The Role of MicroRNAS in Ankylosing Spondylitis

Zheng Li, MD, Sunny H. Wong, PhD, Jianxiong Shen, MD, Matthew T.V. Chan, PhD, and William Ka Kei Wu, PhD

Abstract: Ankylosing spondylitis (AS) is a common and genetically heterozygous inflammatory rheumatic disease characterized by new bone formation, ankylosis and inflammation of hip, sacroiliac joints and spine. Until now, there is no method for early diagnosis of AS and the effective treatment available for AS patients remain largely undefined.

We searched articles indexed in PubMed (MEDLINE) database using Medical Subject Heading (MeSH) or Title/Abstract words (''microRNA'' and ''ankylosing spondylitis'') from inception up to November 2015.

Genetic polymorphisms of miRNAs and their targets might alter the risk of AS development whereas certain miRNAs exhibit correlation with inflammatory index.

Let-7i and miR-124 were upregulated whereas miR-130a was downregulated in circulating immune cells of AS patients. These deregulated miRNAs could modulate key immune cell functions, such as cytokine response and T-cell survival.

miRNA deregulation is key to AS pathogenesis. However, clinical utilization of miRNAs for management of AS patients requires further support from future translational studies.

Abbreviations: AS = ankylosing spondylitis, BASRI = Bath Ankylosing Spondylitis Radiology Index, miRNAs = microRNAs, SNPs = single-nucleotide polymorphisms.

INTRODUCTION

Ankylosing spondylitis (AS) is a common and genetically heterozygous inflammatory rheumatic disease characterized by new bone formation, ankylosis and inflammation of hip, sacroiliac joints and spine.[1–6] It can lead to functional and structural impairments and reduction in quality of life.[7–10] The prevalence of AS is about 0.20% to 0.40% in China.[11–13] Almost 80% of AS influences young adults and the disability rate after 5 years of symptoms onset reaches 40% to 60%.[14–16] Until now, there is no criteria for early diagnosis of AS and the effective treatment available for preventing bone destruction and controlling ankylosis in AS patients remain largely undefined.[17,18]

MicroRNAs (miRNAs) are small noncoding, single-stranded, endogenously expressed RNAs molecules that regulate gene expression by causing translation inhibition and affecting mRNA stability.[19–24] miRNAs play critical roles in many processes, such as cell proliferation, differentiation, apoptosis, and development.[25–30] Increasing evidences have shown that aberrant miRNA expression is linked to different diseases, including cancer, in which miRNAs may act as tumor suppressors or oncogenes.[24,31–35] In this review, we focus on recent discoveries related to miRNAs in AS development and discuss the potential use of miRNAs as prognostic biomarkers or treatment strategies for AS.

METHODS

We searched articles indexed in PubMed (MEDLINE) database using Medical Subject Heading (MeSH) or Title/Abstract words (''microRNA'' or ''miRNA'' and ''ankylosing spondylitis'') from inception up to Nov 2015. There were no limitations imposed on language and study types. We included any study in which the role of miRNAs in AS was examined in relation to disease pathogenesis, diagnosis, prognosis, and treatment. The searching process was conducted by 2 independent investigators. Experts in the field of miRNAs or orthopedics were involved in discussion and analyzing process.

Meta-Analysis

Meta-analysis was conducted for single-nucleotide polymorphisms (SNPs) reported in 2 or more studies. The heterogeneity among studies was assessed with the I^2 test. Odds ratios were calculated with fixed-effects model when $I^2 < 50\%$ or random-effects model when $I^2 > 50\%$. The statistical significance was evaluated by the Z-test with P values transformed from the Z scores.

Ethical Review

The present study is a systemic literature review. We do not involve human beings or experimental subject in this study and no any identifiable private information is collected.

RESULTS AND DISCUSSION

Search Results

A total of 17 papers were found based on the search criteria, in which 11 original studies directly examined the involvement of miRNAs in AS were included and cited in this systematic review. The 6 papers excluded were either not original article, not directly related to AS, lacking in evidence of dysregulation of the studied miRNA in AS or retracted by the authors.

Polymorphisms of miRNAs and Their Target Genes as Genetic Determinants of AS

Chatzikyriakidou et al[36] studied the association of IRAK1 (target of miR-146a) SNPs rs3027898 with AS. They found that strong statistically significant difference was observed in IRAK1 rs3027898 polymorphism distribution between patients with AS and controls. Qi et al[37] also studied the predisposition of common pre-miRNA SNPs with Behcet disease, Vogt–Koyanagi–Harada syndrome, and acute anterior uveitis in association with AS. Their results showed significantly increased frequencies of the miR-196a2/rs11614913 TT genotype and T allele in Behcet disease patients. They also found that a functional variant of miR-196a2 conferred risk for Behcet disease but not for Vogt–Koyanagi–Harada syndrome or acute anterior uveitis-associated AS by modulating the miR-196a gene expression and regulating proinflammatory interleukin-1β and monocyte chemoattractant protein-1 production.

