



Review Article

The role of miRNAs in ankylosing spondylitis: a narrative review

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Abstract

Ankylosing Spondylitis (AS) is a chronic, autoimmune inflammatory disease mainly affecting the axial skeleton and it might lead to functional and structural impairments and cause severe disability, thus, jeopardize quality of life. The last two decades, the potential role of miRNAs in the pathogenesis of AS has been under investigation in numerous studies. The aim of this article is to review the available literature on the involvement of miRNAs in AS pathogenesis. An electronic literature search was conducted by two independent researchers up to 2013. Titles and abstracts of papers were validated by the authors for further inclusion in the present work. At the end, full texts of the included articles were retrieved. SNPs of miRNAs lead to an overexpression of pro-inflammatory cytokines. Deregulated miRNAs enhance the production of pro-inflammatory cytokines, suppress autophagy in T peripheral cells of the blood. MiRNAs through a vast number of research have a potential correlation with AS pathogenesis. Because of their stable structure which easily could be extracted from cells of blood samples, the different expressed levels of miRNAs in peripheral blood and tissues from the joints could be used as potential biomarkers of the activity and the efficacy of the treatment in AS.

Keywords: Ankylosing spondylitis, microRNAs, HLA-B27, SNPs

Introduction

Ankylosing spondylitis (AS) is a chronic, autoimmune inflammatory disease of the axial skeleton which belongs to the broader group of seronegative spondyloarthropathies. Ankylosing spondylitis affects in prevalence 80% mainly young male adults who are in their third decade of their life. The early symptoms of the disease include morning stiffness combined with low back pain, which, besides the fact that it limits the patient's activity, has a negative influence on the patient's psychology. Currently, the most novel approach in AS treatment is based on medication using either anti-inflammatory or immunomodulatory drugs (anti-TNF biologic agents)¹.

microRNAs (miRNAs) consist a group of small, non-coding RNAs with approximately 20-25 nucleotides length that regulate the expression of multiple target-genes mainly at the post-transcriptional level². It is known that miRNAs play critical roles in many processes such as differentiation, cell proliferation and apoptosis. Their crucial role is their function as posttranscriptional regulators of gene

expression through interacting with numerous mRNAs, thus promoting their degradation or decreasing their translation. The last two decades it has become widely known that miRNAs, apart from being important for normal cellular development, are involved in the pathologies of autoimmune diseases, susceptibility to cancer, inflammation and heart diseases as a result of epigenetic modifications such as DNA methylation and nucleosome structural changes via histone modifications³.

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Pathogenesis and pathology

Ankylosing Spondylitis (AS) is currently considered as a genetically determined immunopathological disorder of the axial skeleton. AS is more prominent to family members of patients than to the general population. HLA-B27 blood test is found positive in over 95% of Caucasian patients and in half of their first-degree relatives- and racial groups with an unusually low prevalence of AS also showing a very low prevalence of HLA-B27 (<1% of Japanese people)^{1,4}. Among the various theories referring to the triggering factor that initiates the abnormal immune response, the most dominant hypothesis is the one that investigates the presence of a bacterial antigen, which resembles HLA-B27 accelerating an antibody response, which also targets the HLA-B27. A present theory refers to a possible correlation between (AS) and genitourinary diseases, inflammatory bowel diseases such as Crohn's disease and Reiter's disease causing sacroiliitis and vertebral changes indistinguishable from those of (AS). It has been suggested that the putative organism might be transferred to the spine by local lymphatic drainage.

Regarding to the pathogenesis of (AS), two fundamental lesions emerge: synovitis of arthrodial joints and inflammation at the fibro-osseous junctions of syndesmotic joints and tendons. Synovitis of the sacroiliac and vertebral facet joints cause destruction of articular cartilage and periarticular bone. Inflammation of the fibro-osseous joints affects the intervertebral discs, sacroiliac ligaments and the area of insertions of large tendons. Pathological changes proceed in three separated stages: 1) an overexpressed inflammatory reaction characterized by cell infiltration, granulation tissue formation and erosion of adjacent bone, 2) replacement of the granulation tissue by fibrous tissue and 3) progressive ossification of the fibrous tissue, leading to ankylosis of the joint¹.

