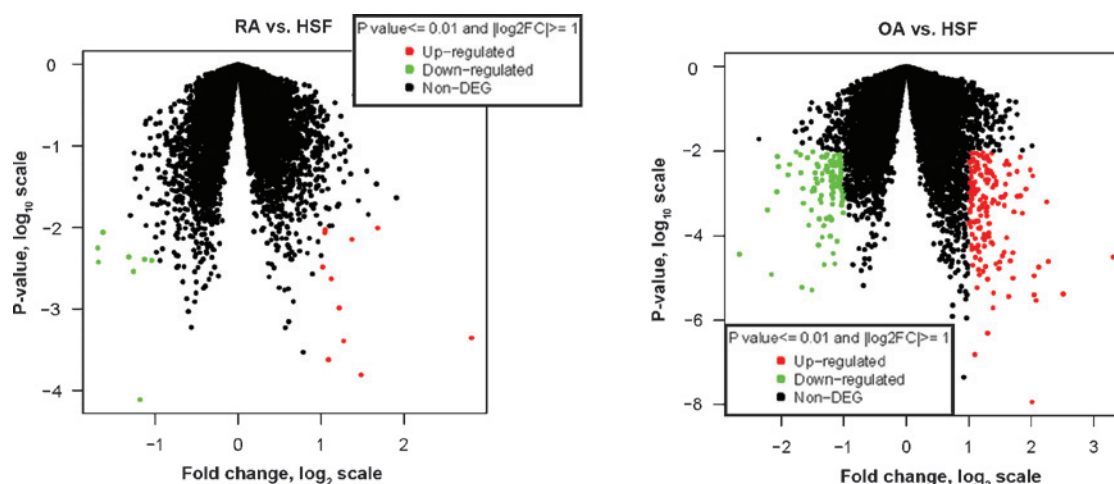


Figure 1. Volcano plots of expression values of all the transcripts. RA, synovial fibroblasts from rheumatoid arthritis group; OA, synovial fibroblasts from the osteoarthritis group; HSF, healthy synovial fibroblasts; DEG, differentially expressed gene.

gender-matched adult healthy donors. The platform was the Agilent-014850 Whole Human Genome Microarray 4x44 K G4112F (Agilent Technologies, Santa Clara, CA, USA).

Microarray data pre-processing. The gene expression profile data were extracted using the Linear Models for Microarray Data (LIMMA) package in R, followed by normexp background correction (14) and subsequent quantile normalization (15). The 95th quantile of all negative controls in each chip was calculated, and only probes with expression values larger than this value in all the samples were retained. According to the annotation platform, the values of probes corresponding to the same transcript were averaged and then defined as the final expression value of a transcript.

Screening of differentially expressed transcripts (DETs). Volcano plots were drawn to display the differential expression profiles of transcripts for each disease group and the control group. Differential expression analysis of transcripts between the RA group and the control group was performed using the *t*-test with the LIMMA package, as well as between the OA group and the control group (16). Transcripts with $|\log_2 \text{fold change (FC)}| > 1$ and $P \leq 0.01$ were screened as DETs. DETs from the two disease groups were compared, and a Venn diagram was used to display the result. A heatmap was also used to exhibit the overall expression profiles of DETs across all samples.

Functional annotation of DETs. The screened DETs were submitted to the online tool Database for Annotation, Visualization and Integrated Discovery (DAVID; <http://david.abcc.ncifcrf.gov/summary.jsp>) to perform functional annotation based on the gene ontology (GO) database using Fisher's exact test (17).

Analysis of interaction networks of DETs. DETs screened from each disease group were submitted to the Search Tool for the Retrieval of Interacting Genes/Proteins (<http://www.string-db.org/>) to construct protein-protein interaction (PPI) networks based on the Biomolecular Interaction Network Database (18).

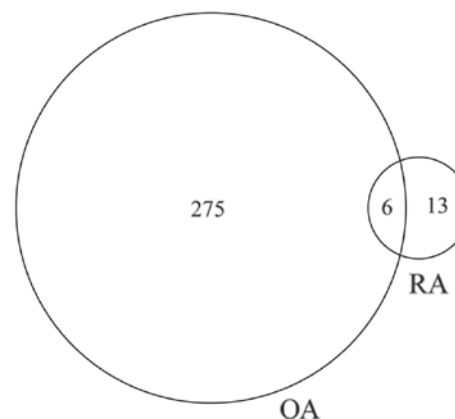


Figure 2. Venn diagram of differentially expressed transcripts between the rheumatoid arthritis group and the osteoarthritis group. RA, synovial fibroblasts from rheumatoid arthritis group; OA, synovial fibroblasts from the osteoarthritis group.