Xu et al[38] explored the association between AS and 2 SNPs, miR-146a rs2910164 and miR-499 rs3746444, in a Han Chinese population. A case–control study consisting of 102 subjects with AS and 105 healthy controls was studied. They found that there is a significant difference in the miR-146a rs2910164 SNP. The frequency of the G allele was markedly higher in the AS patients than in the healthy controls, and the frequency of the GG genotype was higher in AS patients than in controls. However, no significant association was found between the miR-499 rs3746444 variant and susceptibility to AS. In an independent study, Niu et al attempted to replicate the association between miR-146a polymorphisms, including rs2910164, and AS in 611 Chinese patients and 617 controls. Nevertheless, no association between three common miR-146a SNPs and AS was observed in their samples.[39] A meta-analysis of the association between miR-146a rs2910164 and AS from the 2 studies[38,39] indicated that there was a significant heterogeneity between the 2 studies (pQ = 0.007, I^2 = 0.863). Using random-effects model, there was no significant association between with miR-146a rs2910164 (odds ratio = 1.3; P = 0.361) and AS.

Deregulated miRNAs in Relation to Clinical Features in AS

The first study on miRNA expression profiling in AS was performed in T cells from 5 AS patients and 5 healthy controls by Lai et al.[40] Of the 270 miRNAs, they found that the expression of 8 miRNAs (miR-150, miR-342-5p, miR-16, miR-221, miR-99b, let-7b, let-7i, and miR-513-5p) were higher and 5 miRNAs (miR-218, miR-30e, miR-199a-5p, miR-409-3p, and miR-215) were lower in the T cells of AS than in the normal counterpart. Moreover, they confirmed that the expression of miR-16, let-7i, and miR-221 were higher in the T cells of AS than in the normal T cells. They found that the expression of let-7i, miR-221, and miR-16 were correlated with the BASRI (Bath Ankylosing Spondylitis Radiology Index) of the lumbar spine. In addition, after adjusting for gender and age, only the expression level of let-7i and miR-221 was still associated with the BASRI of the lumbar spine. Despite such positive correlations, it is noteworthy that expression of miR-221, let-7i, and miR-16 did not correlate with the sacroiliitis or levels of serum C-reactive protein by radiography in the AS patients.

Huang et al[41] studied the miR-29a expression levels in the PBMCs (peripheral blood mononuclear cells) of the AS, rheumatoid arthritis, and healthy controls by using real-time PCR. They found that there was a higher miR-29a expression was found in the PBMCs of AS patients compared to that in the rheumatoid arthritis patients or healthy controls. They also found that higher miR-29a expression in PBMCs of the AS patients, and miR-29a could be used as a diagnostic marker in the new bone formation but do not reflect the disease activity.

Regulation of Cytokine Response by let-7i and miR-130a

Toll-like receptor-4 (TLR4) stimulation is known to inhibit interferon-γ production in CD3^{+} CD28^{+} T cells. In AS T cells, TLR4 expression was downregulated whereas interferon-γ expression was upregulated when compared with normal T cells. Let-7i, a miRNA upregulated in AS T cells, was found to target TLR4 and derepress interferon-γ production inhibited by LPS (a TLR4 agonist).[40] These findings suggested that increased let-7i expression might facilitate the interferon-γ-mediated T-helper type 1 immune response in T cells. Aside from let-7i, a recent study reported that histone deacetylase (HDAC) 3-mediated repression of miR-130a was observed in PBMCs from AS patients, which was associated with increased expression of its target tumor necrosis factor (TNF) α. In PMBCs, miR-130a overexpression led to a reduction, whereas miR-130a inhibition led to upregulation of TNFα expression.[42] This study highlighted the importance of HDAC3-miR-130a-TNFα in the pathogenesis of AS.

Regulation of T Cell Survival by let-7i and miR-124

Hou et al[43] studied the functional role of let-7i in the T cells survival. Their data demonstrated downregulation of IGF1R (insulin-like growth factor-1 receptor) in the T cells from AS patients. Luciferase reporter method proved that IGF1R was the direct target gene of let-7i. Let-7i overexpression in the Jurkat T cells inhibited the IGF1R expression. Inhibition of IGF1R led to a decrease in mTOR and Akt phosphorylation, upregulation of Bax, Bcl-2 downregulation and cleavage of poly(ADP-ribose) polymerase and caspase 3. Inhibition of IGF1R promoted autophagy while the autophagy induced with overexpression of let-7i protected the cells from apoptosis. Their results suggested that let-7i can control the T cells fates in the AS through targeting IGF1R. Another study demonstrated that miR-124 was upregulated in peripheral blood from AS patients, which was associated with downregulation of ANTXR2. Mechanistically, miR-124-mediated downregulation of ANTXR2 might induce c-Jun NH_2-terminal kinase and autophagy in T cells to participate in AS.[44]

