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Interaction relationships of osteoarthritis and rheumatoid arthritis related genes

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Abstract. – BACKGROUND: Osteoarthritis (OA), also referred to as degenerative joint disease or wear-and-tear arthritis, is caused by the breakdown of joint cartilage. Rheumatoid arthritis (RA) is a chronic, inflammatory type of arthritis. RA is also classified as a kind of autoimmune disease.

AIM: To find the important genes in RA and OA.

MATERIALS AND METHODS: Comprehensively compared 3 datasets of RA with 2 datasets of OA, 98 genes were sifted. We explored protein-protein associations processed for the 98 genes by mining famous gene/protein interaction/association database.

RESULTS: We found most of those genes appear to play a key role in the anti-inflammatory and immunosuppressive effects.

CONCLUSIONS: Our research would play a useful role in the diagnosis and treatment of OA and RA.

Key Words:
Osteoarthritis, Rheumatoid arthritis, Interaction network, Differential gene.

Introduction

Osteoarthritis

Osteoarthritis (OA) is the most prevalent form of arthritis in the elderly. Primary OA is an idiopathic phenomenon, occurring in previously intact joints, with no apparent initiating factor such as joint injury or developmental abnormalities. The disease is characterized by softening, splitting, and fragmentation (fibrillation) of articular cartilage. This process is usually accompanied by subchondral bone sclerosis, bone cysts, and bony outgrowths at the joint margins (osteophytes)[1].

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attacks flexible (synovial) joints[2]. RA affects 1% of the world's population. Patients with RA not only have a progressive and debilitating disease and severe functional impairment, but can also experience a reduced life expectancy due to frequent involvement of the major organ systems[3].

RA is best considered a clinical syndrome spanning several disease subsets. These different subsets entail several inflammatory cascades, which all lead towards a final common pathway in which persistent synovial inflammation and associated damage to articular cartilage and underlying bone are present[4].

Similar as angiogenesis and vascular insufficiency were found in synovial membrane (SM)[5]. Angiogenesis and vascular insufficiency may be caused by somatic cell mutations. Somatic mutations were detected in the p53 tumor suppressor gene in rheumatoid arthritis synovia[6]. Over 50% of risk of developing rheumatoid arthritis is attributable to genetic factors[7].

Different Groups Studies

The mechanisms of RA and OA have not been totally understood yet[8]. The gene expression in disease is often characterized by significant inter-individual variances via specific synchronization/desynchronization of gene expression.

Understanding the genetic contribution to OA has two important clinical implications[9]. First, by finding genes involved in disease risk or involved in progression, we will better understand the molecular pathogenesis of OA, which may open areas for therapeutic intervention. Second, by identifying sets of genetic variants associated with risk for disease or with progression of OA, it will be possible to detect individuals at high risk and to monitor.

To raise awareness of the contribution of the variance to the pathogenesis both in RA and OA, we gathered gene expression data from 5 published studies examining synovial membrane of RA, OA and normal tissues from the Gene Expression Omnibus (GEO) database, and performed an integrated analysis to get an universally gene dataset. Gene expression variances were tested in SM samples of RA patients, OA patients and normal controls (NCs).

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Why we Need Gene Network?

Genome wide expression analysis with DNA microarrays has become a mainstay of genomics research[10]. The challenge lies in interpreting the results to gain insights into biological mechanisms[11]. Single-gene analysis may miss important effects on pathways. Cellular processes often affect sets of genes acting in concert. An increase of 20% in all genes encoding members of a metabolic pathway may dramatically alter the flux through the pathway and may be more important than a 20-fold increase in a single gene[11].

We got a long list of statistically significant genes without any unifying biological theme. A critical challenge is to bring order and understanding into this data.

Functional annotation of differentially expressed genes is a necessary and critical step in the analysis of microarray data[12]. The distributed nature of biological knowledge frequently requires researchers to navigate through numerous web-accessible databases gathering information one gene at a time. A more judicious approach is to provide query-based access to an integrated database that disseminates biologically rich information across large datasets and displays graphic summaries of functional information.

With the analysis of gene network, we can even find the important genes in RA and OA related biological progress, which were not in the list of statistically significant genes sifted via microarray.

