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Genome wide gene expression analysis of macrophages from ankylosing spondylitis patients under interferon-gamma treatment

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Abstract. – BACKGROUND: Ankylosing spondylitis (AS) is a common and highly heritable arthropathy, but the pathogenesis of which is poorly understood, especially the mechanisms in genomics.

AIM: Our work is aim to study the mechanisms of AS in genomics.

MATERIALS AND METHODS: we used microarray dataset GSE11886 from Gene Expression Omnibus (GEO). According to our GSEA approach on the microarray datasets related to AS, we have identified the significantly associated pathways with this disease respectively dependent and independent to the factor of interferon-gamma (IFN-γ).

RESULTS: As a result, we have identified 9 most significant pathways in the comparison of AS patients to control under none treatment, including 5 up-regulated and 4 down-regulated pathways in IFN-gamma-independent study. On the contrary, 11 most significantly up-regulated pathways such as renin-angiotensin system, O-Glycan biosynthesis and gap junction in the comparison of AS patients to control under the treatment of IFN in IFN-gamma-dependent study.

CONCLUSIONS: These may be helpful for understanding the mechanisms of AS regulation under interferon-gamma treatment in genome wide.

Key Words:
Ankylosing spondylitis, Mechanisms, GSEA approach, Interferon-gamma, Genome wide.

Introduction

The ankylosing spondylitis (AS) is a chronic inflammatory disease of the axial skeleton with variable involvement of peripheral joints and nonarticular structures, which is a form of spondyloarthritis and autoimmune disease. It mainly affects joints in the spine and the sacroiliac joint in the pelvis, and can cause eventual fusion of the spine with a strong genetic predisposition[1]. The previous studies were mainly focused on the effects of single genetic factor on AS. The strong genetic association between the genotype factors of HLA-B27and the systemic rheumatic disease of AS has been frequently reported, as well as tumor necrosis factor-alpha (TNF-α) and IL-1[2-4]. The majority of patients with AS exhibit the HLA-B27 antigen and high level of immunoglobulin A (IgA) in the blood. The HLA-B27 antigen is also expressed by Klebsiella bacteria, which are found in high levels in the feces of AS patients. It was suggested that the presence of the bacteria may be a trigger of this disease, and reducing the amount of starch in the diet may be of benefit to AS patients with a test of this diet resulted in reduced symptoms and inflammation in patients with AS as well as IgA levels in individuals with and without AS[5]. However, there is no direct test to diagnose AS and further research is required to determine if diet changes may have a clinical effect on the course of the disease. Although variations of the HLA-B gene increase the risk of developing AS, it is not a diagnostic test[6]. Moreover, no cure is known for AS, although treatments and medications are available to reduce symptoms and pain[7,8]. An IL-6 inhibitor named as tocilizumab, currently approved for the treatment of rheumatoid arthritis, may also show promise for the treatment of AS[9]. A monoclonal antibody against CD20 named as rituximab has also reportedly been effective[10]. Both of these drugs require further investigation, but may one day be viable alternatives for patients who are not responsive to TNF-alpha antagonists.

To evaluate the latent tuberculosis infection in patients with inflammatory arthropathies before treatment with TNF-alpha blocking drugs, a nov-

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el flow-cytometric interferon-gamma (IFN-γ) release assay was used[11]. Low T cell production of TNF-α and IFN-γ in ankylosing spondylitis was considered due to its relation to HLA-B27 and influence of the TNF-308 gene polymorphism[12]. Moreover, the production of tumour necrosis factor alpha and interferon gamma was reported as up regulated by T cells in ankylosing spondylitis during treatment with etanercept[13]. All of these have shown that IFN-γ may play an essential role in the regulation of AS. Therefore, to measure the expression of the majority of genes simultaneously associated with IFN-γ has revealed patterns in complex biologic samples that are reflective of AS disease processes. Here, we employed microarray datasets associated with AS or IFN-γ treatment from the public database library of Gene Expression Omnibus (GEO, www.ncbi.nlm.nih.gov/geo/) and applied the method of gene set enrichment analysis (GSEA) on these datasets. Our goal is to uncover the regulatory mechanisms in pathway level of AS dependent or independent to IFN-γ, which is essential for both the diagnosis and the treatment of AS.