Potential Regulation of Bone Loss by miR-21

Bone loss is an obvious feature of AS. In this regard, miR-21-mediated repression of programmed cell death 4 (PDCD4) has been implicated in the activation of osteoclasts. Huang et al[45] studied the difference in the whole-blood miR-21 levels between patients of AS and the healthy controls, and analyzed the relationship between miR-21, bone erosion, and PDCD4 in the AS patient. When compared with the controls, AS patients had higher expression of miR-21, PDCD4, and CTX (C-telopeptide; one marker of bone turnover). The expression miR-21 was negatively associated with the PDCD4 expression in patients with AS without taking neither disease-modifying antirheumatic drugs nor nonsteroidal antiinflammatory drugs. Positive correlations of CTX and miR-21 level were found in patients of AS with active disease and disease duration less than

TABLE 1. Reported Roles of miRNAs in Ankylosing Spondylitis

miRNA Gene, miRNA Target Gene, or miRNA	Association With AS or Dysregulation in AS	Associated Function	Report Region	Sample Size	References
Gene association studies					
IRAK1 (miRNA-146a target)/ rs3027898	Genotypes AA+AC had strong association with AS vs CC genotype	NA	Greece	Controls n=66, AS n=49	36
miR-196a2/rs11614913	No association with AAU+ AS	NA	China	Controls n=400, AAU+ AS n=209	37
miR-499/rs3746444					
miR-149/rs2292832					
miR-585/rs62376935					
miR-146a/rs2910164	Genotypes GG+GC had strong association with AS vs CC genotype	Genotype not associated with mRNA expression levels	China	Controls n=105, AS n=102	38
miR-499/rs3746444	No association	NA			
miR-146a/rs2910164	No association	NA	China	Controls n=611, AS n=617	39
miR-146a/rs2431697					
miR-146a/rs57095329					
miRNA expression studies					
let-7i	Upregulated in AS T-cells in both discovery and validation	Induced TLR4 downregulation; induced autophagy to protect T cells from apoptosis by targeting IGF1R	Taiwan, China (IGFR1 study)	Controls n=5, AS n=5 for discovery; Controls n=18, AS n=22 for validation (Ref.[40]); Controls n=26, AS n=31 (Ref.[43])	40,43
miR-16					
miR-221					
miR-99b	Upregulated in AS T-cells in discovery	NA			40
let-7b					
miR-513-5p					
miR-150	Upregulated in AS T-cells in discovery but not validation				
miR-342-5p					
miR-30e	Downregulated in AS T-cells in discovery				
miR-199a-5p					
miR-215					
miR-218					
miR-409-3p					
miR-29a	Upregulated in AS PBMC	NA	China	Controls n=30, AS n=30	42
miR-130a	Downregulated AS PBMC	Derepressed TNF-1α expression in PBMC	China	Controls n=20, AS n=20	41
miR-21	Upregulated in AS whole blood	Negatively correlated with PDCD4 mRNA in AS patients taking neither NSAID nor DMARD	Taiwan	NA	45
miR-124	Upregulated in AS whole blood	Promoted JNK activation and autophagy in T cells by targeting ANTXR2	China	Controls n=23, AS n=23	44

The bioinformatic study by Zhao et al. was not included since the level of significance of its evidence could not be assessed.
AAU = acute anterior uveitis, AS = Ankylosing Spondylitis, DMARD = disease-modifying antirheumatic drugs, NASID = nonsteroidal antiinflammatory drugs, PDCD4 = programmed cell death 4, PMBC = peripheral blood mononuclear cells, TLR4 = Toll-like receptor 4.

7 years. These data suggested that miR-21 expression might have an important role in the AS development.

Novel miRNA-Target Relationships Identified by Systems Biology

A recent study by Zhao et al[46] using systems biology approach for analysis of existing expression data of AS patients revealed 4 upregulated (CISD2-miR-134, CLEC4D-miR-106a/miR-20a/miR-106b) and 15 downregulated (HNRNPR-miR-335/miR-433, EP300-miR-212/miR-132, AAK1-miR-203/miR-34b/miR-381/miR-448/miR-498, GOLGA8A-miR-182, CECR1-miR-495, BCL11B-miR-363/miR-20b/miR-519d, CDC25B-miR-214) miRNA-target relationships in AS. Nevertheless, whether the dysregulation of these miRNAs in AS was statistically significant remains uncertain.

No statistics was applied for meta-analysis of miRNA deregulation in AS since all studies listed above except the one by Lai et al[40] followed a candidate-gene approach with different miRNAs being investigated in different studies, rendering quantitative analysis across studies not applicable.