The sub-networks within each network were then detected by clustering analysis using clusterONE (19), and genes involved in each cluster were then subjected to functional enrichment analysis with DAVID ($P < 0.05$ as cut-off value).

Results

Screening of differentially expressed transcripts. The differential expression profiles between each disease group and the control group were displayed in Volcano plots (Fig. 1). In total, 19 DETs were screened from the RA group, with $|\log_2 \text{FC}|$ values of 1-2.8, among which eight genes were downregulated and 11 genes were upregulated. Furthermore, 281 DETs were screened from the OA group, with $|\log_2 \text{FC}|$ values of 1.0-3.3, among which 113 genes were downregulated and 168 were upregulated. As illustrated by the Venn diagram (Fig. 2), the OA group contained a greater number of DETs than the RA group, while the two groups had six DETs in common (Table I). In addition, the heatmaps indicated that the disease samples may be separated from the control samples using the identified DETs (Fig. 3).

Table I. Common differentially expressed transcripts between the RA group and the OA group.

Transcript	Gene symbol	RA group		OA group	
		logFC	P-value	logFC	P-value
NM_182734	<i>PLCB1</i>	-1.18252	7.74×10^{-5}	-1.03053	7.02×10^{-3}
NM_000211	<i>ITGB2</i>	2.81476	4.41×10^{-4}	3.31223	3.08×10^{-5}
NM_001853	<i>COL9A3</i>	-1.12924	4.07×10^{-3}	-1.03848	5.18×10^{-3}
NM_139125	<i>MASPI</i>	-1.31698	4.33×10^{-3}	-1.27800	3.61×10^{-3}
NM_002522	<i>NPTX1</i>	-1.69186	5.62×10^{-3}	-1.48981	9.55×10^{-3}
NM_014638	<i>PLCH2</i>	1.37193	7.12×10^{-3}	1.29122	7.54×10^{-3}
A_32_P4882	NA	-1.62833	8.70×10^{-3}	-2.66329	3.58×10^{-5}

RA, the rheumatoid arthritis; OA, the osteoarthritis; FC, fold change.