Clinical features

The onset of symptoms starts insidiously; a young adult complains of low back pain and stiffness, mainly at the morning, recurring at intervals over a number of years. This is usually diagnosed as 'simple mechanical back pain' but the symptoms are worse after inactivity and during the night rest. Gradually pain and morning stiffness become unceasing and additional symptoms appear; fatigue, swelling of joints, tenderness at the insertion of the *Achilles tendon*¹.

In late-diagnosed cases, the posture is characteristic manifesting progressive loss of the normal lumbar lordosis, increased thoracic kyphosis and a forward thrust of the neck. Spinal mobility is repressed in all directions among them the loss of extension is the first in appearance. Additionally, peripheral joints (hips, knees and shoulders) are involved in over a third of the patients with the appearance of inflammatory arthritis, effusion and loss of mobility. There might also be tenderness of tendon insertions close to a large joint.

Given that (AS) is an inflammatory disease, patients are

prone to extra-skeletal manifestations. Fatigue and loss of weight are usually appear with the onset of the symptoms. Acute anterior uveitis occurs in about 25% of the patients which if neglected might lead to glaucoma. Other extra-skeletal disorders, such as aortitis, carditis and pulmonary fibrosis are rare and present late in the disease⁴.

Diagnosis

Diagnosis is usually easy in patients with loss of spinal mobility and typical deformities, but it is often missed in those with unusual forms of presentation. In over 10%, AS manifests as an asymmetrical inflammatory arthritis -usually of the ankle, knee or hip- and might be many years until back pain and stiffness appear. Atypical onset is more common in female patients. HLA-B27 blood test is positive in over than 90% of patients. A positive medical history of autoimmune disease in a close member of the family is strongly suggestive.

As regard to imaging scanning, spinal X-ray is the most commonly used initial radiologic examination. The first vertebral change is flattening of the normal anterior concavity of the vertebral body. Later, ossification of ligaments around of the intervertebral discs produce bridges (syndesmophytes) between adjacent vertebral giving finally the appearance of a "*bamboo spine*"⁵. Magnetic Resonance Imaging (MRI) of lumbar spine may have significant features such as exaggerated bone inflammation at vertebrae and sacroiliitis. Diagnostic and classification criteria for AS are not totally defined. The first organized attempt to conduct diagnostic criteria include the modified criteria of New York (1984), which are used broadly mainly because of their simplicity⁶.

The first standardized classification criteria were conducted by the European Spondyloarthritis Study Group in 1991. Moreover, this classification has been continued by the Assessment of Spondyloarthritis International Society (ASAS), who have published recommendations for clinical trials and management of AS. In 2009, the ASAS published a statement on the classification of axial AS, which was formed with the objective of validating the classification/diagnostic criteria of axial AS. The brief criteria from this publication have a sensitivity of 82.9% and a specificity of 84.4%.

Treatment of AS

Treatment of AS consists of a wide range of interventions, which target individual pathways associated with the progression of the disease. The most common treatment methods involve repression of the inflammation, which is the primary complication resulting from AS, physical therapy and surgical interventions to address deformities of the spine^{1,4}.

Anti-inflammatory treatment

The primary target of the anti-inflammatory therapy in AS patients with increased disease activity is the inhibition of the tumor necrosis factor α (TNF- α). This treatment intervention is mainly effective in the primary stages of the

disease, where reduction of inflammation could prevent deformities of the skeleton. TNF- α inhibitor drugs are anti-TNF antibodies and TNF receptors. Adalimumab and infliximab are monoclonal antibodies, while etanercept is a TNF receptor drug; both of these drugs are approved for use in AS treatment^{7,8}. Nonsteroidal anti-inflammatory drugs (NSAIDs) are another line of therapeutic intervention used in AS. NSAIDs are more mainly used to alleviate morning back pain and increase spinal mobility in patients with AS, however, several studies have shown that NSAIDs are also effective in reducing progression of the disease.

