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Identification of a two-loci epistatic interaction associated with susceptibility to rheumatoid arthritis through reverse engineering and multifactor dimensionality reduction

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Abstract

Altered synovial fibroblast (SF) transcriptional activity is a key factor in the disease progression of rheumatoid arthritis (RA). To determine the transcriptional regulatory network associated with SF response to an RA proinflammatory stimulus we applied a CARRIE reverse engineering approach to microarray gene expression data from SFs treated with RA synovial fluid. The association of the inferred gene network with RA susceptibility was further analyzed by a case–control study of promoter single-nucleotide polymorphisms, and the presence of epistatic interactions was determined using the multifactor dimensionality reduction methodology. Our findings suggest that a specific NF-κB transcriptional regulatory network of 13 genes is associated with SF response to RA proinflammatory stimulus and identify a significant epistatic association of two of its genes, *IL6* and *IL4I1*, with RA susceptibility.
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Rheumatoid arthritis (RA) is a chronic inflammatory disease with a prevalence of approximately 1% that primarily affects diarthrodial joints, in which synovial inflammation leads to cartilage and bone destruction. The synovial membrane, a rather acellular tissue in normal conditions, becomes hypertrophic and is composed mainly of synovial fibroblasts (SFs) [1]. SFs in RA display an activated phenotype, which significantly contributes to disease initiation and progression [2,3]. Although several transcription factors like AP-1 [4], NF-κB [5], or p53 [6] have been previously associated with SF altered activity, no precise transcriptional regulatory network has been associated with RA pathophysiology. With the advent of microarray technology, global gene expression data can now be used to model transcriptional networks associated with molecular disease mechanisms.

Modeling transcriptional regulatory networks is considered a *reverse engineering* problem. By reverse engineering we understand the process of determining the structure of a system by reasoning backward from observations of its behavior [7]. Different methods have been recently described to determine functional networks from microarray gene expression data. After providing success with lower eukaryotes [8] they are also proving successful in defining regulatory networks in the first studies with human gene expression data [9].

Microarray analysis of cultured SFs treated with a single factor can be useful to study molecular mechanisms relevant to RA [10,11]. However, the synovial environment in RA is extremely complex, with the interplay of cytokines, chemokines, matrix-degrading enzymes, growth factors, and immune

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cell particles [12]. Furthermore, several RA proinflammatory factors like TNF and IL1β can regulate gene transcription via convergent signaling pathways. Synovial fluid is known to contain most of the proinflammatory factors associated with RA pathophysiology. Thus we hypothesize that SF in vitro treatment with a complex proinflammatory stimulus like RA synovial fluid can help to identify the specific SF transcriptional network associated with this disease.

Transcriptional regulatory networks are theoretically prone to the presence of epistatic effects [13]. Epistasis, or more specifically, genetic epistasis, can be defined as the nonindependent effect of genetic polymorphisms over a particular trait in an individual [14]. Until now, the analysis of epistatic effects on human diseases has been limited by the exponential number of combinations to be analyzed in multilocus models. Recently, however, data mining approaches for dimensionality reduction in the analysis of gene × gene and gene × environment interactions have proven useful in the detection and characterization of epistatic effects in human diseases [13,15].

The present study was therefore designed to determine, first, whether a particular transcriptional regulatory network is involved in SF response to RA synovial fluid stimulation and, second, whether promoter polymorphisms in the genes of this network are associated with susceptibility to RA via epistatic interactions. To answer these questions we studied the differential gene expression profiles from cultured synovial fibroblasts with and without RA synovial fluid stimulation. We applied CARRIE, a new method of transcriptional network ascertainment that couples gene expression analysis with promoter sequence information to infer regulatory relationships [16], to the results. After defining the associated transcriptional network, we analyzed the presence of epistatic interactions associated with RA susceptibility between promoter single-nucleotide polymorphisms (SNPs) from the coregulated genes by using the multifactor dimensionality reduction (MDR) method [17].