CONCLUSION AND FUTURE PERSPECTIVES

In this systematic review, we summarized the current evidences regarding the involvement of miRNA in the pathogenesis of AS in relation to their clinical utilities as biomarkers. One of the limitations of this review is that not all papers assessed stated clearly how AS was diagnosed in their studies, which may contribute to inter-study variations in miRNA discovery. Moreover, the limited number of related studies in the literature may preclude the complete depiction of the role of miRNA in AS.

miRNAs are a class of regulatory RNA that functions as a master control of gene expression. Their dysregulation has been observed in different diseases, including AS (see Table 1 for the summarized roles of miRNAs in AS). The deregulation of immune cell functions, including proinflammatory pathway (eg, Toll-like receptor 4, c-Jun NH_2-terminal kinase), cytokine expression (eg, TNFα), and T-cell prosurvival signaling (eg, IGF1R, PDCD4), by miRNAs seems to play important roles in the pathogenesis of AS. Another emerging theme is T-cell autophagy, which has been shown to be involved in the modulation of immune response.[47] Focused investigations of miRNAs known to regulate these pathways may lead to further discovery of novel AS-related miRNAs. Importantly, the function and downstream molecular pathways of several key deregulated miRNAs, such as miR-16, miR-221, and miR-29a, remain to be elucidated. Dysregulation of these miRNAs has been confirmed with a reasonably large sample size.[40,42] Pertinent
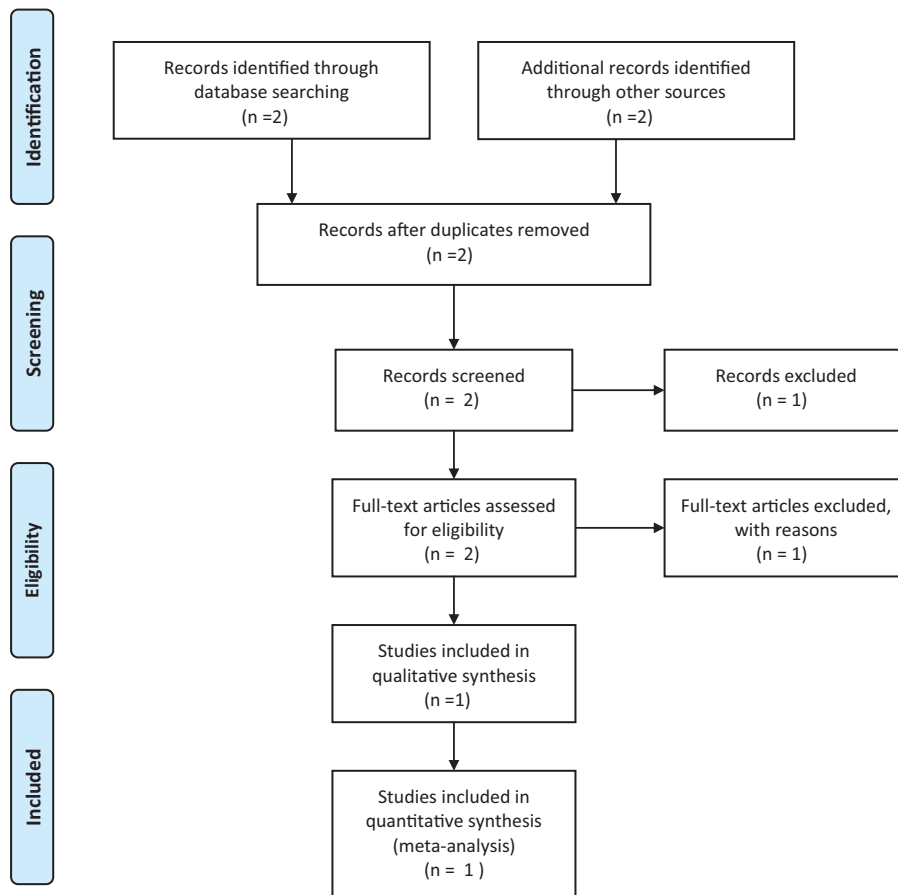
to disease susceptibility and prognostication, genetic polymorphisms of miRNAs and their targets might dictate the risk of AS development whereas certain miRNAs exhibit correlation with inflammatory index. Nevertheless, the clinical use of miRNAs as disease susceptibility or prognostic markers for AS remains in its infancy. Validations with larger sample sizes are required for establishing the clinical values of these markers. Furthermore, functional characterization has to be performed for rs3027898 in *IRAK1* to confirm its relationship with miRNA-146a.[36] For AS treatment, with more research efforts being put forth to the development of miRNA-based therapeutics and delivery system, it is hopeful that miRNAs will achieve clinical utility for AS at last.

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