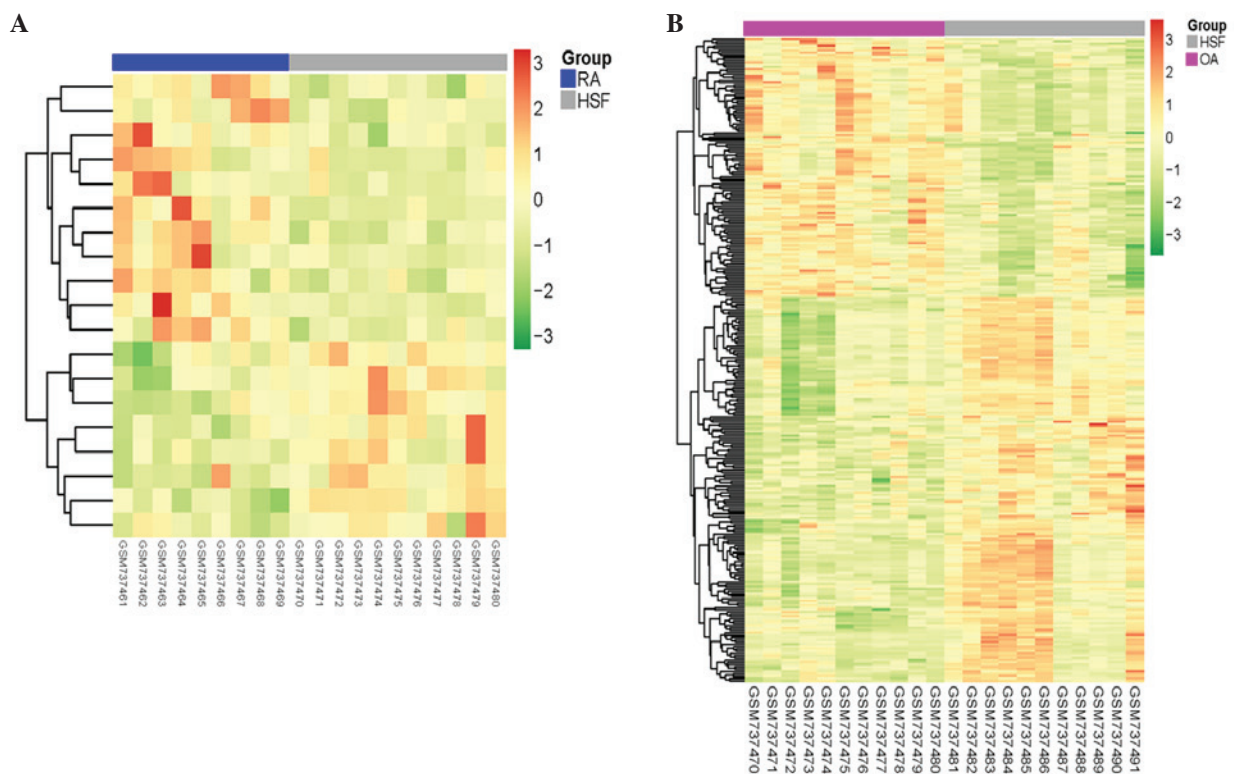


Figure 3. Clustering analysis of samples based on the screened differentially expressed transcripts. (A) Clustering analysis was based on the expression levels of 19 transcripts differentially expressed between the RA group and the healthy controls. (B) Clustering analysis based on the expression levels of 281 transcripts differentially expressed between the synovial fibroblasts in the OA group and the healthy controls. Each row represents a single gene and each column represents a tissue sample. The colored bar at the top indicates the group: Gray, HSF; blue, synovial fibroblasts from RA group; pink, synovial fibroblasts from OA group. Expression levels were indicated in a heat-map style with dark red indicating high expression and dark green low expression. RA, rheumatoid arthritis; OA, osteoarthritis; HSF, healthy synovial fibroblasts.

Functional annotation of DETs from each disease group. According to functional annotation, DETs from the RA and OA groups were enriched in 8 and 130 GO terms, respectively. Certain DETs from the RA group (e.g. *PLCH2*, *PLCB1*, *NPTX1* and *MASPI*) and from the OA group (e.g. *F2RL2*, *PLCB4*, *PLCH2*, *PLCB1* and *PLCXDI*) were commonly enriched in four GO terms, including two molecular function terms associated with phospholipase C activity (Table II). In addition to the four common GO terms, DETs screened from the RA patients were also associated

with immune response (e.g. *MASPI*, *IL27RA* and *ITGB2*), while those from the OA group were predominantly associated with the cell cycle and chromosomes, including *NEK2*, *TTK*, *PTTG2*, *MAF*, *CENPN* and *SGOL2*.

Analysis of PPI networks of DETs. The interaction networks of DETs from the RA group and the OA group are shown in Figs. 4 and 5, respectively. It was discovered that the PPI network based on DETs from the OA group was more complex than that of the RA group. The interaction network of the RA

Table II. Common GO terms between the RA group and the OA group.

GO term and function	Differentially expressed genes	
	RA group	OA group
GO:0004435 - Phosphoinositide phospholipase C activity	<i>PLCH2, PLCB1</i>	<i>F2RL2, PLCB4, PLCH2, PLCB1</i>
GO:0004629 - Phospholipase C activity	<i>PLCH2, PLCB1</i>	<i>F2RL2, PLCB4, PLCH2, PLCB1, PLCXD1</i>
GO:0005509 - Calcium ion binding	<i>NPTX1, MASP1, PLCH2, PLCB1</i>	<i>F10, MASP1, NRXN2, PCDHB3, PCDHB2, VWCE, COLEC12, GRIN3A, C9ORF140, SLIT2, NPTX1, CDH15, CLGN, PLCB4, HPSE, PLCH2, PCDHB16, PLCB1, THBS1, DTNA</i>
GO:0007267 - Cell-cell signaling	<i>NPTX1, WISP1, ITGB2</i>	<i>FGF18, ACHE, NTF3, PCDHB3, DLGAP5, EFNB2, MME, PCDHB2, ITGB2, RIMS1, NPTX1, PCDHB16, DTNA, HTR2A</i>

RA, rheumatoid arthritis; OA, the osteoarthritis; GO, gene ontology.