Results

Differentially expressed genes and significant Gene Ontology (GO) terms

Using conservative criteria for differential expression we obtained a total of 157 genes differentially expressed between treatment groups (Supplementary Table S1). A partial list (fold change >3) of differentially expressed genes is shown in Table 1.

To evaluate the global gene expression changes on SF in response to an RA synovial fluid stimulus we compared GO terms from differentially expressed genes. Statistically overrepresented GO terms ($p < 0.05$) were immune response (GO: 0006955), response to biotic stimulus (GO: 0009607), defense response (GO: 0006952), receptor binding (GO: 0005102), cytokine activity (GO: 0005125), and response to wounding (GO: 0009611). The complete list of genes associated with each differentially expressed GO can be found in Supplementary Table S2.

Table 1
Three-fold differentially expressed genes in cultured synovial fibroblasts after RA synovial fluid treatment

Accession No.	Gene	Description	Fold change [a]
NM_016584	*IL23A*	Interleukin 23, α subunit p19	7.9
NM_000641	*IL11*	Interleukin 11	6.7
NM_006329	*FBLN5*	Fibulin 5	5.7
NM_000963	*PTGS2*	Prostaglandin-endoperoxidase synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	5.4
NM_000759	*CSF3*	Colony stimulating factor 3 (granulocyte)	4.4
NM_000675	*ADORA2A*	Adenosine A2a receptor	4.1
AK058127	—	*Homo sapiens* cDNA FLJ25398	3.8
NM_000758	*CSF2*	Colony stimulating factor 2 (granulocyte–macrophage)	3.7
NM_005623	*CCL8*	Chemokine (C–C motif) ligand 8	3.6
NM_001432	*EREG*	Epiregulin	3.6
NM_006443	*C6orf108*	Chromosome 6 open reading frame 108	3.6
NM_002192	*INHBA*	Inhibin, βA (activin A, activin ABα polypeptide)	3.5
NM_000346	*SOX9*	SRY (sex determining region Y)-box 9 (campomelic dysplasia, autosomal sex-reversal)	3.5
NM_033035	*TSLP*	Thymic stromal lymphopoietin	3.4
NM_031437	*RASSF5*	Ras association (RalGDS/AF-6) domain family 5	3.4
NM_021724	*NR1D1*	Nuclear receptor subfamily 1, group D, member 1	3.3
NM_004049	*BCL2A1*	BCL2-related protein A1	3.2
NM_002089	*CXCL2*	Chemokine (C–X–C motif) ligand 2	3.1
NM_001682	*ATP2B1*	ATPase, Ca^{2+} transporting, plasma membrane 1	3.0
NM_002692	*POLE2*	Polymerase (DNA directed), ε2 (p59 subunit)	−3.1
NM_001684	*ATP2B4*	ATPase, Ca^{2+} transporting, plasma membrane 4	−3.1
NM_139314	*ANGPTL4*	Angiopoietin-like 4	−3.2
NM_000331	*SAA1*	Serum amyloid A1	−3.2
NM_006283	*TACC1*	Transforming, acidic coiled-coil-containing protein 1	−3.3
NM_002084	*GPX3*	Glutathione peroxidase 3 (plasma)	−3.4
NM_012242	*DKK1*	Dickkopf homolog 1 (*Xenopus laevis*)	−3.8
NM_006006	*ZBTB16*	Zinc finger and BTB domain containing 16	−4.5

[a] $p < 0.00001$.

Analysis of transcriptional regulatory networks

Determination of significant transcription factor matrices

We determined those transcription factors that most likely control the response of SFs to RA synovial fluid using CARRIE. We found that, from all significant matrices (Fig. 1), the NF-κB distribution matrix stands out as the most clearly associated. NF-κB has a p value four orders of magnitude more significant than the immediate associated transcription factor (TF).

Determination of NF-κB regulatory network

We inferred the transcriptional regulatory network of NF-κB involved in the SF response to RA synovial fluid using CARRIE (Fig. 2). Although no significant expression change was observed for NF-κB itself, a significant relationship with 13

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