Chapter

Pathogenic Role of microRNA in Rheumatoid Arthritis

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Abstract

Rheumatoid arthritis (RA) being a chronic inflammatory disease can be affected by both genetic and environmental factors. Abnormal functioning of immune response is the main underlying cause of RA. A growing number of studies on related diseases uncovered that microRNA (miRNA) may influence the pathogenesis of RA, such as the promotion of proliferation of fibroblast-like synoviocytes and secretion of cytokines by highly expressed miRNAs. A large number of studies have reported the aberrant expressions of miRNAs during the entire phase of RA, from the preclinical to terminal stages. These dynamic changes can be potentially developed as a bio-marker for predicting the risk, diagnosis and clinical management of RA. This chapter aims to summarize and discuss miRNAs' roles and mechanisms in the process of RA development, differential diagnosis from other diseases, clinical management and refractory RA. Therefore, miRNA demonstrates future perspectives of diagnosis and treatment of clinical RA under the support of newly discovered theoretical basis.

Keywords: Rheumatoid arthritis, microRNA, bio-marker, diagnosis, refractory rheumatoid arthritis

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune disease, which causes joint deformity and disability in patients. RA can occur at any age, particularly with a high incidence in women aged 30–50 [1]. It has been shown that the average lifespan of RA patients is 3 to 18 years shorter than that of healthy people [2]. Patients with RA have high mortality rate and extra-articular complications, such as cardiovascular diseases, becoming the greatest challenge [2]. Proliferation of synovial tissue, infiltration of inflammatory factors, imbalance of immunity system, and destructions of bone and cartilage are the main common pathological characteristics of RA [3]. However, the current understanding of RA etiology and pathology are far yet to be elucidated. Some opinions on RA etiology illustrated high risk factors including but not limited to gene background, gender difference, smoking, obesity and environment factors. During the last decade, a growing number of evidence has shown that the epigenetic mechanism of microRNA (miRNA) regulation contributes remarkably to RA pathogenesis.

MiRNAs belong to the non-coding RNA family, with about 22 nucleotides in length. The processes of miRNAs biogenesis and maturation take place in the nucleus.

The transcription of primary miRNAs (pri-miRNAs) from DNA molecule is the first step. After the recognition of these pri-miRNAs by an enzyme-protein complex, they are cleaved into precursor miRNAs (pre-miRNAs) with 70–100 nucleotides in length. Subsequently, the pre-miRNAs mature in the cytoplasm. Mature miRNAs finally regulate the post-transcriptional gene expression by binding to 3′-untranstaled region (3'UTR) of target mRNAs. Interestingly, the same gene can be modulated by multiple miRNAs, which collectively fine tune the expression of a certain gene. One-third of human genes of note is regulated by miRNA [4]. In addition, miRNAs participate the regulation of cell bio-behaviors, such as apoptosis, proliferation and invasion. Cytokine signaling is commonly known to regulate immune system, which is associated with the pathogenesis of RA. A large number of evidence showed that miRNAs participate in regulations of both innate and adaptive immunities by modulating cytokine signaling [5], such as the upregulations of miR-146 and miR-155 in LPS-mediated innate immune response. Besides, a high expression level of miR-155 during thymic differentiation can increase Treg sensitivity to IL-2 and growth factors [6]. Given their important roles in cell regulation mechanisms and immunity responses, miRNAs have been frequently studied as potential bio-markers in diagnosis, target treatment, activity monitoring and therapy for RA disease. For example, during the early stages of undifferentiated arthritis, a high expression level of miR-483 was only found in patients who finally developed RA. In this chapter, we aim to review the different roles of miRNAs in RA, from the pathogenesis to clinical impact.

