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Aberrant methylation patterns affect the molecular pathogenesis of rheumatoid arthritis

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ABSTRACT

This study aims to investigate DNA methylation signatures in fibroblast-like synoviocytes (FLS) from patients with rheumatoid arthritis (RA), and to explore the relationship with transcription factors (TFs) that help to distinguish RA from osteoarthritis (OA). Microarray dataset of GSE46346, including six FLS samples from patients with RA and five FLS samples from patients with OA, was downloaded from the Gene Expression Omnibus database. RA and OA samples were screened for differentially methylated loci (DMLs). The corresponding differentially methylated genes (DMGs) were identified, followed by Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO) enrichment analysis. A transcriptional regulatory network was built with TFs and their corresponding DMGs. Overall, 280 hypomethylated loci and 561 hypermethylated loci were screened. Genes containing hypermethylated loci were enriched in pathways in cancer, ECM-receptor interaction, focal adhesion and neurotrophin signaling pathways. Genes containing hypomethylated loci were enriched in the neurotrophin signaling pathway. Moreover, we found that CCCTC-binding factor (CTCF), Yin Yang 1 (YY1), v-myc avian myelocytomatosis viral oncogene homolog (c-MYC), and early growth response 1 (EGR1) were important TFs in the transcriptional regulatory network. Therefore, DMGs might participate in the neurotrophin signaling pathway, pathways in cancer, ECM-receptor interaction and focal adhesion pathways in RA. Furthermore, CTCF, c-MYC, YY1, and EGR1 may play important roles in RA through regulating DMGs.

1. Introduction

Rheumatoid arthritis (RA) is a chronic systematic immune disease characterized by inflammation of the capsule around the joints, synovial hyperplasia, and destruction of articular cartilage. RA affects approximately 1% of the population, and accounted for 49,000 deaths in 2010 [1]. Apart from decreased quality of life and shortened life span, RA contributes to lung fibrosis, renal amyloidosis, and heart diseases [2]. Current therapies for RA primarily include pharmacological treatments, physical therapy, and complementary and alternative medicine, and are marked by limited efficacy and variable side effects [3]. Thus, there is a pressing need for novel effective therapies with minimum side effects in the management of RA.

Fibroblast-like synoviocytes (FLS) form the synovial intimal lining and produce cytokines, proteolytic enzymes and inflammation mediators, ultimately resulting in the degradation of the extracellular matrix (ECM) and cartilage destruction [4]. In RA, FLS exhibits an aggressive phenotype that promotes the destruction of joint cartilage via the strengthening of inflammation [5]. The mechanisms underlying the aggressive FLS phenotype of RA involve complement and coagulation, Toll-like receptors, NOD-like receptors and cell adhesion pathways [6,7].

DNA methylation, a well-defined epigenetic determinant, plays a pivotal role in regulating gene expression and transcription [8]. The pro-inflammatory cytokine, interleukin-1, contributes to DNA methylation in RA FLS by regulating the expression of DNA methyltransferases [9]. Recently, genomic studies have revealed a list of differentially methylated genes (DMGs) that might play important roles in the pathogenesis of RA [7]. Moreover, a characteristic DNA methylation signature distinguishing RA FLS from osteoarthritis (OA) FLS and normal FLS was identified [7].

More recently, the RA methylation signatures were assessed in an increased number of OA and RA cell lines, as well as normal synoviocytes, and the DNA methylation signature was demonstrated to be relatively stable [10]. However, previous studies have failed to pay enough attention to the transcription factors (TFs) that regulate the DMGs in RA, which help to distinguish RA from OA. To address this issue, differentially methylated loci (DML) between RA FLS and OA FLS were screened. Additionally, we built a transcriptional regulatory network accompanied by topological analysis. Taken together, the results presented here extend our understanding of the pathogenesis of RA.

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2. Materials and methods

2.1. Microarray data and preprocessing

Microarray dataset of GSE46346 [7] was downloaded from the Gene Expression Omnibus (GEO) base on a GPL16304 Illumina Human Methylation 450 BeadChip [UBC enhanced annotation v1.0] platform. The dataset consisted of six FLS samples isolated from the synovial tissues of patients with RA and five matched FLS samples from patients with OA (control group). As described previously [7,10], the diagnosis of RA was consistent with the American College of Rheumatology 1987 revised criteria [11]. Clinical information on two RA and two OA patients was limited because the samples were de-identified. In addition, of the four RA patients with clinical information, three were seropositive for serum rheumatoid factor and/or anti-cyclic citrullinated peptide antibody. Four were treated with low dose prednisone, two with methotrexate, two with a tumour necrosis factor (TNF) blocker and one with leflunomide. OA patients were treated with acetaminophin and narcotics for pain. FLS were isolated from synovial tissues obtained from the RA and OA patients at the time of joint replacement. Here, all six FLS samples from patients with RA and five FLS samples from patients with OA were included for analysis.

