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Original Research

Potential regulatory factors in the pathogenesis of ankylosing spondylitis

Hongxi Liu*, Jun Zhou, Junwei Bi, Wenbo Yang

Department of Osteomyelitis, Gansu Provincial Hospital of TCM, Lanzhou 730050, China

*Correspondence to: 13008737521@163.com

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Abstract: Ankylosing spondylitis (AS) is a chronic rheumatic disease that mainly affects the spinal joints (vertebrae). Spondylitis means inflammation of the spine, and ankylosing spondylitis means that bones tend to fuse. The AS causes the vertebrae to swell in the spine. Therefore, based on protein interaction network analysis, we conducted in-depth research on the molecular mechanism of key regulatory factors in the AS disease process. We carried out a differential analysis of the expression of miRNAs in disease samples and miRNAs in normal samples. Protein network interaction analysis is performed according to a group of target genes regulated by significant differentially expressed miRNAs and clustered into an interaction module. In addition, enrichment analysis of functions and pathways was performed on these modular genes. Based on the predictive analysis of multidimensional regulators, we identified a range of regulatory factors that have potential regulatory effects on AS, such as endogenous genes and transcription factors. We obtained 20 differentially expressed miRNAs and 7082 target genes and clustered into 11 modules. Enrichment results showed that these modular genes are mainly involved in the functions and pathways of protein polyubiquitination, neutrophil activation involved in immune response, and Wnt signaling pathway. We revealed ten transcription factors (MYC, NFKB1, and TP53). After network connectivity analysis, we obtained 12 internal drive genes (UBE2D1, CCNF, and NEDD4). These core genes are thought to be potential regulators of AS. MYC is also considered to be a core factor that inhibits SART3 phosphorylation and plays a vital role in the immunological pathogenesis of AS. The combination of the above analysis results can provide a new idea for biologists and medical scientists to study the immune pathogenesis of AS and can provide a valuable reference for subsequent treatment options.

Key words: Ankylosing spondylitis; Expression of dysregulated miRNA; MYC; SART3 phosphorylation; A key regulator.

Introduction

Ankylosing spondylitis (AC) or rheumatoid arthritis is a progressive, chronic, autoimmune joint disease that usually affects the spine and pelvic bones. Due to inflammation and stiffness of the spine, the patient may "lean forward." Inflammation involves the pelvic joints, lumbar spine, chest and neck. Men are more susceptible to infection, and the onset of this disease usually occurs in the second decade or early in the third decade of life. The AS is a chronic systemic inflammatory disease whose etiology is unknown and is also complex systemic rheumatism (1, 2), which mainly affects the ankle and spine (3, 4). As a chronic inflammation, AS severely compromises patients' quality of life and work efficiency (5, 6), and also causes young people to suffer from severe bone pain and bone stiffness (7). Arrhythmias have been observed in AS patients in recent years, and it affects 1% of the world's population (8). With clinical features of inflammatory back pain and peripheral joint pain, AS is also a hereditary disease that can lead to disabling inflammation and immune diseases (9-12). It has been reported that AS involves axial skeletal arthritis (13). AS affects the ankle joint, shaft joint, and multiple extra-articular organs, thus bringing about structural and functional damage. Early diagnosis, exercise, and health education can improve the prognosis of AS patients (14). Besides, AS is also the basis of progressive joint swelling, but its specific cause is vague (15). It has long been considered chronic inflammatory arthritis of unknown etiology, and its autoimmune origin has been proposed, but it has never been confirmed (16). However, because of the high risk of morbidity and mortality in AS patients, the world's major medical scientists have been seeking active treatments (17). After treatment, there is a chance of cardiovascular and respiratory complications in AS patients (18, 19).

Endothelial progenitor cells have been found to have the potential to repair endothelial damage and reduce cardiovascular risk so that they can be used as therapeutic targets for the prevention of cardiovascular diseases such as AS (20). More and more data show that HLA-B27 has a strong correlation with AS and has a therapeutic effect (21). At the same time, HLA-B27 positivity is the most likely virulence factor in the development of AS (22). There is some evidence that bacterial infection may play an essential role in the pathogenesis and progression of AS, especially mesenchymal stem cell dysfunction may be critical pathogenesis of many rheumatic diseases (23). In this study, we present a comprehensive approach to identify essential regulators of AS based on protein interaction network analysis. The analysis results of the article provide not only new insight into the treatment of AS but also provide abundant resources for biologist experiments (24-41).