Materials and Methods

Data Collection

Here, we used microarray dataset GSE11886 from GEO, which was contributed by Smith et al[14]. In this experiment, macrophages were derived from the peripheral blood of 8 AS patients (median disease duration 13 years [range < 1-43 years]) and 9 healthy control subjects over 7 days with the use of granulocyte-macrophage colony-stimulating factor. Cells were stimulated for 24 hours with interferon- (IFN; 100 units/ml), were left untreated for 24 hours, or were treated for 3 hours with lipopolysaccharide (LPS; 10 ng/ml). RNA was isolated and examined by microarray and real-time quantitative reverse transcription-polymerase chain reaction analysis. The microarray platform was chosen as Affymetrix Human Genome U133 Plus 2.0 Array (HG-U133_Plus_2, containing 54675 total probe sets). The raw data were available and can be downloaded from the website. 9 biological replicates for healthy control subjects with IFN-γ treatment (named as from CT-IFN-1 to CT-IFN-9, from GSM300389 to GSM300397) and 9 replicates for healthy control subjects with none treatment (named as from CT-NO-1 to CT-NO-9, from GSM300398 to GSM300406) and 7 replicates for AS patients with IFN-γ treatment (named as from AS-IFN-1 to AS-IFN-7, from GSM300407 to GSM300413) and 8 replicates for AS patients with none treatment (named as from AS-NO-1 to AS-NO-8, from GSM300414 to GSM300421) were used. There are two studies in our re-analysis, including IFN-γ-dependent and IFN-γ-independent study by comparison of AS patients to healthy control subjects.

Pre-Processing

We then performed data pre-processing on our local server with expanded memory specification (EMS) memory of 64G using R version 2.10.1 software & Bioconductor 2.5.0. Each Affymetrix dataset was background adjusted, normalized and log2 probe-set intensities calculated using the Robust Multichip Averaging (RMA) algorithm in Affy package[15].

Gene Set Enrichment Analysis

Category package in version 2.6.0 of Bioconductor with R software was used to perform our gene set enrichment analysis (GSEA) of pathways and genes included in our two interested studies with the pathway database named Kyoto Encyclopedia of Genes and Genomes (KEGG)[16]. The gene sets represented by less than 10 genes were firstly excluded in our analysis. Then the t-statistic mean of the genes between disease samples and normal control samples was computed in each pathway. Lastly, we used a permutation test with 1000 times for calculating the corrected p values of each pathway, and set the cutoff of significance level as 0.01 for identifying the significant pathways in each study (Figure 1). The annotation of significant genes in each pathway was performed by using biomaRt package, BioMart v 0.8 rc3 (version of 0.8 release candidate 3).

Next, in order to group each identified pathway into different functional classes, we made the following classification of identified pathways based on the KEGG pathway maps br08901 of BRITE Functional Hierarchies in KEGG database (http://www.genome.jp/kegg-bin/get_htext?br08901.keg). In this database, the KEGG pathways are mapped into 8 functional groups, including Global Map, Metabolism, Genetic Information Processing, Environmental Information Processing, Cellular Processes, Organismal Systems, Human Diseases and Drug Development.