Physical therapy

Physical therapy targets to increase muscle strength and spinal mobility and alleviate back pain in patients with AS. A wide amount of exercise methods have been used in the rehabilitation of AS patients, including weight training, cardiovascular training, and aquatic exercises. Generally, physical exercise methods lead to similar treatment outcomes, which result in an progressive increase in spinal mobility, repression of back stiffness, as well as a decrease in pain⁹. Among the different types of exercise methods cardiovascular training have been shown to increase fitness status in (AS) patient while aquatic exercise has been more effective in reducing morning back pain. In our days, spa therapies and home exercise programs after hospitalization have been organized.

Surgical intervention

Progression of AS might lead to irreversible deformities in the axial skeleton, thus jeopardize normal cardiovascular activity of the patients. The most common deformity in AS patients is the fixed thoracolumbar kyphotic deformity (TLKD) of the spine. The sole treatment method of this deformity is surgical. There are three surgical techniques used to alter TLKD, known as the opening wedge osteotomy (OWO), closing wedge osteotomy (CWO) and polysegmental wedge osteotomy (PWO). Generally, there is preference in choosing between the three techniques. However, CWO and PWO have been shown to be more effective for the patients, compared to the OWO⁷. Other complications that arise from AS such as spinal fractures in the thoracolumbar region are used to be treated surgically.

microRNAs

miRNAs form a group of endogenous, small, non-coding RNAs with approximately 20-25 nucleotides length. The vast majority of miRNAs are transcribed by two enzymes which belong to the larger group of RNA polymerase II enzymes, and their upstream regulatory regions include canonical core enhancers and promoters which are being strictly regulated by transcription factors¹⁰.

microRNAs are processed in two different nuclear steps by two members of the RNA polymerase II family of enzymes. microRNAs, which derive mostly from independent genes

or represent introns of protein-coding genes in less than 1%, processed from precursor molecules (pri-miRNAs)³. In the first step of the canonical pathway, which take place at the nuclear, the Drosha-DGCR8 complex processes pri-miRNA into a pre-miRNA (70 nucleotide precursor hairpin) which is exported to the cytoplasm through Exportin-5 transmembrane protein. In the cytoplasm area during the second step of miRNA processing, enzyme Dicer assisted by TRBP yields a miRNA/miRNA* duplex. Following processing, one strand of the miRNA/miRNA* duplex (the guide strand) is incorporated into a miRNA-induced silencing complex (mRISC) and the other strand (passenger strand) is released to the cytoplasm and degraded. In general, the retained strand is the one with the less stability at the 5' end in the miRNA/miRNA* duplex. Once the mRISC with the guide strand is assembled, the miRNA guides the complex to its target by base-pairing with the target mRNA. In contrast to the procedure that is shown in plants, most investigated animal microRNAs bind to multiple, partially complementary sites in the 3'-untranslated region of the target mRNA. The complementarity is usually focused on the nucleotides 2-8 in the 5' end of the miRNA. This small sequence at the 5' end of the miRNAs is known as the "seed" sequence, implying that it nucleate binding between miRNA and target mRNA¹¹. The fate of the mRNA depends on the existence or not of perfect complementarity between the two sequences which are interacting. A miRNA is about to direct destruction of the mRNA target if has near-perfect or perfect complementarity to the target¹². Moreover, the existence of multiple, partially complementary areas in the target mRNA will probably direct the inhibition of protein accumulation without decreasing mRNA levels³. Upon binding to the 3'-untranslated region of a target mRNA the miRNA-mRNA complex directs the mRNA to the P-bodies. P bodies have the role of storage place where mRNAs, after the disassembly of ribosomes, are degraded or stored until repression is released and re-enter translation¹³. It is commonly known that their function is basically as posttranscriptional regulators of gene expression by specifically interacting with certain mRNAs and repressing genes translation or inducing genes degradation¹⁴.