Table 1. Basic information about GSE118806.

Data	GSE118806
Time	
Submission date	Aug 20, 2018
Last update date	Nov 28, 2018
Contact name	Hui Chun Yu
Address	
Organization name	Dalin Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation
Department	Medical research
Street address	No. 2, Min-Sheng Rd, Dalin Town
City	Chia-Yi
Country	Taiwan
ZIP/Postal code	62247
Organism	Homo sapiens
Experiment type	Non-coding RNA profiling by array
Platforms	Agilent-070156 Human_miRNA_V21.0_Microarray 046064 (gene name version)

Materials and Methods

Differential expression analysis

Firstly, we logged in the gene expression omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo/) and used the keyword of AS for searching (Table 2). We screened MiRNA and selected the microarray with the number of GSE118806 (42). A total of 6 samples (GSM3348017 Cells_IL2320 μg/ml_ 72 hrs, replicate 1, GSM3348018 Cells_IL23 20 μg/ml_ 72 hrs, replicate 2, GSM3348019 Cells_IL23 20 μg/ml_ 72 hrs, replicate 3, GSM3348020 Cells culture medium_3 days, replicate 1, GSM3348021 Cells_culture medium_3 days, replicate 2, GSM3348022 Cells_culture medium_3 days, replicate 3). For the difference in the collected disease samples, we used the R language limma package (43). For the microarray data, we use the background correction function to perform background correction and standardization. The control probe and the low-expressed probe are filtered based on the normalize Between Arrays function, and the high-quality normalized data is obtained. The lm Fit and eBayes functions of the limma package were analyzed with default parameters to identify differentially expressed genes with a p-value> 0.05.

Protein interaction network analysis

Within the observation module, the target interactions of genes can help us understand the core molecules that drive the function and dysfunction of the module. We looked for 7082 target genes regulated by miRNAs in the module and constructed protein interaction networks (PPIs) for each module (44). We import the interaction data into Cytoscape for visualization.

Function and pathway enrichment

For the study of the molecular mechanism of disease, signal pathway exploration is an effective means. The function and pathway involved in the module gene can characterize the dysfunction mechanism of the module during the disease process. Therefore, regarding the Go function (p-value cutoff = 0.01, q value Cut off = 0.01) and the KEGG path (p-value cutoff = 0.05, q value Cut off = 0.2) (45), we use the R language Cluster profiler package for enrichment analysis. Based on the functions and pathways involved in the module's genes, we identified it as a potential dysfunctional module for AS.

Predicting the multifactorial regulation of modules

We downloaded all human transcription factor target data in the TRRUST v2 database (46) to identify regulators that regulate the module's genes. Pivot analysis is then performed based on these interaction data to determine the regulatory effects of these transcription factors on the module. Pivot analysis refers to the search for a driver pair with at least two pairs of modules in a target pair. Finally, statistical analysis of pivots, pivots that have a regulatory effect on more dysfunctional modules was identified as core pivots. We use Cytoscape (47) for presentation and network analysis. Finally, screening for the gene with the highest connectivity is considered to be the core molecule for the regulation of module progression and identified as an internal drive gene. These internal drive genes characterize potential key disorders of AS.

Results

Determining the time-series expression of axonal spondylitis

To explore critical regulators that play an essential role in the immunogenic pathogenesis of AS, we screened the expression profiles of AS and obtained 20 differentially expressed miRNAs (Table 2). These differentially expressed miRNAs may be directly or indirectly related to AS, and they may play an essential regulatory role in the development of the disease.

Construction of a protein interaction network for AS

To systematically study the mechanism of action of AS-related genes in patient samples, we conducted extensive analytical studies. We looked for the target gene regulated by the miRNA in the module, and there were a total of 7082. The construction of protein interaction network PPIs for them resulted in 11 modules (Figure 1).

Biological networks characterize global underlying pathogenic mechanisms.

Each module characterizes a potential mechanism by which the core gene imbalance drives the module

Table 2. The first 20 miRNAs with the most significant difference.

