Analysis of critical molecules and signaling pathways in osteoarthritis and rheumatoid arthritis

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Abstract. Osteoarthritis (OA) and rheumatoid arthritis (RA) are the most prevalent forms of arthritis in the elderly. This study aimed to explore the molecular mechanisms of these diseases and identify underlying therapeutic targets. Using GSE1919 microarray data sets downloaded from the Gene Expression Omnibus database, we screened differentially expressed genes (DEGs) in OA and RA cells. The underlying molecular mechanisms of these crucial genes were investigated by Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis. Small molecule expression and SNP analysis were also conducted by searching CMap and dbSNP databases. More than 320 genes changed in the arthritic cells and there were only 196 DEGs between OA and RA. OA and RA activated the classic mitogen-activated protein kinase signaling pathway, insulin signaling pathway, antigen processing and presentation and intestinal immune network for IgA production. Graft-versus-host disease and autoimmune thyroid disease-related pathways were also activated in OA and RA. Parthenolide and alsterpaullone may be treatments for OA and RA and insulin-like growth factor 1, collagen α2(I) chain and special AT-rich sequence-binding protein 2 may be critical SNP molecules in arthritis. Our findings shed new light on the common molecular mechanisms of OA and RA and may provide theoretical support for further clinical therapeutic studies.

Introduction

Osteoarthritis (OA) is a group of states associated with defective articular cartilage and changes in the underlying bone. OA is divided into erosive or non-erosive. Erosive OA is more abrupt and commonly exhibits subchondral bone erosions (1). Pathological changes in articular cartilage and subchondral bone result from chondrocyte imbalance in the extracellular matrix. Although numerous studies have reported probable chemical or mechanical causes of cartilage destruction (2,3), this area of research requires more detailed investigation.

Rheumatoid arthritis (RA) is a complex, chronic multi-systemic autoimmune disease, which affects the synovial membranes of multiple joints, cartilage and bone as well as bursa and tendon sheaths (4). RA is a prevalent chronic inflammatory joint disease affecting 0.5-1% of the world's population (5). RA leads to severe morbidity and disability if incorrectly treated, imposing a substantial economic burden on the affected individuals and society. The inflammatory process associated with RA is primarily observed in the synovial tissue. Synovial hyperplasia results from synovial outgrowths or synovial villi, comprised of macrophages, synovial lining cells, lymphocytes and blood vessels (6). Joint destruction occurs when the synovial pannus produces enzymes resulting in cartilage penetration, cartilage damage and joint erosion (7).

Although RA and OA share similar symptoms, it has been demonstrated that RA follows an alternative inflammatory pathway of pathogenesis to OA. Diagnosis and assessment of RA and OA is largely based on semi-quantitative methods of diagnosis, including symptoms, joint damage and physical function (8). At present, no cure exists for RA and OA and the management of these diseases depends upon early detection and aggressive treatment. Therefore, it is increasingly important to explore the molecular mechanisms of these diseases and analyze the associated signaling pathways, in order to uncover an effective therapeutic approach. In this study, we analyzed gene expression profiles of OA and RA cells to determine differentially expressed genes (DEGs) in the two forms of arthritis. Furthermore, through comparison we determined changed metabolic and non-metabolic pathways, small bioactive molecules and SNP corresponding genes associated with RA and OA.

Materials and methods

Gene expression profiles of synovial tissue samples from RA and OA patients and normal donors. The transcription profile of GSE1919 was obtained from the National Center for Biotechnology Information Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/) and was based on the Affymetrix Human Genome U95A Array (Santa Clara, CA, USA). A total of 15 chips were used in this study, including

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5 OA tissue chips, 5 RA tissue chips and 5 normal donor (ND) tissue chips (9). The study was approved by the Ethics Committee of the Charité Universitätsmedizin Berlin and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. All patients gave their informed consent prior to their inclusion in the study.