Materials and MethodS

RA Related Genes

An integrated analysis method was combined with the results of 3 studies that address a set of related research hypotheses. Meta-analyses of gene expression profiles integrating multiple microarray studies have been particularly useful to identify conserved genetic signatures of human disease[13]. We applied this meta-analysis method to 3 independent rheumatoid arthritis related gene profiling studies that compared RA versus normal control (Table I).

In 2008, Huber et al[14] made an analysis of gene expression in RA, OA (osteoarthritis), and normal control (NC) samples was carried out using Affymetrix U133A/B oligonucleotide arrays, and the results were validated by real-time reverse transcription-polymerase chain reaction (PCR). For the comparison between RA and NC, 568 probe sets (about 533 genes) with significantly different variances in the two groups ($p \leq 0.05$; Bonferroni/Holm corrected Brown-Forsythe version of the Levene test) were selected. Disease-relevant or even disease-specific pathways/complexes are characterized by broad intra-group inter-individual expression variances, which indicated RA pathogenesis in different individuals may depend to a lesser extent on common alterations of the expression of specific key genes.

In 2010, Ungethuem et al[8] identified a set of 736 gene tags (about 717 genes) significantly deregulated in pair-wise comparisons between RA and normal donors (ND), based on an false discovery rate (FDR) of 2.5 by SAM (Significance Analysis of Microarrays).

In 2012, Smiljanovic et al[15] analyzed pair-wise comparisons between 8 RA patients and 12 normal donors. Transcriptional profiles of monocytes isolated from RA patients identified 1,627 probe sets (about 1,070 genes) as differentially expressed compared with normal donors.

OA Related Genes

Similar to the above, we analyzed 2 independent OA related gene profiling studies that compared OA versus normal control (Table II).

In the first study (Ungethuem et al)[8], 736 genes were identified as significantly differentially expressed in pair-wise comparisons between OA and ND, based on an FDR of 2.5 by SAM.

In the second study (Del Rey et al)[16], synovial fibroblasts (SF) were obtained from 11 patients

Table I. Three independent RA related gene profiling studies.

Affymetrix array type, year	RA sample	Control	GEO accession	Diff gene	Reference	Country
HG-U133A/B, 2008	12	10	GSE12021	533	14	Bethesda, USA
HG-U95A, 2010	5	5	GSE1919	717	8	Berlin, Germany
HG-U133A/plus2, 2012	4	12	GSE38351	1070	15	Berlin, Germany

Table II. Two independent OA related gene profiling studies.

Array type, year	RA sample	Control	GEO accession	Diff gene	Reference	Country
Affymetrix HG-U95A, 2010	5	5	GSE1919	726	8	Berlin, Germany
Agilent-014850 HG, 2012	9	11	GSE29746	587	16	Madrid, Spain

with OA (OASF). Samples were collected under similar sub-confluent conditions 24h after serum addition. More than 20 microarray data were used for determine the statistical significance (*p* value) of the differences in gene expression. Over 587 genes were identified after examining the numbers of transcripts down-regulated or up-regulated at least twofold with an uncorrected *p* value of < 0.05.

Integrated Analysis

When different groups study the same biological system, the list of statistically significant genes from the multiple studies may show their overlap.

Comprehensively compared the 3 datasets of RA with the 2 datasets of OA, frequency of 98 genes were greater than 3 among the all 5 datasets (Figure 1).

Gene Network

Genes can form a variety of functional connections with each other, including stable complexes, metabolic pathways and a bewildering array of direct and indirect regulatory interactions. These connections can be conceptualized as networks and the size and complex organization of these networks present a unique opportunity to view a given genome as something more than just a static collection of distinct genetic functions.

We explored protein-protein associations processed for the 98 genes by mining famous gene/protein interaction/association database like KEGG, Gene Ontology, BioGRID and Reactome[17-20].

Then, Figure 2 was created by Cytoscape, a software environment for integrated models of biomolecular interaction networks[21].