DEG	logFC	AveExpr	t	P.Value	adj.P.Val	B
hsa-miR-8069	21.224	326.195	3.872	1.290E-02	0.550	-4.578
hsa-miR-642a-3p	7.077	19.288	4.986	4.756E-03	0.550	-4.576
hsa-miR-6090	4.330	21.827	3.129	2.779E-02	0.550	-4.581
hsa-miR-1229-5p	3.298	2.906	4.598	6.605E-03	0.550	-4.577
hsa-miR-7107-5p	3.254	14.437	5.393	3.434E-03	0.550	-4.576
hsa-miR-7110-5p	3.129	10.075	4.225	9.227E-03	0.550	-4.578
hsa-miR-6797-3p	2.807	4.409	2.802	4.003E-02	0.550	-4.582
hsa-miR-1207-5p	2.352	14.719	3.446	1.980E-02	0.550	-4.580
hsa-miR-6088	2.021	11.287	2.973	3.301E-02	0.550	-4.582
hsa-miR-7704	1.505	5.890	2.736	4.319E-02	0.550	-4.583
hsa-miR-4484	-2.750	17.323	-3.699	1.530E-02	0.550	-4.579
hsa-miR-324-3p	-2.755	18.270	-2.658	4.729E-02	0.550	-4.583
hsa-miR-21-3p	-3.079	12.737	-3.612	1.671E-02	0.550	-4.579
hsa-miR-1287-5p	-3.405	1.805	-5.083	4.391E-03	0.550	-4.576
hsa-miR-6085	-9.517	64.513	-3.187	2.609E-02	0.550	-4.581
hsa-miR-27a-3p	-12.509	80.402	-2.628	4.898E-02	0.550	-4.583
hsa-miR-378i	-14.012	134.306	-2.915	3.521E-02	0.550	-4.582
hsa-miR-17-3p	-15.392	76.806	-2.831	3.874E-02	0.550	-4.582
hsa-miR-4284	-106.096	1472.228	-2.680	4.605E-02	0.550	-4.583
hsa-miR-1246	-138.960	917.739	-2.925	3.484E-02	0.550	-4.582

Notes: DEG: Differential gene; logFC: Log fold change; AveExpr: Average expression; t: t test statistic; P.value: test value; adj.P.Val: corrected p value, b: β coefficient.

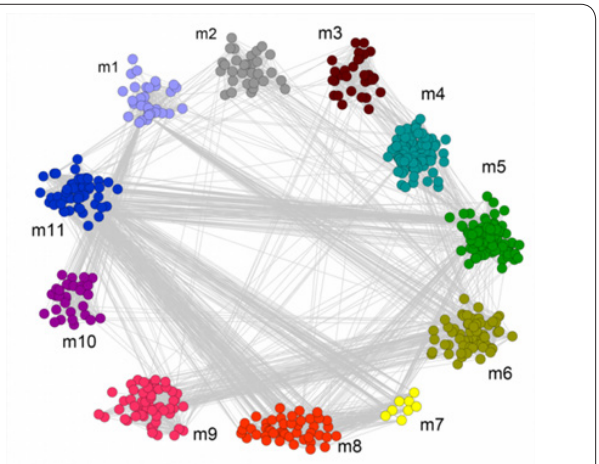

Figure 1. The protein interaction network map is characterizing the underlying pathogenesis of ankylosing spondylitis. Different colors describe different modules, a total of 11 color modules.

to dysregulate, causing global dysfunction and mediating disease development. Therefore, these 11 functional modules may participate in different functions and paths. It represents a regulatory mechanism that mediates the onset of AS.