2. The functions of miRNA in RA development

Studies showed that synovial hyperplasia is a main pathological feature of RA, but the pathogenesis of RA is not fully elucidated. Fibroblast-like synoviocyte (FLS) is a major cell type found in the structure of synovial intima [7]. The most important step in the development of RA is the transformation of FLS by over-activation to RAFLS [8]. This process makes RA to present a characteristic, aggressive, and active clinical phenotype. It has been reported that RAFLS can recruit inflammatory cells through autocrine and paracrine methods to maintain the inflammatory state [7]. At the same time, compared with FLS, RAFLS has the characteristics of anti-apoptosis, predominant cell proliferation, invasion and metastasis. It also secretes inflammatory factors and promotes erosion of bone matrix (e.g. matrix metalloproteinases, MMPs). These secreted cytokines form a complex network system that affects each other, leading to an imbalance between synovial cell proliferation and apoptosis. This process therefore plays an important role in the progression of RA disease. Controlling the local proliferation of synovial cells and inducing their apoptosis are the key towards improvement of RA prognosis. Recent results showed that the activated phenotype of RAFLS is underpinned by epigenetic mechanisms—DNA methylation, histone modifications, and miRNA activity [9]. Newly emerging evidence suggested that dysregulated miRNA expressions in RA synovial tissues, especially in RAFLS, may generally contribute to the molecular mechanism of disease. Comparing miRNAs expression in FLS between RA and osteoarthritis (OA) patients, miR-124a was only down-regulated in RAFLS [10]. Further experiments revealed that overexpression of miR-124a can to suppress RAFLS proliferation. In contrast to miR-124a, miR-203 was up-regulated in RAFLS compared with healthy FLS [11, 12]. Importantly, a high level of miR-203 can target NF-κB signaling pathway, promote IL-6 and MMPs secretions, and support RAFLS invasion and migration [12]. Besides, there are lots of miRNAs like miR-126 [13], miR-152 [14], miR-137 [15], miR-199a-3p [16] and miR-338-5p [17], controlling the development of RA via regulating RAFLS.

RA is a well-known autoimmune disease, and both innate and adaptive immunities are the crucial steps for RA development. The role of miRNAs in regulating immune response has been reported in the literature. Alternations of miRNAs level can control the differentiation and immunological functions of various immune cells (monocytes, macrophages, and T cells) [18]. Many changes of miRNAs in these cells in RA patients have been reported. Chronically activated T cells are considered to be the trigger and key to RA. They are also the crucial link in inducing and aggravating RA immunological inflammatory response. On the one hand, they can induce activation of synovial macrophages and RAFLS. On the other hand, they contribute to T-Treg imbalance, which is a predominant mechanism of RA. A great number of studies have confirmed that there are various miRNA expressions modulating T cells, such as miR-17 [19] and miR-146a [20]. Additionally, RA patients showed the increases of miR-16, miR-103a, and miR-222 in peripheral blood mononuclear cells (PBMCs) of RA patients, which promoted cytokine secretion and disturb T-Treg balance [20]. The main miRNAs changes in different cells of patients compared with healthy controls were summarized in **Table 1**.

miRNA	Regulation	Sample	Target	Effects	Ref.
miR-203	↑	RAFLS	NF-κB pathway	IL-6↑, MMPs↑	[12]
miR-126	↑	RAFLS	PI3K/AKT pathway	proliferation↑, apoptosis↓	[13]
miR-338-5p	↑	RAFLS	NFAT5	proliferation↑, invasion↑, migration↑	[17]
miR-155	↑	RAFLS	JAK2/ATST3	IL-6 mediated inflammation↓, invasion↓, proliferation↓, MMPs↓	[21, 22]
		Synovial tissue	FOXO3a	IL-1β↑, IL-6↑,TNF-α↑, RAFLS proliferation ↑	[23]
miR-125b	↑	Synovial tissue	NF-κB pathway	NF-κB mediated inflammation↑	[24]
miR-301a	↑	PBMCs	PIAS3	Th17 differentiation↑, cytokines↑	[25]
miR-124a	↓	RAFLS	CDK2,MCP1	proliferation ↑, chemotaxis↑	[10, 11]
miR-199a-3p	↓	RAFLS	RB1	proliferation↑, apoptosis↓	[16]
miR-152	↓	RAFLS	ADAM10	proliferation↑, apoptosis↓	[26]
miR-137	↓	RAFLS	CXCL12	proliferation↑, migration↑, pro-inflammatory cytokines↑	[27]
miR-22	↓	Synovial tissue	SIRT1	proliferation↑, proinflammatory cytokine↑	[28]
miR-192	↓	RAFLS	Caveolin 1	proliferation↑, apoptosis↓	[29]
miR-21	↓	PBMCs	STAT3	Th17↑, Treg↓	[30]
miR-548a	↓	PBMCs	TLR-4/NF-κB	NF-κB mediated inflammation↑	[31]

Table 1.
Changes in miRNA level in RA patients compared to healthy individuals.