Analysis of DEGs. Raw data were normalized using the robust multichip average method (10) with the default settings implemented in the R affy package (version 2.13.0). The Limma (linear models for microarray data) method was used to identify DEGs (11). The original expression datasets from all conditions were extracted into expression estimates and used to construct the linear model. Significance of gene expression differences between OA, RA and ND cells were tested by classical t-test and P-values were adjusted for multiple comparisons using the false discovery rate (FDR) of Benjamini and Hochberg (12). FDR-corrected P<0.05 was considered to indicate a statistically significant difference.

Pathway analysis. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database is a collection of manually drawn pathway maps of the molecular interaction and reaction networks. A total of 130 pathways, involving 2,287 genes, were collected from KEGG (updated 2011/06). The Database for Annotation, Visualization and Integrated Discovery (DAVID), a high-throughput and integrated data-mining environment, analyzed gene lists derived from high-throughput genomic experiments. The DAVID (13,14) was used to identify over-represented pathways based on the hypergeometric distribution. Pathways with P<0.05 and count >2 were considered to be significant.

Small molecule expression analysis. CMap (the connectivity map) is a collection of gene expression profiles from cultured human cells treated with bioactive small molecules. The database contains 6,100 bioactive small molecule-interfering tests and 7,056 corresponding gene expression profiles (15). DEGs were analyzed through the CMap database to identify small bio-active molecules which resulted in similar or adverse gene expression. Probes of DEGs were converted to the accession number of GeneBank and then probe numbers for use in the CMap.

RA- and OA-related SNP analysis. RA- and OA- related SNPs were obtained following a dbSNP database search (http://www.ncbi.nlm.nih.gov/projects/SNP/) using the keywords 'osteoarthritis' and 'osteoporosis'. SNPs of RA and OA, which were suitable for probing, were acquired through the comparison of their corresponding genes with DEGs under pathological conditions.

Results

Recognition of DEGs in different samples. Analysis of GSE1919 using the Limma method identified a total of 460 DEGs in OA tissues compared with normal tissues. In RAt issues 1,148 DEGs were identified in comparison with ND (P<0.05; Fig. 1). Only 196 DEGs were identified when we compared the gene expression in OA tissues with that of RA. These results indicate similarities in the molecular mechanisms of OA and RA.

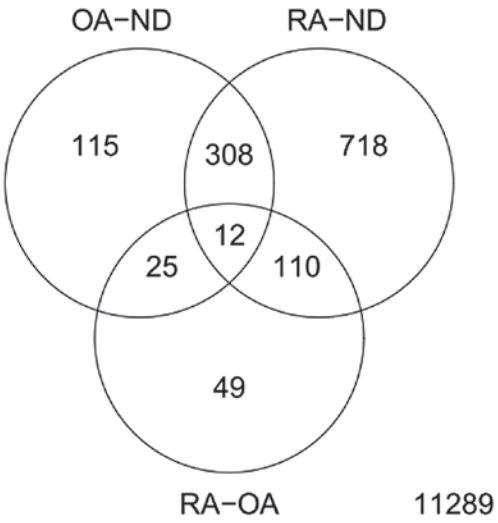

Figure 1. Venn diagram indicates the number of overlapping genes differentially expressed between normal and arthritic tissues. Of the 11,289 screened genes from micorarray, 1337 were differentially expressed between normal and arthritic tissues, including 460 genes between OA and ND, 1148 genes between RA and ND, and 196 genes between RA and OA. -, comparison between two groups. OA, osteoarthritis; RA, rheumatoid arthritis; ND, normal donor.

Analysis of pathways induced by arthritis. In arthritic tissues, gene expression profiles were significantly changed compared with the normal tissues. Therefore, DEGs were adopted to perform KEGG sub-pathway enrichment analysis. Significantly changed pathways with ≥2 genes included and P<0.05 in arthritis were obtained. It was demonstrated that the majority of pathways were involved in metabolic and non-metabolic processes, indicative of a large number of changes in arthritic tissues compared with normal tissues (Table I). These results are likely to be important for drug discovery for the treatment of arthritis.