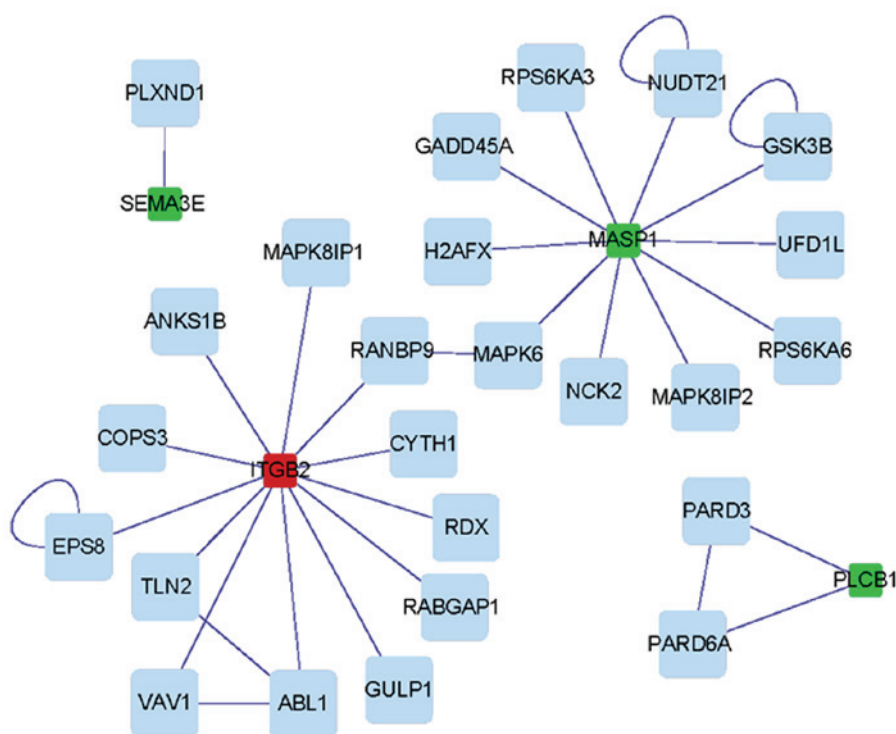


Figure 4. Interaction network of differentially expressed transcripts screened from synovial fibroblasts of patients with rheumatoid arthritis. Green squares represent downregulated DEGs and red squares indicate upregulated DEGs. DEG, differentially expressed gene.

group contained one sub-network, while that of the OA group had four sub-networks. DEGs including *PSMB9*, *MAF*, *HTR2B* and *HTR2A* were the hubs of corresponding PPI sub-networks of DEGs from the OA group, while *PLCB1* was a hub of the PPI sub-network of DEGs from the RA group (Table III).

Discussion

The present study revealed that in OA patients, the number of DEGs was higher compared to that in RA patients, which was consistent with the findings of Del Rey *et al* (13). This

previous study identified 2,050 DEGs, several of which were also identified in the screening performed in the present study, such as *ITGB2*, *PIP4K2C* and *NRXN2*, although with different magnitudes of differential expression. Notably, the present study aimed to unravel the mechanisms underlying RA and OA from the perspective of PPI by building PPI networks, the construction of which was not conducted in the previous study by Del Rey *et al* (13).

Two DEGs, *PLCH2* and *PLCB1*, screened from the RA patients as well as the OA patients, were enriched in two PLC (phospholipase C) activity-associated GO terms, which may

Table III. Identified sub-networks and functional annotation.

Variable	OA group				RA group
	Cluster1	Cluster2	Cluster3	Cluster4	Cluster1
Nodes (n)	4	4	3	6	3
Density	1	0.667	0.667	0.6	1
Quality	1	1	1	0.529	1
P-value	0.008	0.011	0.026	0.046	0.016
Genes	<i>ABCD3, PEX19,</i> <i>ABCD2, ABCD1</i>	<i>BACH1, MAF,</i> <i>MAFB, ATF4</i>	<i>HTR2A, MPDZ,</i> <i>HTR2B</i>	<i>NCOA3, ESR1,</i> <i>NCOA1, PSMB9,</i> <i>GRIP1, ESR2</i>	<i>PLCB1,</i> <i>PARD3,</i> <i>PARD6A</i>
Top significant GO terms	Peroxisomal membrane	Sequence-specific DNA binding	Serotonin binding	Steroid hormone receptor signaling pathway	Occluding junction

RA, rheumatoid arthritis; OA, osteoarthritis.