Having a clear understanding of miRNAs in the regulation of RA pathogenesis provides a new direction and strategy for RA treatment. In some animal models, miRNA mimics or silencers were used to regulate miRNAs expressions, thereby reversing the inflammatory reaction or joint damage. One example is the amelioration of arthritis severity by reducing RAFLS's population via intra-articular injections of miR-124 and miR-140 mimics [32, 33]. Furthermore, intra-peritoneal injection of miR-223 silencer showed the same effect on relieving arthritis severity [34]. In a CIA mice model, intravenous administrations of miR-146a [35] and miR-708-5p [36] mimics were beneficial to prevent synovial hyperplasia and structural joint damage. Taken together, further investigations on the role of miRNAs in the pathogenesis of RA are of utmost importance for the treatment and delaying progression of RA, as well as developing novel targeted drugs.

3. MiRNA as a potential bio-marker in RA diagnosis

RA often begins insidiously with chronic developments of signs and symptoms, which may vary in intensity over many weeks. For most patients with new-onset RA, there is no obvious symptom in the early stage. Most of them show joint discomfort, which is difficult to distinguish RA from other diseases. In clinical practice, using rheumatoid factor (RF) and cyclic citrullinated peptide (CCP) antibodies as diagnostic indicators are not sufficient [37]. Notably, the sensitivity and specificity of CCP antibodies in RA diagnosis were ~72% and ~92% respectively [38]. In some special cases, the CCP antibodies' titers cannot reach the diagnostic thresholds. Moreover, genetic and environmental risk factors, together with systemic immunization, affect the multi-stage development of RA. Identifying patients with RA and providing them a proper treatment can prevent 90% of patients in early-stage period from the progression of joint damage, and improve prognosis [39]. Therefore, there is an urgent need for identifying novel bio-markers to screen high-risk individuals and those with early stage of RA.

Single nucleotide polymorphism (SNP) variants residing within boundaries of genes encoding miRNAs is a common phenomenon, which may affect multiple major human disorders including RA [40]. The associations between miRNA-linked SNP and RA susceptibility have been studied extensively, such as rs11761231 in miR612, rs615672 in miR-541, rs2837960 in miR-509/602, rs6684865 in miR-181, rs9550642 in miR-1238 and rs6920220 in miR-519 [41]. Furthermore, the association of variations of miRNA target genes with RA was exemplified by the discovery of SNP rs3027898 variant in miR-146a target gene, IL-1 receptorassociated kinase (IRAK-1), in RA patients. Collectively, the alterations of miRNA gene and its target gene may increase the risk of developing RA.

The current understanding of the role of miRNAs in RA pathogenesis is limited, especially in the preclinical phase of RA. Some serum miRNA expression profiles from different people were evaluated to determine mechanisms underpinning the progression of RA onset in at-risk individuals. Among those miRNA expressions, only miR-103a-3p specifically increased in both RA patients and their seropositive first-degree relatives [42]. Patients who have symptoms of non-differential arthritis and a high serum miR-22 expression, finally developed RA [20]. Recently, a study examined circulatory miRNAs in RA patients, and further investigations illustrated that miR-221-3p, let-7d-5p, miR-431-3p, miR-130a-3p, miR-126-3p and miR-24-3p were significantly elevated in subjects "at risk" of developing RA [42]. Particularly, the elevated whole blood level of miR-103a-3p may become a powerful bio-marker for positive anti-citrullinated peptide antibodies (ACPA) individuals who have possibility to develop RA [42].

Early stage RA (ERA) is defined as a disease duration less than 12 months. Several clinical studies have shown that ERA is a "window of opportunity" for disease-modifying anti-rheumatic drug (DMARD) therapy. Most patients at this stage will get long-term remissions or even complete remissions after systematic treatments. There are no golden diagnostic criteria in ERA to date, although this stage is important in clinical practice. During the last decade, multiple studies have demonstrated miRNA as a powerful tool for identifying molecular bio-markers for diagnosis in ERA. One highlight example is the analysis of highly expressed miR-22 level for distinguishing ERA patients from healthy individuals. Besides, miR-16, miR-146a, miR-223 and miRNA-155 were significantly down-regulated in ERA, and even lowered in established RA and healthy controls [43–45]. Generally, these miRNAs possibly improve early diagnosis of RA, especially in sero-negative patients.