Analysis of small molecules resulting in RA and OA. DEGs were first divided into upregulated and downregulated genes and then enriched with significantly changed genes obtained from treatment of small molecules from the CMap database. Targeted molecules observed to induce similar effects to arthritis were selected and the 20 targeted molecules with the lowest P-values were enumerated (Table II). Parthenolide and alsterpaullone were identified in OA and RA tissue analysis and are highly relevent molecules.

Analysis of RA- and OA-related SNPs. A total of 10 SNPs were obtained from the dbSNP database with the keyword 'osteoarthritis' and 15 were obtained with the keyword 'osteoporosis'. Following comparison of these acquired SNP corresponding genes with DEGs, we revealed that no arthritis-related SNP corresponding genes were the same as the previously identified DEGs and three osteoporosis-related SNP corresponding genes were identified in the DEGs (Table III).

Discussion

During the development of OA and RA, significant changes in gene expression occur. The present study demonstrated that more than 320 genes changed in OA and RA. Study of these

Table I. Difference in signaling pathways between normal and arthritic tissues.

Arthritic tissue	Term	P-value
OA-ND	hsa04612:Antigen processing and presentation	7.07E-04
	hsa05322:Systemic lupus erythematosus	7.72E-04
	hsa04910:Insulin signaling pathway	0.001034
	hsa05332:Graft-versus-host disease	0.002426
	hsa03320:PPAR signaling pathway	0.003083
	hsa04940:Type I diabetes mellitus	0.003567
	hsa05416:Viral myocarditis	0.00369
	hsa05330:Allograft rejection	0.008703
	hsa04640:Hematopoietic cell lineage	0.011659
	hsa04010:MAPK signaling pathway	0.014961
	hsa05310:Asthma	0.019068
	hsa04514:Cell adhesion molecules	0.019897
	hsa04142:Lysosome	0.024332
	hsa04672:Intestinal immune network for IgA production	0.030296
	hsa04920:Adipocytokine signaling pathway	0.032485
	hsa05320:Autoimmune thyroid disease	0.035251
RA-OA	hsa04060:Cytokine-cytokine receptor interaction	2.55E-06
	hsa05340:Primary immunodeficiency	1.52E-05
	hsa04062:Chemokine signaling pathway	0.002685
	hsa04630:Jak-STAT signaling pathway	0.003031
	hsa04650:Natural killer cell-mediated cytotoxicity	0.004972
	hsa04660:T cell receptor signaling pathway	0.007133
	hsa04672:Intestinal immune network for IgA production	0.00735
	hsa02010:ABC transporters	0.032296
	hsa04510:Focal adhesion	0.040002
	hsa04640:Hematopoietic cell lineage	0.04746
RA-ND	hsa04666:Fcγ R-mediated phagocytosis	7.49E-10
	hsa04612:Antigen processing and presentation	2.56E-07
	hsa04062:Chemokine signaling pathway	8.32E-07
	hsa04672:Intestinal immune network for IgA production	8.82E-07
	hsa05330:Allograft rejection	1.71E-06
	hsa04514:Cell adhesion molecules	2.55E-06
	hsa04940:Type I diabetes mellitus	2.58E-06
	hsa05416:Viral myocarditis	4.06E-06
	hsa05332:Graft-versus-host disease	5.22E-06
	hsa05322:Systemic lupus erythematosus	8.12E-06
	hsa04650:Natural killer cell-mediated cytotoxicity	2.57E-05
	hsa05310:Asthma	3.00E-05
	hsa04662:B cell receptor signaling pathway	3.72E-05
	hsa04660:T cell receptor signaling pathway	3.87E-05
	hsa05340:Primary immunodeficiency	4.24E-05
	hsa04670:Leukocyte transendothelial migration	1.71E-04
	hsa04640:Hematopoietic cell lineage	2.68E-04
	hsa04620:Toll-like receptor signaling pathway	3.16E-04
	hsa05320:Autoimmune thyroid disease	5.90E-04
	hsa04664:Fcε RI signaling pathway	6.59E-04
	hsa04010:MAPK signaling pathway	0.001477
	hsa04060:Cytokine-cytokine receptor interaction	0.001906
	hsa04520:Adherens junction	0.004307
	hsa04142:Lysosome	0.005237
	hsa04722:Neurotrophin signaling pathway	0.019966
	hsa05210:Colorectal cancer	0.022059
	hsa04512:ECM-receptor interaction	0.022059
	hsa04910:Insulin signaling pathway	0.02415
	hsa04510:Focal adhesion	0.031928
	hsa05221:Acute myeloid leukemia	0.039772
	hsa04810:Regulation of actin cytoskeleton	0.04152
	hsa05120:Epithelial cell signaling in *Helicobacter pylori* infection	0.048088