Methylation levels at each locus were calculated for each sample using GenomeStudio software [12]. Methylation loci with $p > 0.05$ or located on the X chromosome were filtered out. The remaining 398,614 methylation probes were normalized using the Illumina Methylation Analyzer package of Bioconductor in R language, as described previously [13].

2.2. Identification of differentially methylated genes

DMLs between RA and OA samples were screened using the Student's *t*-test followed by multiple testing correction with Benjamini-Hochberg adjustment [14]. Adjusted p-values <0.05 were considered statistically significant. Genes containing more than one DML were defined as DMGs based on annotation information obtained from the GPL16034 platform.

2.3. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

GO analysis has been used to annotate genes, gene products and sequences [15]. GO terms are classified into three categories: cellular components, molecular functions, and biological processes. The KEGG database serves as a repository for the systematic analysis of gene function in numerous biochemical pathways [16]. The Database for Annotation, Visualization and Integrated Discovery (DAVID) is a web-accessible program for classifying functionally associated genes into a manageable number of biological modules, followed by a systematic analysis of gene modules in biological context [17].

GO and KEGG pathway enrichment analysis was performed for DMGs using the DAVID online tool. A gene count of ≥ 5 or a p-value <0.05 was set as the strict threshold for GO and KEGG pathways analyses.

2.4. Construction of a transcriptional regulatory network

The University of California Santa Cruz (UCSC, http://genome.ucsc.edu) genome browser is an interactive website that provides users with access to genome sequence data from numerous organisms [18] (http://genome. ucsc.edu/). To determine how the identified DMLs regulate the transcription of DMGs, ultimately affecting the progression and development of RA and OA, a transcriptional regulatory network was built using UCSC. First, all known and predicted associations between TFs and corresponding target genes were downloaded from the UCSC genome browser. Then, the DMGs were mapped to the downloaded associations. Only TFs that could bind to the DMGs, were screened. A transcriptional regulatory network, comprised of TFs and their corresponding DMGs, was constructed and visualized with Cytoscape software [19]. In the network, the degree of a gene or TF corresponds to the number of its interactions. The genes or TFs with high degree were regarded as particularly important for the pathogenesis of RA.

3. Result

3.1. DMLs identification

We identified a total of 841 loci that were differentially methylated in the RA and OA groups. These DML include 280 hypomethylated loci and 561 hypermethylated loci.

3.2. GO enrichment analysis

GO enrichment analysis was performed for genes containing hypermethylated or hypermethylated loci. Genes containing hypermethylated loci were primarily associated with the regulation of cell proliferation, positive regulation of cellular component organization, and the regulation of vesicle-mediated transport (Table 1). Genes that containing hypomethylated loci were predominately associated with the regulation of cell development, regulation of nervous system development, cell adhesion, biological adhesion, skin development, and regulation of neuron differentiation (Table 2).

3.3. KEGG pathway enrichment analysis

KEGG pathway enrichment analysis revealed that genes containing hypermethylated loci were primarily enriched in pathways in cancer, ECM-receptor interaction, focal adhesion, small cell lung cancer, and neurotrophin signaling pathways. Genes containing hypomethylated loci were significantly enriched in the neurotrophin signaling pathway and hypertrophic cardiomyopathy (HCM) pathways.

3.4. Construction of a transcriptional regulatory network

A transcriptional regulatory network including 51 TFs, 460 genes, and 1248 interactions was constructed (Fig. 1), Within the network, TFs and genes were ranked by degree of interaction in descending order. The top 10 TFs, or genes, include CCCTC-binding factor (CTCF, degree = 260), Yin Yang 1 (YY1, degree = 159), v-myc avian myelocytomatosis viral oncogene homolog (c-MYC, degree = 120) and early growth response 1 (EGR1, degree = 120) (Table 3). Of the 10 TFs or genes, CTCF possessed the highest degree of interaction (degree = 260).

4. Discussion

RA is a chronic systematic immune disease. FLS aggravates the progression of RA by perpetuating inflammation and exacerbating the joint damage. FLS associated with RA could be affected by the regulation of gene expression via DNA methylation. Here, we examined differential methylation patterns in RA FLS and OA FLS and identified 280 hypomethylated loci and 561 hypermethylated loci.

GO functional annotation analysis revealed that genes containing hypermethylated loci were mainly involved in cell regulation. Additionally, GO analysis revealed that genes containing hypomethylated loci were involved in a variety of biological processes, including cell adhesion, skin development, regulation of cell development, nervous system development, and neuron differentiation. Consistently, the role of cell adhesion has been reported in RA FLS [6].

The neurotrophin signaling pathway was found to be involved in RA using a novel method, identifying genes and pathways related to RA