RA diagnosis not only distinguishes the different phases of RA, but also differentiates RA from other diseases, such as systemic lupus erythematousus (SLE), OA, multiple sclerosis (MS). Those diseases show similar symptoms to RA at the beginning. Several studies have established analyses of different expressions of miRNAs among those indistinguishable diseases. Compared with healthy people, miR-146a and miR-155 were up-regulated in PBMCs of RA cases, and conversely, they had low expressions in PBMCs of SLE patients. In addition, miRNA-516a-3p, miRNA-629 and miRNA-525-5p levels in PBMCs were significantly up-regulated in active SLE patients compared with those in healthy controls [46], but all these miRNAs have no specific expressions in RA patients. A recent study revealed that the expressions of miR-371b-5p and miR-5100 also increased notably in the serum of SLE compared with healthy control and RA [47]. Further results revealed that miRNA-346 in synovial tissues was only specifically elevated in RA [48]. Another seven miRNAs expressions in macrophages from patients with active RA and OA were also recently determined. MiR-99a, miR-100, miR-125b, miR-199-3p, miR-199-5p, miR-152 and miR-214 were down-regulated in macrophages in RA, while only miR-223 was up-regulated, compared with OA samples [49]. One more example is that the expression level of miR-34a-3p in RAFLS was generally lower than that in OAFLS [50].

Clearly, the observable changes in miRNAs and their molecular networks are of great values for determining new mechanisms related to the onset of RA, and also being used as bio-markers to predict the onset of preclinical RA and distinguish RA from other diseases.

4. The application values of miRNAs in RA clinical management

4.1 MiRNAs' functions in activity monitoring of RA

The clinical management strategy of RA is "treat-to-target" [51]. In other words, patients can achieve remission or at least low disease activity state within 6 months after effective treatment. If RA is insufficiently treated, extra-articular manifestations, such as the most frequently occurring rheumatoid nodules and even cardiovascular disease, may occur. Notably, this kind of cardiovascular disease is more closely associated with RA disease activity rather than traditional cardiovascular risk factors. Furthermore, either manifestation of RA or complication of RA therapies (e.g. MTX and leflunomide) may lead to interstitial lung disease (ILD). This affirms the importance of activity monitoring from different aspects. Hence, it is necessary to develop new treatment strategies to retard RA progression by quick identification of conditions of RA remission before irreversible damage in joint

[39]. Currently, clinical indicators for RA activity monitoring are mainly based on clinical, laboratory and physical examinations, including simplified disease activity index (SDAI), disease activity score 28 (DAS28), erythrocyte sedimentation rate (ESR), C reaction protein (CRP) [52]. These indicators can be affected by subjective and objective factors, such as OA, fibromyalgia, and assessor's experience. Both ESR and CRP are non-specific markers of inflammation, which are commonly affected by age, anemia, immunoglobulin and other factors. Therefore, these markers are not specific enough to RA patients. In view of the clinical demands, it is particularly important to develop effective, precise and accurate biological markers for the evaluation of RA disease activity. Recent studies demonstrated that miRNA, a potential bio-marker, can be aberrantly expressed in different stages of RA progression, and thus allowing to monitor disease activity.

The correlations of miRNA levels (miR-125b, miR-21, miR-155, miR-346, miR-223 and miR-146a) in PBMC of RA patients with clinical characteristics and inflammation markers in RA patients were reported [53]. The expression levels of miR-146a and miR-155 were positively related to ESR, DAS28-CPR and cytokines (IL-1β, IL-17α, IL-6 and TNF-α). On the contrary, miR-21 was negatively related to DAS28 and those cytokines. Another study found that miR-125b was inversely correlated with RA activity [54]. The studies on miR-24 in patients' serum with active RA disease uncovered that the miR-24 level increased with ESR and the DAS28 [55]. Besides, miR-5571-3p and miR-135b-5p levels were found to be positively correlated with the disease activity and the inflammation level of RA [56]. Notably, the upregulated expressions of hsa-miR-432-5p and especially hsa-miR-194-5p in serum were associated with relapse in RA patients [57]. Increasing serum level of miR-223 was also found in remission patients several days before RA relapse [20]. Moreover, blood samples from 76 RA patients illustrated that lowering the levels of miR-548a-3p can promote RA relapse or increase disease activity [31]. In some cases, RA patients without proper treatment were accompanied by extra-articular symptoms, together with changes in some miRNAs levels. Analyzing abnormal expressions of miRNAs can assist in diagnosis of RA-related diseases. For example, some researchers collected miRNAs (e.g. let-7c-5p, miR-30a-5p, miR-30e-5p, miR-125a-5p, miR-126-3p, miR-126-5p, miR-425-5p, miR-3168, and miR-4446-3p) in a panel to predict cardiovascular disease in patients with RA [58]. Other findings found that differences in circulating miR-200c levels can distinguish RA patients with and without ILD [59]. More examples of the relationship between miRNAs expression and RA activity were shown in **Table 2**.