Regarding to the online database known as miRBase, a mature miRNA could bind to numerous mRNA targets, and at least one-third of human protein-encoding genes appears to be regulated by microRNAs. Among all the microRNAs discovered since our days, two miRNAs miR-146a and miR-499 have been received remarkable attention in this field. MiR-146a is encoded by chromosome 5q33. Mature miR-146a are capable of binding to 3'-untranslated regions of many target mRNAs, including tumor necrosis factor receptor-associated factor 6 (TRAF-6), interleukin-1 receptor-associated kinase 1 (IRAK-1) and other transcripts related to inflammatory response^{2,15}. It has been proposed that miR-146a participates in cytokine signaling and Toll-like receptor thus modifying the immune response. The miR-499 gene was found in 20q11.22¹⁶. Targets of miR-499 are

genes responsible for the expression of pro-inflammatory cytokines such as IL-6, IL-2RB, IL-23a, IL-21, IL-2, IL-18R and regulatory factor X 4 (RFX4).

Polymorphisms of miRNAs and their target genes in AS

Single nucleotide polymorphisms (SNPs) have been shown to be the most common type of genetic variation in the human genome. SNPs which are embedded in miRNAs regions can alter miRNAs expression thus affect their function in three stages: through the transcription of the primary transcript, through pri-miRNA and pre-miRNA maturation, and by regulating miRNA-mRNA interactions. The common miR-146a polymorphism rs2910164 involves a G>C nucleotide replacement. It could lead to the change from a GU pair to a CU mismatch in the stem structure hence altering the structure of the miR-146a precursor. The miR-499 rs3746444T>C polymorphism is located in the stem region of the miR-499 gene and results in an AU to GU mismatch in the stem structure of the miR-499 precursor. The majority of studies which have been published since our days tried to shed light on the potential relationship between SNPs in miRNAs regions and the pathogenesis of inflammatory diseases such as OA or RA. None has studied since our days the potential association between AS and miR-146a rs2910164G>C or miR-499 rs3746444T>C¹⁷.

According to the previous statement a quite interesting research was conducted by Xu et al. (2015)¹⁸ between March 2013 and April 2014 in a Chinese province researching the possibility of a significant correlation between SNPs -located in miR-14a and miR-499- and ankylosing spondylitis. The study was composed by two groups. In the first one 102 AS patients- whose disease was diagnosed according to the modified New York criteria- were enrolled in this study and the latter was composed by 105 healthy controls. Researchers found a significantly high association between the miR-146a rs2910164 G>C variant and AS patients. Among the AS patients, the G allele frequency was remarkably higher than in the healthy controls (49.0% vs. 35.2%, $P=0.005$, $P_c=0.01$, $OR=1.767$). However, the miR-499 rs3746444 T/C SNP showed no correlation between the susceptibility of AS at the allele level (75.0% vs. 85.2%, $P=0.369$, power=15%).

The second field of research was more restricted on analyzing genotype frequency distribution. Furthermore, researchers found a significant association between the polymorphism miRNA-146a rs2910164 and the appearance of AS. The frequency of the GG genotype was remarkably higher in AS patients than in healthy controls (GG vs. GC+CC, $P=0.014$, $P_c=0.042$, $OR=2.516$). However, no significant difference was detected in the distribution of miR-499 rs3746444 T>C genotypes between patients with AS and controls ($P_c>0.05$, power=37%). The latter part of this study focused on the potential existence of association between microRNA polymorphisms and disease activity parameters with the imminent results rather not to