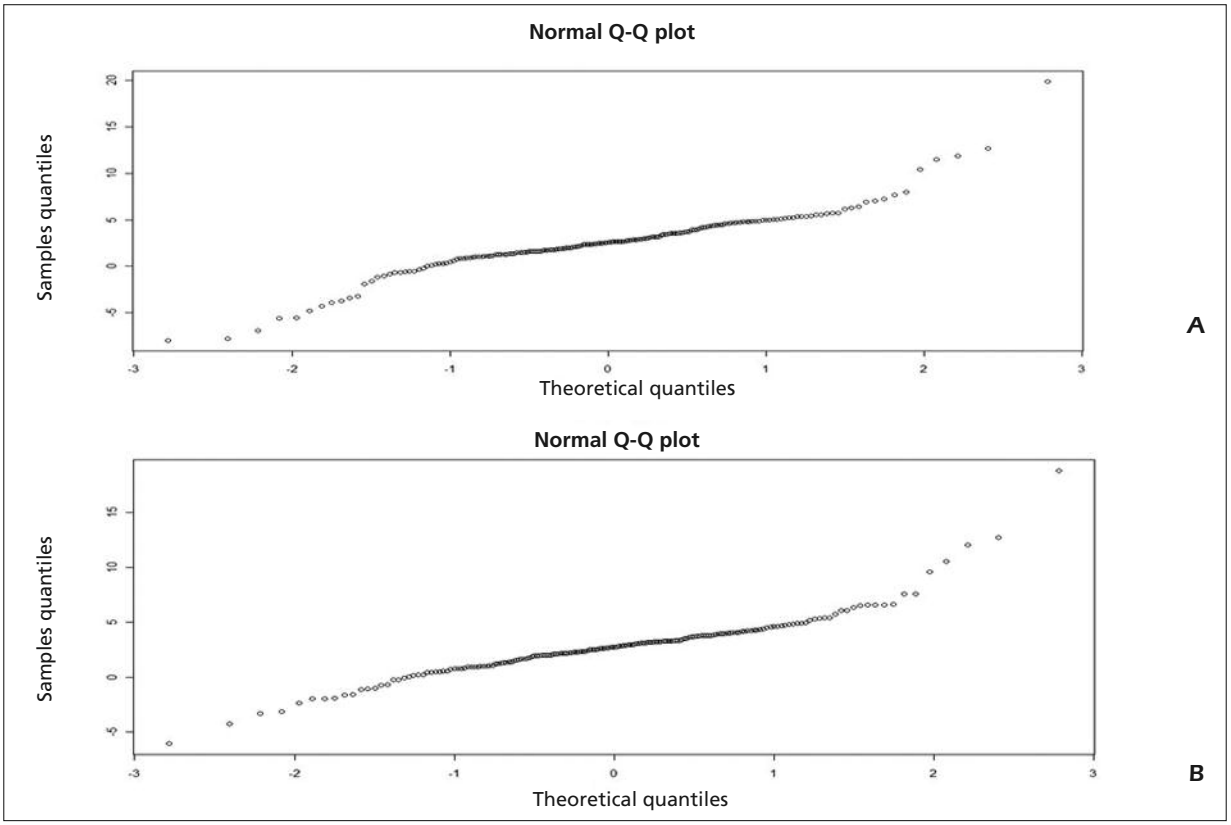

Figure 1. The QQ plots of significant pathways in the comparisons under none or IFN treatment. ***A,*** Is for the comparison under none treatment. ***B,*** Is for the comparison under IFN treatment.

Results

Significant Pathways in IFN-γ-Independent Study

Based on our GSEA approach on the datasets of IFN-γ-independent study, we have identified 9 most significant pathways in the comparison of AS patients to control under none treatment, including 5 up-regulated and 4 down-regulated pathways. The details of these pathways were shown in Table I. Among these 5 up-regulated pathways, 3 pathways named as non-homologous

Table I. The most significant pathways in the comparison of AS patients to control under none treatment.

Pathways	Corrected *p* values	KEGG map1	KEGG map2
Up pathways			
03450: Non-homologous end-joining	3.00E-03	Genetic information processing	Replication and repair
03430: Mismatch repair	5.00E-03	Genetic information processing	Replication and repair
03030: DNA replication	6.00E-03	Genetic Information processing	Replication and repair
04150: mTOR signaling pathway	7.00E-03	Environmental information processing	Signal transduction
05110: Vibrio cholerae infection	9.00E-03	Human diseases	Infectious diseases
Down pathways			
04940: Type I diabetes mellitus	2.00E-03	Human diseases	Endocrine and metabolic diseases
05330: Allograft rejection	4.00E-03	Human diseases	Immune diseases
05332: Graft-versus-host disease	4.00E-03	Human diseases	Immune diseases
05340: Primary immunodeficiency	8.00E-03	Human diseases	Immune diseases

end-joining, mismatch repair and DNA replication were mapped into the group of Genetic Information Processing related to Replication and Repair; mTOR signaling pathway involved in the group of Environmental Information Processing was related to Signal Transduction; only one human disease related pathway named as Vibrio cholerae infection was associated with Infectious Diseases. On the contrary, all of these 4 down-regulated pathways were related to human disease. The pathway of type 1 diabetes mellitus was associated with Endocrine and Metabolic Diseases and other 3 pathways including Allograft rejection, Graft-versus-host disease and Primary immunodeficiency were associated with Immune Diseases. Furthermore, we have identified the significant genes in each IFN-γ-independent pathways, which were detailed in Additional file 1.

Significant Pathways in IFN-γ-Dependent Study

By contrast, we have identified 11 most significant pathways in the comparison of AS patients to control under the treatment of IFN according to our GSEA approach on the datasets of IFN-γ-dependent study, only including up-regulated pathways. The details of these pathways were shown in Table II. Furthermore, we have also identified the significant genes in each IFN-γ-dependent pathways, which were detailed in Additional file 2. There were 3 Human Diseases related pathways including two Infectious Diseases related pathways named as vibrio cholerae infection and epithelial cell signaling in Helicobacter pylori infection and one cancer related pathway of bladder cancer. 4 metabolism related pathways were also up-regulated, including O-Glycan biosynthesis, Sulfur metabolism, Galactose metabolism and Aminophosphonate metabolism. The up-regulated pathways of Renin-angiotensin system and GnRH signaling pathway were associated with the function of Organismal Systems. The pathway of Gap junction was related to Cell Communication in the functional group of Cellular Processes. Calcium signaling pathway was related to Signal Transduction in the functional group of Environmental Information Processing.