Identification of dysfunction modules for AS

Studying the functions and pathways involved in genes is an important means to identify their pathogenesis. In order to investigate the possible dysfunction of modular gene dysfunction, we performed an enrichment analysis of GO function and KEGG pathway for 11 modules of AS. We collected 1265 cell composition entries, 1378 molecular functional terms and 8891 biological processes (Figure 2A). Based on the analysis, we observed that related functional modules tend to enrich a variety of disease-related functions, including protein

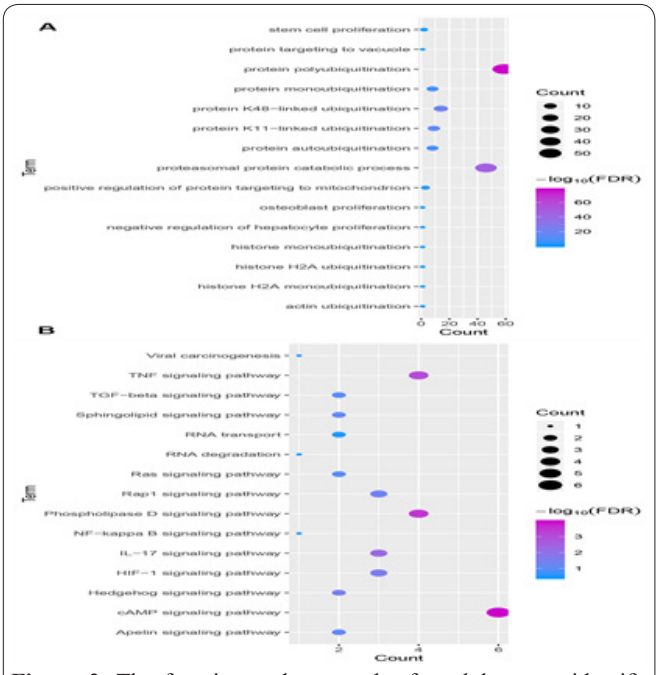

Figure 2. The function and approach of modular gene identification involving the ankylosing spondylitis dysfunction module; A. Enrichment analysis of module gene GO function. The darker the color, the higher the richness. The larger the circle, the more important the proportion of genes in the module representing GO function. B. Enrichment analysis of the KEGG pathway of modular genes. The darker the color, the higher the richness. The larger the circle, the more significant the proportion of the gene in the KEGG pathway entry.

polyubiquitination, neutrophil activation involved in immune response, and neutrophil-mediated immunity. The enrichment results of the 286 KEGG pathways reflect the signaling pathways involved in functional modular genes, including the Wnt signaling pathway, NOD-like

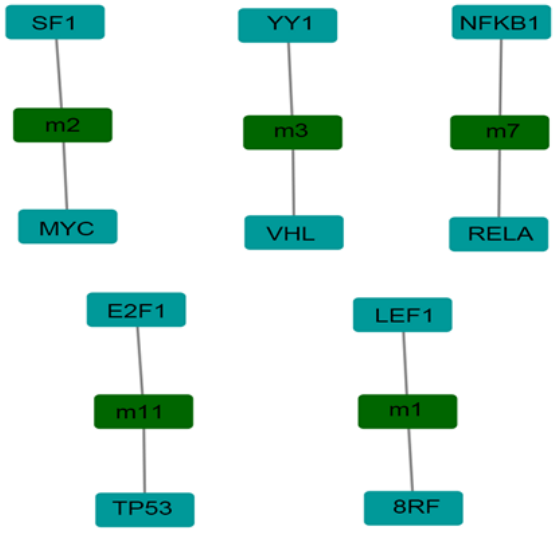

Figure 3. Transcription factor regulatory network map of ankylosing spondylitis; The green rectangle represents the module, and the blue rectangle represents the corresponding ncRNA.

receptor signaling pathway, and SNARE interactions in vesicular transport (Figure 2B). Given the enrichment of the module gene, the functional and pathway results we obtained are closely related to AS. Therefore, we identified these 11 modules as dysfunction modules. Module genes can regulate a range of functions and pathways, and module dysregulation is likely to be a significant cause of morbidity. We may find that these functions and pathways may be closely related to the development of AS. In the process of driving AS transcription factors and endogenous genes are considered to be critical factors regulating the occurrence and development of the disease. Based on the TF-based pivot analysis, we explored the regulator that caused the module to be dysfunctional.