OA, osteoarthritis; RA, rheumatoid arthritis; ND, normal donor.

common DEGs may help identify potential broad-spectrum anti-arthritis drugs. Furthermore, only 196 DEGs were identified between OA and RA, including interleukin 3 receptor α (*IL3RA*), transforming growth factor β receptor III and *CRYAB,* indicating that the two diseases are correlated and drugs that simultaneously treat these diseases may exist.

Cluster analysis of DEGs demonstrated several common pathways associated with these diseases, including the classic mitogen-activated protein kinase (MAPK) signaling pathway and the insulin signaling pathway. The signaling pathways leading to MAPK activation have been linked to various catabolic responses in diseases, including arthritis (16,17). Two immune pathways, antigen processing and presentation and intestinal immune network for IgA production, were also activated in the arthritic tissues, indicating that the immune response is involved in these diseases. Inflammation and cytokines play significant roles in RA and in certain cases of OA (17). Furthermore, changes of several cell adhesion molecules, including integrin β2 (*ITGB2*) (18) and protein tyrosine phosphatase receptor

Table II. Intersection of gene expressions between small bioactive molecules and the differentially expressed genes of arthritis.

Arthritic tissue	CMap name	P-value
OA vs. ND	Doxorubicin	0
	H-7	0
	Alsterpaullone	0
	GW-8510	0
	Anisomycin	0
	Thapsigargin	0
	MG-262	0
	Parthenolide	0
	Withaferin A	0
	Cephaeline	0
	15-delta prostaglandin J2	0
	Mitoxantrone	0.00002
	Valinomycin	0.00006
	Disulfiram	0.00016
	Lomustine	0.00024
	Terfenadine	0.00026
	Lanatoside C	0.00026
	Gossypol	0.00052
	5224221	0.00056
	5194442	0.00062
RA vs. ND	Thapsigargin	0
	Parthenolide	0
	Niclosamide	0
	Alsterpaullone	0.00002
	Helveticoside	0.00016
	Valinomycin	0.0003
	Fluticasone	0.00097
	5194442	0.00105
	Cephaeline	0.00134
	Diphenylpyraline	0.00179
	Tiapride	0.00192
	Methylergometrine	0.00219
	Metixene	0.00292
	Methyldopate	0.00294
	Lanatoside C	0.00326
	CP-320650-01	0.00329
	Enoxacin	0.00394
	Procainamide	0.004
	CP-690334-01	0.00445
	Tranylcypromine	0.00449

OA, osteoarthritis; RA, rheumatoid arthritis; ND, normal donor.

type c (*PTPRC*) (19) and lysosome-related molecules, including phospholipase A2 group XV (*PLA2G15*) (20) and adaptor-related protein complex 1β (*AP1B1*) (21) in OA and RA cells demonstrated that the two diseases altered their microenvironment and removed the exogenous substances by the lysosome. In addition, graft-versus-host (22) and autoimmune thyroid disease-related pathways were also activated in OA and RA (23). Further investigation of these pathways is likely to be shed light the network of signal pathways under OA and RA. More studies on the DEGs between OA and RA may provide useful information to differentiate the molecular mechanisms associated with OA and RA.