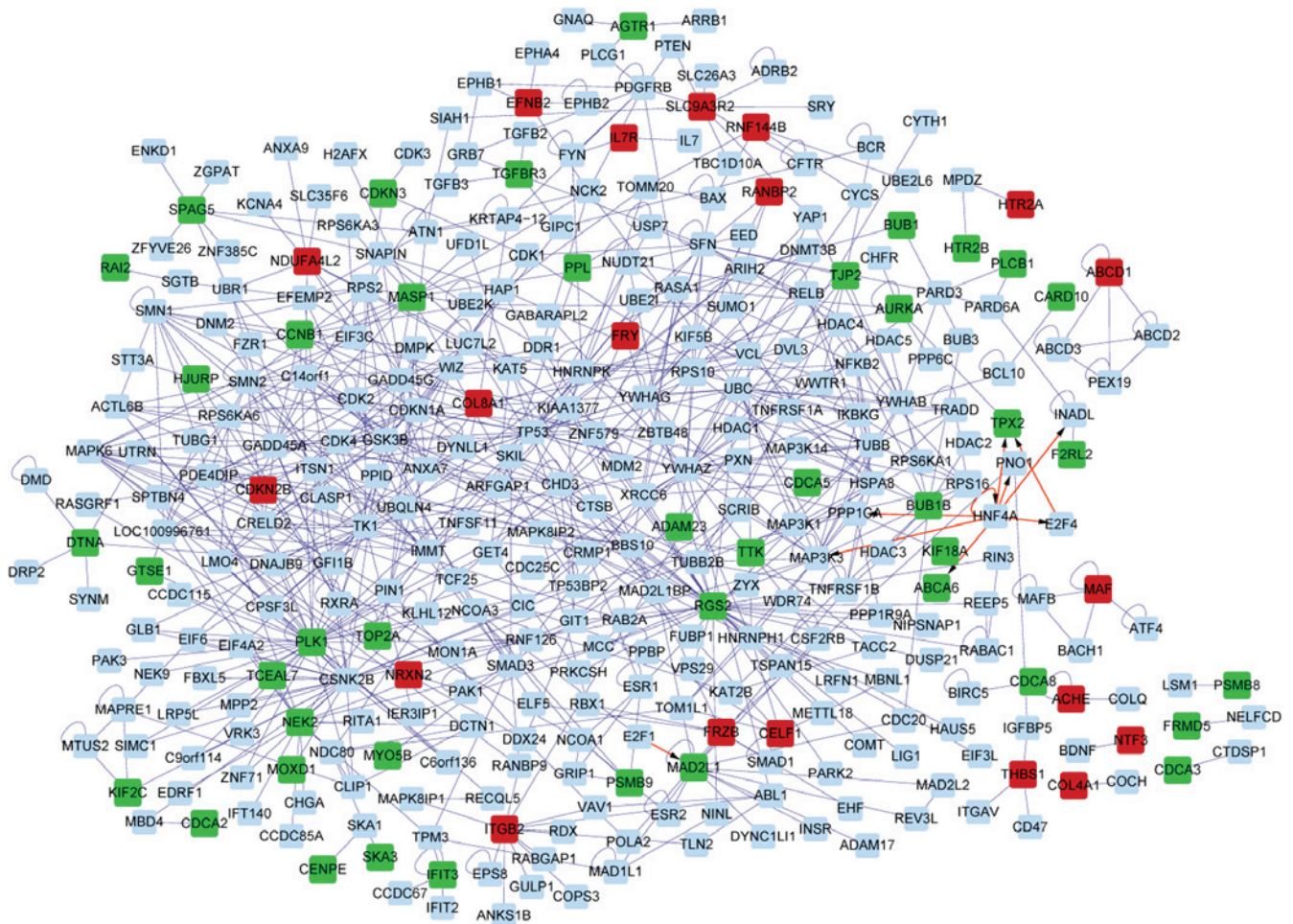


Figure 5. Interaction network of DEGs screened from synovial fibroblasts from patients in osteoarthritis group. Green squares represent downregulated DEGs and red squares indicate upregulated DEGs. DEG, differentially expressed gene.

infer that the two arthritis types share certain common mechanisms regarding phospholipase C activity. *PLCH2* and *PLCB1* encode two members of the phosphoinositide-specific PLC superfamily, PLC-eta 2 and -beta 1, respectively, and PLC catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate

into inositol 1,4,5-trisphosphate and 1,2-diacylglycerol (20). Previous studies have reported a notable elevation of the pro-inflammatory enzyme PLA2 (phospholipase A2), another phospholipase type catalyzing the hydrolysis of membrane glycerophospholipids to release arachidonic acid and

lysophospholipids in synovial fluids and sera of RA patients, and its expression has been proven to positively correlate with the disease activity in RA (21,22). Vignon *et al* (23) observed that PLA2 activity in RA and OA patients was similar, implying that pathological changes mediated by PLA2 are common in RA and OA. However, to the best of our knowledge, PLC activity has not been previously reported in RA or OA. As the two phospholipase types hydrolyze phospholipids at different sites, PLC may presumably also have a role in RA and OA, which requires further experimental validation.