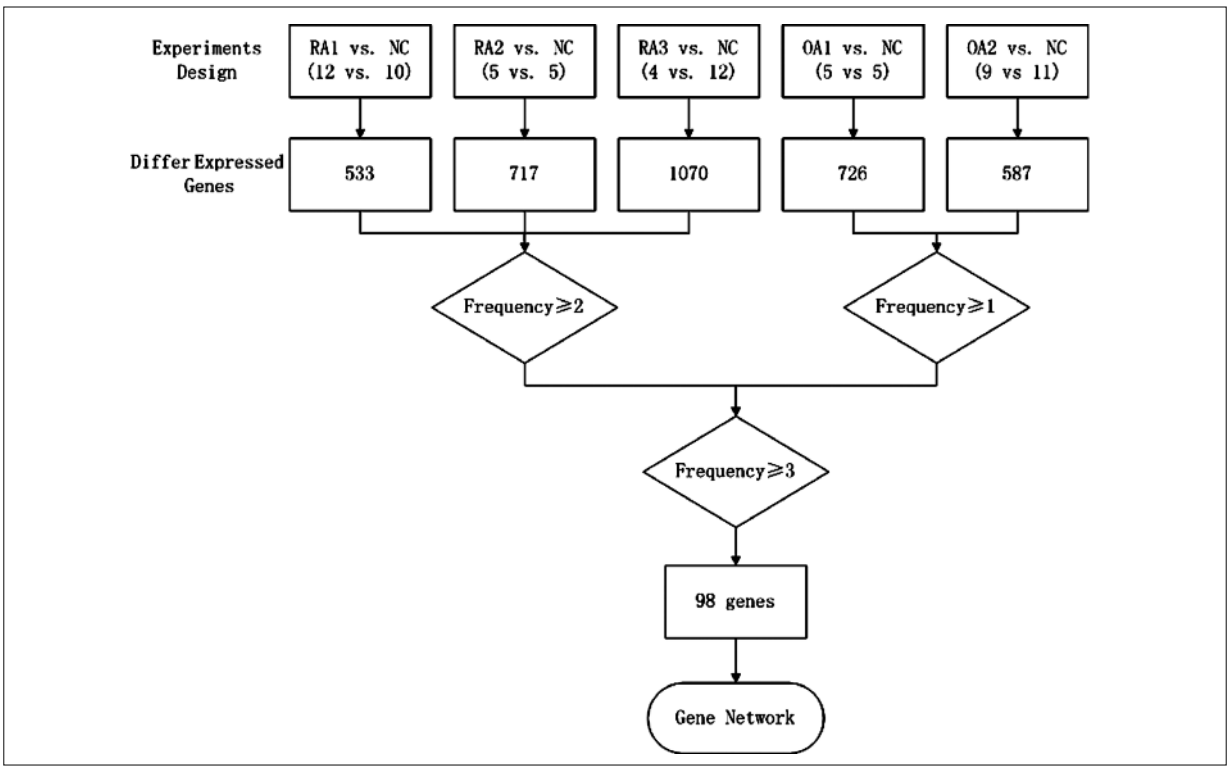

Figure 1. Pipeline of OA & RA related gene analysis. In Figure 1, an *arrowhead* means one data flow. Comprehensively compared the 3 datasets of RA with the 2 datasets of OA, frequency of 98 genes were greater than 3 among the all 5 datasets.