be notably. Researchers found no significant differences in the levels of ESR and CRP in blood samples among different miR-146a genotype groups of AS patients, nor were there significant differences among miR-499 polymorphism groups. The two miRNA polymorphisms had no association with the AS activity level. As a conclusion, Xu et al. (2015)¹⁸ through their research found that the miR-146a rs2910164 variant was related with increased risk of AS at both the allelic and genotypic stages. The G allele and GG genotype were more prevalent in the patients than in the control group. Nonetheless, the miR-499 rs3746444 T>C variant was not associated with the risk of AS. The SNP which is located on miR-146a rs2910164 G>C might serve as a promising candidate marker for identification of AS. Moreover, the researchers shed new light on the significant relationship between natural genetic variations in miRNA genes such as SNPs and human diseases such as AS with the promising hypothesis that an assessment of the rs2910164 polymorphism during examinations of high-risk individuals could lead to the primary detection of AS.

Another study which has a significant reference value was published by Niu et al. (2015)¹⁹. In this case, researchers tested frequencies of three common MIR 146a SNPs: rs2910164, rs2431697 and rs57095329 between 611 patients with AS (diagnosis according to 1984 New York Modified Criteria) and 617 healthy controls. The results were contradictory to the previous study addressed by Hu et al. (2013)¹⁸. Thus, researchers who used different and wider cohorts found no significant difference between case and control groups of three these SNPs. Minor allele frequencies in cases and controls were 17.5% vs. 17.9% for rs2431697 C, 19.7% vs. 19.2% for rs57095329 G, and 41.2% vs. 41.1% for rs2910164 G, respectively. The lowest $p=0.439$ was observed in rs57095329 for allele test. And allele frequencies of rs2910164 G in case and control groups were nearly equal. For genotypic tests, SNP rs2910164 GG homozygote frequencies in cases and controls were 17.8% and 15.2%, respectively. Chi-square p-value for rs2910164 genotype GG vs. GC+CC was 0.235 (Odds Ratio=1.20, 95% Confidence Intervals 0.89–1.63). Genotype frequencies of all SNPs were in Hardy-Weinberg equilibrium. The statistical power was 0.19 for rs2431697, 0.15 for rs57095329 and 0.17 for rs2910164. Chatzikyriakidou et al. (2010)²⁰ in their study investigated the possible correlation between polymorphism rs3027898 in IRAK1 gene and the polymorphism rs2910164 in miR-146a with AS susceptibility. They figure out that there was significant association between polymorphism rs3027898 in IRAK1 gene, polymorphism rs2910164 in miR-146a and the existence of AS.

The pathogenesis of AS has still not been clearly identified but the aberrant expression of SNPs in miR-146a gene might be associated with the development of AS. Normally, miR-146a has a critical role as a crucial regulator, thus preventing an overexpressed inflammatory response. IRAK-

1(IL-1-receptor-associated kinase) and TRAF-6 (tumor necrosis factor receptor-associated factor 6), which are involved in the inflammatory reaction, are targets of miR-146a transcript. miR-146a through the down-regulation of IRAK1 and TRAF6 (mRNA target genes) inhibits the expression of NF- κ B targets genes such as IL-8, IL-6, IL-1 and TNF- α ^{2,16,20}.

Furthermore, recent studies have proved that dysregulation of miR-146a expression has a crucial role to the generation of imbalance between Th1/Th2 cells of the peripheral blood, thus promoting Th1 differentiation and hyperactive chronic inflammatory response²¹. As a result, abnormalities related to miR-146a structure and decreased level of expression contribute to either increased osteoclast activity via TNF- α , IL-17, IL-1 β , NF- κ B overexpression or overexpressed inflammation in the joints via Th1/Th2 imbalance. Polymorphism rs2910164 G>C in miR-146a, which produces a CU mismatch in the miR-146a precursor, is located on the stem region opposite of the mature miR-146a sequence. There is a theory established by recent studies that this change at the sequence alter the stability and the level expression of the mature miR-146a. Nevertheless, the same studies conclude to controversial results regarding to which of two allele, G allele or C allele, leads to a more stable and active mature miR-146a^{22,23}.