Furthermore, in the present study, two PLC-regulating genes, *HTR2B* and *HTR2A*, which encode two members of the 5-hydroxytryptamine 2 receptor family that binds to the neurotransmitter serotonin, were also observed to be specifically differentially expressed in OA patients. These receptors activate PLC to initiate PLC-mediated signal transduction pathways (24). Of note, *HTR2A* was significantly upregulated, while *HTR2B* was significantly downregulated, implying their different roles in regulating PLC. The two genes were observed to be enriched in the GO biological process terms phosphoinositide-mediated signaling and second-messenger-mediated signaling as well as in the cellular component term plasma membrane-associated processes. This suggests that alterations in PLC-associated biological functions may be the predominant aberrations in OA patients.

In addition, DEGs screened from OA samples were also specifically and predominantly enriched in GO terms associated with the cell cycle and chromosomes. Among the abundance of DEGs screened from the OA samples, *PSMB9* and *MAF* were the key hubs of the sub-PPI network. *PSMB9*, which was downregulated in OA, encodes a member of the proteasome B-type family, and proteasomes cleave peptides via an adenosine triphosphate/ubiquitin-dependent pathway. This result was in accordance with previous studies, as the ubiquitin-proteasome pathway has been implicated in the pathogenesis of OA (25), and Rollin *et al* (26) has reported that *PSMB9* is aberrantly expressed in chondrocytes of OA patients. As the present study indicated that *PSMB9* was mainly enriched in the GO biological process terms protein metabolic processes and cell cycle, it may be hypothesized that the downregulation of *PSMB9* expression induces OA by disturbing normal protein metabolism and the cell cycle. *MAF*, a homolog of the avian musculoaponeurotic fibrosarcoma oncogene *V-Maf*, encodes a DNA-binding, leucine zipper-containing transcription factor. The increase in its expression in chondrocytes from patients with OA has been validated by Li *et al* (27), and it has been suggested that the upregulation of *c-MAF* expression may alter the phenotype of chondrocytes via regulating corresponding target genes (28), or interacting with other genes associated with chondrogenic differentiation (29). As in the present study, *c-MAF* was mainly enriched in chromosome-associated GO cellular component terms, it is presumed that it causes abnormal chondrogenic differentiation via inducing chromosomal abnormalities in OA patients.

Compared to those of OA, DETs screened from RA samples were exclusively enriched in immune response-associated GO biological process terms in addition to the four

common GO terms, implying that the immune system has a particularly critical role in the occurrence of RA, which is consistent with the fact that RA is a systemic autoimmune disease. *MASPI* encodes a serine protease that functions as a component of the lectin pathway of complement activation (30). The upregulation of *MASPI* expression, which was also observed by Rioja *et al* (31), indicated enhanced activation of the lectin pathway as well as the activation of the complement system in RA. Enhanced complement activation has been indicated to be potentially associated with the occurrence and/or augmentation of inflammation in RA (5). Activated synovial fibroblasts and upregulation of the expression of various adhesion molecules that mediate their attachment to the cartilage have been confirmed in rheumatoid arthritis (32). Among these adhesion molecules, the expression of integrin α group proteins, including VLA-3, -4 and -5 has been indicated to increase most significantly (33,34). *ITGB2* encodes integrin β 2, the expression of which was observed to be upregulated in the present study, which was also reported in RASFs by Del Rey *et al* (35); it is therefore indicated that *ITGB2* is linked with RA.

In conclusion, the present study indicated that genes involved in PLC activity, including *PLCH2* and *PLCB1*, and its regulation, including *HTR2A* and *HTR2B*, are aberrantly expressed in RA as well as in OA. Alterations in the expression of genes associated with the cell cycle, including *PSMB9* and *MAF*, were indicated to be linked with OA, while genes participating in the immune response were linked with RA.

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