The results showed that a total of 10 transcription factors have significant transcriptional regulation of the onset of AS, involving 10 Pivot-Module interaction pairs (Figure 3). Statistical analysis of these transcription factor regulatory pairs revealed that ten transcription factors (e.g., MYC, NFKB1, and TP53) exerted a specific regulatory effect on one dysfunction module. Besides, we performed network connectivity analysis based on 11 dysfunction modules and identified 12 critical internal drive genes, including UBE2D1, CCNF, and NEDD4. These internal drive genes have high connectivity in the network module, and also have significant regulatory effects on the immune mechanism of AS. In summary, it was also found that MYC that affects the critical gene may promote the development of AS by targeting SART3 to regulate the improved function of histone deubiquitination.

Discussion

AS is a chronic inflammatory disease that affects the spine and surrounding joints (48). Joint stiffness, pain, and gradual loss of spinal activity can lead to severe functional limitations that subsequently negatively impact the patient's quality of life. These symptoms not only interfere with physical health but also interfere with social life and mental health (9). Therefore, the research on the treatment mechanism of AS has become a top priority. Fortunately, many biologists and medical researchers have invested in the pathogenesis of AS. To gain an insight into the key genes that induce AS, we have combined a range of analytical methods to identify key disorders of AS. We constructed a full-expression profile of AS samples for differential analysis and screened for 20 differential miRNAs that may be considered to be potentially pathogenic genes for AS. We searched for the 7082 target genes regulated by miRNAs in the module and performed protein interaction network analysis. As a result, we obtained 11 functional barrier modules and performed functional enrichment analysis. Based on the results of enrichment analysis, we found that two modules are mainly involved in protein polyubiquitination. Studies have shown that protein-ubiquitinating active enzymes are involved in the pathogenesis of AS in immune-activated dendritic cells (49). Y. enterocolitica infection may induce ubiquitin antibodies in a portion of patients with AS. It can be explained by a ubiquitin-dependent mechanism involving discoveries related to the virulence of Y. enterocolitica (50). The enrichment of the pathway revealed that the functional module gene is mainly involved in the Wnt signaling pathway causing AS. Related studies have shown that levels of Wnt regulatory proteins may be potential biomarkers of AS (51, 52).

The Wnt pathway may play a key role in the unique pathology of AS, including T cell activation and differentiation, and bone marrow lipogenesis (53, 54). Subsequently, we explored a range of drivers for these dysfunction modules, including transcription factors (MYC, NFKB1, and TP53) and endogenous genes (UBE2D1, CCNF, and NEDD4). In terms of transcription factor regulation, ten transcription factors all regulate the functional modules and affect the development of AS. On the one hand, according to reports by scholars such as Li YK, AS fibroblasts are characterized by an imbalance between proliferation and apoptosis (55). On the other hand, according to transcriptome network analysis, NFKB1 is present in the progression of AS, such as apoptosis, and plays an important role (56). However, some studies have shown that the NFKB1 promoter polymorphism may be associated with certain autoimmune and inflammatory diseases, so it may also be involved in the immune response mechanism of AS (57-59). TP53 is also thought to be involved in the pathogenesis of AS (60). Therefore, the transcription factors mentioned above can be considered to be involved in the different developmental stages of AS and maybe a core factor in inhibiting the role of phosphorylation in the immunological pathogenesis of AS.

We also screened a series of genes with the most connectivity, which is considered to be the core molecules of the regulation module, and identified as endogenous genes. Twelve internal drive genes characterize potential key disorder molecules that induce AS. We also found that MYC, which affects the key gene, may promote the development of AS by targeting SART3 to regulate the positively regulated function of histone deubiquitination. These functions and pathways involved in the modular gene produce a comprehensive network effect that comprehensively regulates the dysfunction module of AS and mediates the pathogenesis of

the disease. Based on the analysis results of this study, we obtained a more detailed pathogenic module of AS and a series of key factors that have a strong regulatory effect on the disease. In addition to the above-mentioned key factors, other unmentioned transcription factors and endogenous genes have the potential to play a role in the pathogenesis of AS, which requires further exploration. Compared with other studies (61, 62), in this study, we first screened differential miRNAs in the GEO database and analyzed the co-expression of GO, KEGG, and PPI by predicting the target genes downstream of miRNA. Such a comprehensive analysis is helpful for us to find the key treatment to AS and lays a foundation for future studies. Conclusively, the study provides a theoretical basis for inhibiting phosphorylation to play a negative feedback control role in the immunological pathogenesis of AS.

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