Discussion

AS is one of highly heritable arthropathies, but the pathogenesis is not well understood. The previous studies were mainly focused on the genetic loci and polymorphisms associated with the susceptibility of AS. The genes within or near the major histocompatibility complex (MHC) has been proved as a disease-causing ankylosing spondylitis (AS) gene, and the genes within this region contribute directly to the genetic susceptibility for AS[17]. The role of germline polymorphisms of the T-cell receptor A/D and B loci in susceptibility to AS was investigated by linkage studies using microsatellite markers in 215 affected sibling pairs[18].

Table II. The most significant pathways in the comparison of AS patients to control under the treatment of IFN.

Pathways	Corrected *p* values	KEGG map1	KEGG map2
Up pathways			
04614: Renin-angiotensin system	2.00E-03	Organismal systems	Endocrine system
00512: O-glycan biosynthesis	5.00E-03	Metabolism	Glycan biosynthesis and Metabolism
04540: Gap junction	5.00E-03	Cellular processes	Cell communication
05110: Vibrio cholerae infection	5.00E-03	Human diseases	Infectious diseases
00920: Sulfur metabolism	6.00E-03	Metabolism	Energy metabolism
05219: Bladder cancer	7.00E-03	Human diseases	Cancers
00052: Galactose metabolism	8.00E-03	Metabolism	Carbohydrate metabolism
05120: Epithelial cell signaling in helicobacter pylori infection	8.00E-03	Human diseases	Infectious diseases
00440: Aminophosphonate metabolism	9.00E-03	Metabolism	Metabolism of other amino acids
04912: GnRH signaling pathway	9.00E-03	Organismal systems	Endocrine system
04020: Calcium signaling pathway	1.00E-02	Environmental information processing	Signal transduction
Down pathways			
None			

The tumor necrosis factor (TNF) beta and heat shock proteins (HSP) genotype frequency were significantly different between AS patients and random controls[19,20]. Previous linkage and association studies have suggested the presence of a susceptibility gene for AS close to or within the cytochrome P450 2D6 gene (CYP2D6) located at chromosome 22q13.1[21].

Currently, the genomic and proteomic studies using chip technology are in an early phase but have potential both as diagnostic or prognostic tools and as a further hypothesis-free tool to investigate AS pathogenesis, especially for demonstrating the involved pathways in the regulation of AS[22,23]. A genome-wide screen for susceptibility loci in ankylosing spondylitis also confirmed the strong linkage of the MHC with AS and provided suggestive evidence regarding the presence and location of non-MHC genes influencing susceptibility to the disease[24]. In our study, we performed GSEA approach on the microarray datasets related to AS either with or without the treatment of IFN-γ in order to mine the significantly associated pathways in IFN-γ-dependent or -independent study. Ankylosing spondylitis monocytes has been shown upregulation of proteins involved in inflammation and the ubiquitin proteasome pathway[25]. Activation of the toll-like receptor 4 (TLR4) signaling pathway may induce the release of proinflammatory cytokines such as tumour necrosis factor (TNF)-alpha and interleukin (IL)-12, which was considered to play an important role in pathogenesis of immune-mediated diseases, such as the disease of AS[26].

To the significantly associated pathways, the renin angiotensin system (RAS) may be one of the several factors involved in bone metabolism, which plays an important role in regulating blood volume, total body sodium and systemic vascular resistance[27]. The alterations in RAS could alter the regulation of blood flow to bone, impacting on bone turnover due to the important role of vasculature in bone remodelling. Based on the high-throughput glycomic techniques, some specific glyco-biomarkers have been proved to be significant in relation to autoimmunity and some bone diseases, in particular AS[28]. The biosynthesis of the O-glycan may play an important role in the regulation of AS. The molecular pathway of gap junction was indicated to be activated by mechanical stimulation during osteoblastic growth, differentiation and activity in health, and its role of mechanostimulatory approaches in treating various bone pathophysiologies was also considered[29].

Conclusions

Ankylosing spondylitis (AS) is a common and highly heritable arthropathy, but the pathogenesis of which is poorly understood, especially the mechanisms in genomics. According to our GSEA approach on the microarray datasets related to AS, we have identified the significantly associated pathways with this disease respectively dependent and independent to the factor of IFN-γ. As a result, we have identified 9 most significant pathways in the comparison of AS patients to control under none treatment, including 5 up-regulated and 4 down-regulated pathways in IFN-γ-independent study. On the contrary, 11 most significantly up-regulated pathways in the comparison of AS patients to control under the treatment of IFN in IFN-γ-dependent study. These may be helpful for understanding the mechanisms of AS regulation under interferon-gamma treatment in genome wide.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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