Table III. Corresponding differently expressed genes of disease-related SNPs.

Arthritic tissue	Gene	SNP ID
OA-ND	IGF1	121912430
	COL1A2	72658152
RA-ND	IGF1	1E+08
	SATB2	1E+08

OA, osteoarthritis; RA, rheumatoid arthritis; ND, normal donor. SNP ID represents the identification number from the dbSNP database. SNP, single nucleotide polymorphism.

Based on the DEGs and data from the CMap database, we acquired a series of small molecules. Two of these small molecules, parthenolide and alsterpaullone, demonstrated significant similarity in OA and RA tissues ($P<0.05$) and required additional analysis to determine their suitability as broad spectrum anti-arthritis drugs.

Parthenolide is a sesquiterpene lactone of the germacranolide class which occurs naturally in the plant feverfew (*Tanacetum parthenium*). Parthenolide modulates the NF-κB-mediated inflammatory responses in experimental atherosclerosis (24) and blocks lipopolysaccharide-induced osteolysis through suppression of NF-κB activity (25). Parthenolide induces apoptosis in acute myelogenous leukemia cells, leaving normal bone marrow cells relatively unscathed (26). Pharthenolide also exhibits microtubule-interfering activity (27), anti-inflammatory and anti-hyperalgesic effects (28) and activity against the parasite *Leishmania amazonensis* (29). Since numerous cases of OA and RA result from the interruption of immune responses, including inflammation, it is probable that parthenolide is a suitable therapeutic for these ailments.

Alsterpaullone is a potent, ATP-competitive inhibitor of the cell cycle regulating cyclin-dependent kinases CDK1/cyclin B ($IC_{50} = 0.035\ \mu M$) and an inhibitor of GSK-3β and the neuronal CDK5/p25 (30). In addition, alsterpaullone induces apoptosis by activation of caspase-8 and -9 followed by disruption of mitochondrial potential (31).

Through analysis of RA- and OA-related SNPs, we identified that three osteoporosis-related SNP corresponding genes (*IGF1, COL1A2* and *SATB2*) were differentially expressed. Insulin-like growth factor 1 (IGF-1), also called somatomedin C, is a protein encoded by the IGF1 gene in humans (32,33). IGF-1 is expressed and produced by chondrocytes and is one of the anabolic growth factors associated with cartilage (34) and thus is involved in arthritis. Collagen α2(I) chain is a protein encoded by the *COL1A2* gene in humans (35,36). Mutations in this gene are associated with osteogenesis imperfecta, Ehlers-Danlos syndrome, idiopathic osteoporosis and atypical Marfan syndrome. Special AT-rich sequence-binding protein 2

(SATB2), also known as DNA-binding protein SATB2, is a human protein encoded by the SATB2 gene (37). SATB2 has been identified to be disrupted in two unrelated cases with *de novo* apparently balanced chromosome translocations associated with cleft palate and Pierre Robin Sequence (38). The present study also demonstrated that these DEGs were often mutated under arthritis and thereby more studies should focus on their roles in OA and RA.

The present findings shed new light on the molecular mechanisms of OA and RA. Results revealed more than 320 DEGs in both diseases which may be involved in OA and RA development via MAPK and insulin signaling pathways, antigen processing and presentation, intestinal immune network, graft-versus-host disease and autoimmune thyroid disease-related pathways. Notably, parthenolide and alsterpaullone were identified as important small molecules involved in the induction of anti-inflammatory and apoptosis-related gene expression and thus we suggest that these molecules may be suitable anti-arthritis drugs for OA and RA. Furthermore, mutations of *IGF1, COL1A2* and *SATB2* genes were critical for the pathogenesis of OA and RA. However, there are specific limitations in our study. The pathway enrichment was only based on the connection between genes and therefore genes without strong neighbors are likely to be excluded from the analysis (13). In addition, further experimental analysis is required to confirm the conclusions of the present study.

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