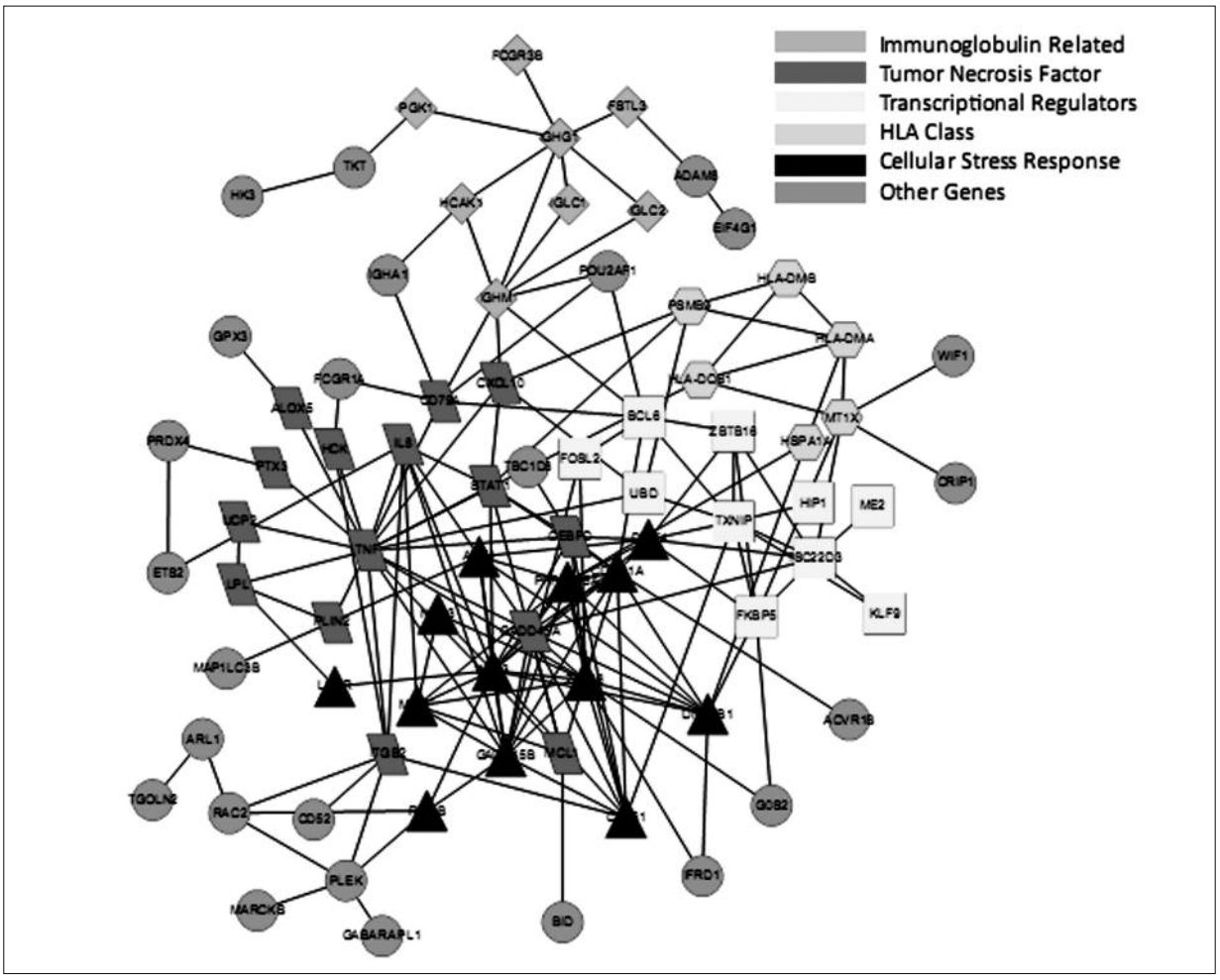

Figure 2. Gene Network.

Results

The whole gene network consists of several sub-network/clusters in Figure 2. Functional links between proteins can often be inferred from genomic associations between the genes that encode them. Different color means different groups of genes that are required for the same function tend to show similar species coverage, are often located in close proximity on the genome (in prokaryotes), and tend to be involved in gene-fusion events.

It is known that IGHG1 (immunoglobulin heavy constant gamma 1), IGLC2 (Ig lambda-2 chain regions), IGLC1 (immunoglobulin lambda constant 1) and IGHM (Immunoglobulin heavy constant mu) genes are immune related[22]. They are immunoglobulin which recognize foreign antigens and initiate immune responses such as phagocytosis and the complement system. Data is the green flag in Figure 2.

Gene with the purple flag in Figure 2 encodes a multifunctional pro-inflammatory cytokine that belongs to the tumor necrosis factor (TNF) superfamily. STAT1 (signal transducer and activator of transcription 1) in response to cytokines and growth factors, STAT family members are phosphorylated by the receptor associated kinases, and then form homo or heterodimers that translocate to the cell nucleus where they act as transcription activators. This suggests that OA and RA are similar to tumor.