miRNAs and Autophagy in AS

Autophagy is a normal nuclear activity during which dead cells and pathogens are removed in order to maintain cellular activity. In autophagy targeted cytoplasmic components are isolated from the rest of the cell within a double-membraned vehicle known as autophagosome. The autophagosome fuses with the lysosomes and the contents are degraded and recycled. Recent studies have shown that autophagy might play an important role in the pathogenesis of AS²⁴. Beclin 1 regulates autophagy, LC3 executes autophagy and ATG5 is involved in the maturation of autophagy. The expression of these three crucial for the autophagy genes tried to correlate with AS Wang et al. (2017)^{25,26} in their study. Moreover, Wang et al. (2017)^{25,26} through their study aimed to investigate whether miR-199a-5p takes part in the pathogenesis of AS and whether it affects autophagy. To figure out how miR-199a-5p affects T cells autophagy, bioinformatics tools were used to search for potential targets of miR-199a-5p. Among the targets of miR-199a-5p, Rheb (Ras Homolog Enriched in Brain) is an upstream positive regulator of mTOR signaling in mammalian. Researchers speculate that miR-199a-5p influence cell autophagy by regulating Rheb expression. Wang et al. (2017)^{25,26} found that the increased level of miR-199a-5p to the group of the patients downregulate the levels of Rheb, thus, decreasing autophagy genes Beclin 1, LC3 and ATG5 and increasing at the same time the expression of pro-inflammatory cytokines such as IL-1 β , IL-4, IL-23 and IL-17.

On the other hand, increased expression of miRNAs seems

to have a protective effect in AS pathogenesis. Hou et al. (2014)²⁷ address a study to investigate the functional role of miRNA let7i in the T cell survival. Their results demonstrate significant down-regulation of IGF1R (insulin-like growth factor 1 receptor) in the T cells from AS patients. IGF1R proved to be direct target of let-7i. Let-7i overexpression in the Jurkat T cells inhibited the IGF1R expression. Inhibition of IGF1R factor led to a significant decrease in mTOR, upregulation of Bax, downregulation of Bcl-2 and cleavage of caspase 3. Moreover, this study conclude that overexpression of let-7i protect the cells from apoptosis by upregulating the expression of IGF1R and promoting cell autophagy. Their results suggested that let-7i could be an important regulator of T cells in AS through targeting IGF1R. One more study, which conducted by Xia Y et al. (2015)²⁸, demonstrated that miR-124 was overexpressed in peripheral blood sample from AS patients, which was correlated with downregulation of ANTXR2. miR-124-mediated downregulation of ANTXR2 may induce c-Jun NH2-terminal kinase and autophagy in T cells to participate in AS.

miRNAs as potential biomarkers in AS

Despite composing small nuclear molecules, miRNAs seem to have an extremely stable structure through which might be able to be isolated from samples taken from peripheral blood samples. Altered expression levels of miRNAs in peripheral blood mononuclear cells and synovial fluid concentration have been described under various pathological condition, including autoimmune diseases²⁹.

Wang et al. (2017) through their research demonstrated that miR-31 was significantly up-regulated in PBMCs of 40 AS patients compared with 40 healthy controls^{25,26}. Moreover, researchers found a significant association between ESR, index which demonstrates the activity of the disease, and miR-31 in the group of AS patients. Lai et al. (2018)³⁰ in their research found that the expression levels of five miRNAs(miR-29b-1-5p, miR-4449, miR-211-3p, miR-1914-3p and miR-7114-5p) were significant higher in AS T cells of peripheral blood sample from patients with AS using a microarray that contained 2549 miRNAs expression profiles. Lai et al. (2013)³¹ in a previous research demonstrated that the expression of three miRNAs(miR-16, miR-221 and let-7i) was increased in T cells from AS patients compared to those from healthy controls. In the same study the elevated expression of the two miRNAs (miR-221 and let-7i) showed a potential correlation between BASRI index for lumbar spine in AS patients. In another interesting study, Zhang et al. (2018)³² found that the expression of miR-16a in peripheral blood of AS was significantly higher compared to control people. Furthermore, in the same study AS patients at active phase had remarkably higher miR-16a levels, compared to stable phase. In addition, serum levels of IL-4 and IL-10 in AS patients in Zhang et al. (2018)³² research where significant increased, thus, miR-16a expression was positively correlated with IL-4/IL-10 or