Genes with the yellow flag in Figure 2, the key nodes like ZBTB16 and TSC22D3 are function as transcriptional regulators. They appear to play a key role in the anti-inflammatory and immunosuppressive effects[23].

The gene datasets with the light blue flag in Figure 2 belong to the HLA class and play a central role in the immune system by presenting peptides derived from extracellular proteins[24].

Within the dark blue flag in Figure 2, key note ATF3 (Activating Transcription Factor-3) is induced by a variety of signals, including many of those encountered by cancer cells, and is involved in the complex process of cellular stress response. Multiple transcript variants encoding different isoforms have been found for this gene.

The secreted protein encoded by CYR61 (Cysteine rich angiogenic inducer 61) is growth factor-inducible and promotes the adhesion of endothelial cells. The encoded protein interacts with several integrin and with heparin sulfate proteoglycan. This protein also plays a role in cell proliferation, differentiation, angiogenesis, apoptosis, and extracellular matrix formation.

The expression of CDKN1A (Cyclin-dependent kinase inhibitor 1A) is tightly controlled by the tumor suppressor protein p53, through which this protein mediates the p53-dependent cell cycle G1 phase arrest in response to a variety of stress stimuli. This protein can interact with proliferating cell nuclear antigen (PCNA), a DNA polymerase accessory factor, and plays a regulatory role in S phase DNA replication and DNA damage repair.

This gene encodes a multifunctional pro-inflammatory cytokine that belongs to the tumor necrosis factor (TNF) superfamily. STAT1 in response to cytokines and growth factors, STAT family members are phosphorylated by the receptor associated kinases, and then form homo or heterodimers that translocate to the cell nucleus where they act as transcription activators. This suggests that OA and RA are similar to tumor.

Discussion

The mechanisms of RA and OA have not been totally understood yet[8]. The gene expression in disease is often characterized by significant inter-individual variances via specific synchronization/desynchronization of gene expression.

Microarray analysis is an effective technique to simultaneously compare the global gene expression between experimental and control groups. However, high-throughput data are generally of high variability, low reproducibility and contain non-specific noise[25], where subgroups of genes within the dataset may be associated with a generalized response to a given stimulus. This effect can be ameliorated using a comprehensive text mining and bioinformatics approach including: BioGRID, Reactome, GO analysis, Dynamic Gene Network analysis and DAVID pathway analysis as an effective method to enrich the most relevant IR (immune response) responsive genes[17-20].

We explored protein-protein associations processed for the 98 genes by mining famous gene/protein interaction/association database like KEGG, Gene Ontology, BioGRID and Reactome.

The PPI (protein-protein interaction) subnetwork was extracted from the total protein-protein interaction network by using 98 overlapping genes as seed genes, which reduced the complexity of the total network. The individual pathway analysis of 98 overlapping genes provided several essential pathways including Cellular Stress Response and several immune-related signaling pathways. These results suggest that these pathways are important at the RA and OA.

Some gene encodes a multifunctional proinflammatory cytokine that belongs to the tumor necrosis factor super-family. STAT1 in response to cytokines and growth factors, STAT family members are phosphorylated by the receptor associated kinases, and then form homo or heterodimers that translocate to the cell nucleus where they act as transcription activators. This suggests that OA and RA are similar to tumor.

ACVR1B (Activin receptor type-1B), LRRC15 (Leucine rich repeat containing 15) and STAT1 appear in 5 datasets for 5 times, respectively. This indicates that they have a very important role in both OA and RA.

Nearly no study describes function of ACVR1Band LRRC15 in OA or RA in present. Further research like high throughput target sequencing might clear their role in those diseases.

Conclusions

The total protein-protein interaction networks provide basic information for genetic association studies performed using irradiation, which could act as an initial step for better deciphering the molecular mechanisms of irradiation response together with our microarray results[26]. Protein networks are used to increase the statistical power in human genetics, to aid in drug discovery, to close gaps in metabolic enzyme knowledge and to predict phenotypes and gene functions, to name just a few examples. Our research would play a useful role in the diagnosis and treatment of OA and RA.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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