disease index but not with CRP or ESR. Anti-TNF monoclonal antibodies consist a novel therapeutic approach to AS. Qing Lv et al. (2015)³³ in their research found that the expression levels of two specific miRNAs (miR-126-3p and miR-29a) was significant lower in 40 AS patients than in 50 healthy control which participate in their study. Moreover, Qing Lv et al. (2015)³³ infer that the expressions of miR-126-3p and miR-29a, which was lower in AS group, were significantly up-regulated in AS group after 12-week therapy with etanercept (a known TNF inhibitor) taking baseline as control. Through this study researchers present the potential role of miRNAs as potential biomarkers for AS diagnosis, activity evaluation and curative effect monitoring.

Therapeutic Potential of microRNAs in AS

Current treatment of AS includes physiotherapy interventions, in order to improve spinal mobility, and drugs such as Nonsteroidal Anti-Inflammatory Drugs (NSAIDs), disease-modifying drugs and anti-TNF agents which block the progress of the disease. In severe cases when AS cause spinal ankylosis and heart-lung capacity are jeopardized might be necessary the surgical intervention through vertebral osteotomies^{7,8}.

Studies which were conducted the last two decades revealed a vast number of miRNAs involving in AS pathogenesis, thus, an emerging role for miRNAs as potential therapeutic intervention become a field of research. In a recent study, Ni et al. (2018)³⁴ through their study tried to identify the potential target genes for the treatment of (AS) by using online Dataset GSE25101 including the blood samples from 16 (AS) and 16 normal controls. They reported that NDUFS4 (NADH ubiquinone oxidoreductase iron-sulfur protein4), which is involved in the imbalance of autophagy, differentiation and immune response, was significant higher in blood sample of (AS) because of the overexpression of 14 different miRNAs. Moreover, Ni et al. (2018)³⁴ infer that indomethacin³⁵, a common anti-inflammatory drug in clinical treatment of AS, could have as potential target the NDUFS4, so by analyzing the sample of a (AS) patient for overexpressed NDUFS4 via deregulated miRNAs could figure out whether or not indomethacin is effective. A novel approach in (AS) treatment is the use of antisense oligonucleotides (anti-miRs or antagomirs)³⁶ in order to suppress the miRNAs which are involved in pathogenesis of (AS). A significant advantage of using antisense oligonucleotides is that their administration would prevent side effects as a result of topical injection in the synovial impaired joints³⁷. An example of targeting miRNAs for the treatment of AS is the inhibition of miR-124. The upregulation of miR-124 in osteoblasts results in overexpression of Osterix, RUNX2, β -catenin and downregulation of GSK-3 β , thus, leading to increased osteoblast differentiations and bone formation. Tang et al. (2018)³⁸ demonstrated in their study that silencing of miR-124 with oligonucleotides of antisense miR-124 inhibited the overexpressed osteoblasts differentiation which

participates in cultured in vitro cells from tissue samples of AS impaired hips.

Conclusions

Since the discovery of microRNA in 1993 at Harvard, a great deal of effort had been devoted to annotate their biologic function and the correlation with diseases. Not only had the genomics, mechanism, and function of microRNAs been discovered, but also disorders of microRNAs had been associated with certain human disease and pathological changes of tissue. According to recent research published over the last 10 years miRNAs dysfunctions have a significant relevance to pathogenesis of rheumatic inflammatory diseases such as ankylosing spondylitis. 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. 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.

Authors' contributions

GED: collected literature, drafted the manuscript. GIL: proof-read the manuscript, provided critical review, gave final permission for submission.

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