4.2 MiRNAs as potential bio-markers of therapeutic effectiveness

Despite the great progress of management of RA over the past three decades, anti-rheumatoid drugs (DMARDs), including conventional synthetic DMARDs (csDMARDs) and specific targeted DMARDs (e.g. biologic DMARDs, b-DMARDs, and targeted synthetic DMARDs, tsDMARDs), are still the first-line drugs for RA patients; however, a certain number of patients does not benefit from the treatments with multiple DMARDs [65]. For those patients, biological treatments targeting inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukins (ILs), and B or T cells may give a better outcome [66–69]. Nevertheless, ~20–30% of patients fail to respond to these biological agents. Therefore, it is necessary to explore novel bio-markers for predicting the clinical responses of RA patients to DMARDs or other therapies. The current indicators for assessing the therapeutic effectiveness involve inflammatory factors, disease activity and patient response outcome (PRO) [70]. These evaluation indicators, however, are easily affected by many factors, resulting in certain deviations. Emerging research showed

miRNA	Correlation with RA activity	References
miR-146a	Positive	[60]
miR-155	Positive	[60]
miR-132	Positive	[60]
miR-21	Positive	[61]
miR-5571-3p	Positive	[56]
miR-135b-5p	Positive	[56]
miR-432-5p	Positive	[57]
miR-194-5p	Positive	[57]
miR-223	Positive	[62]
miR-206	Positive	[63]
miR-125b	Negative	[54]
miR-548a	Negative	[31]
miR-16	Negative	[62]
Let-7a	Negative	[64]

Table 2.
The relationship between miRNAs expression and RA activity.

that the prediction of RA treatment was possibly achieved by monitoring the alterations of miRNA levels. This encourages the development of novel therapeutic strategies for RA via identifying molecular mechanisms of miRNAs.

Several findings demonstrated that almost all miRNAs expressions were changed during therapy. Using MTX significantly decreased the expressions of miR-155 and miR-146a, but increased the expression of miR-34 in rat tibiotarsal tissues [71]. Clinical research proved that RA patients who responded to MTX had lower expressions of specific miRNAs, including not only hsa-miR-155-5p and hsa-miR-146a-5p, but also the newly reported hsa-miR-132-3p [60]. The circulating miR-10a in RA patients was markedly decreased, but was up-regulated when treated with MTX [72].

Although miR-223 and miR-16 were shown to be overexpressed in synovial tissues of RA patients, their expressions were decreased after treated with csDMARDs [62]. More importantly, disease severity was reduced when miR-223 was silenced in experimental arthritis [34]. Another finding demonstrated that miR-125b expression showed more alternations between patients in terms of good response and poor response [73]. Its expression was relatively low in patients with early RA, but increased markedly after 3 months of conventional therapy [54]. Thus, these miRNAs could become potential bio-markers in both csDMARD and bDMARD therapies.

Furthermore, some miRNAs were good candidates for predicting the treatment of RA with anti-TNF therapy. A placebo-controlled, double-blind and prospective study of patients with early RA showed that the highly expressed miR-886.3p in combination with lowly expressed miR-22 were associated with the probability of EULAR good response (~95%) [74]. This may indicate the responses of miR-22 and miR-886.3p to adalimumab treatment in RA. RA patients before TNF-α therapy showed a relatively higher miRNA-5196 expression than those treated with anti-TNF-α therapy and healthy controls [75]. Studies implied that an increase of miR-155 may result in the upregulation of membrane TNF expression on monocytes and the defect of monocyte capacity to differentiate into M2-like anti-inflammatory

macrophages, which were the clinical characteristics specific to RA. Notably, increased miR-155 could be partially reversed by monoclonal anti-TNF antibodies [76]. Besides, the expressions of miR-126, miR-148a, miR-29c, miR-30c, miR-17, miR-21, miR-223 and let-7b in neutrophil of RA patients were declined after treatment with anti-TNF-α drugs [77]. Obviously, these miRNAs could be the potential bio-markers for DMARDs therapy in RA.

Taken together, dynamic changes of miRNAs were not only associated with disease activity, but were also affected by therapeutic effects. This reflects the potential clinical values of miRNAs expression as novel prognostic markers for RA patients, in terms of RA management.

5. MiRNA in refractory rheumatoid arthritis

Most patients achieve remission or low disease activity state with effective therapies and treatment strategies. However, about 20–25% of the patients do not reach a state of low disease activity, and the causes of refractory rheumatoid arthritis (RRA) have not been identified. The RRA may attribute to the epigenetic changes accumulated by chronic RA. A large amount of evidence indicated that changes in miRNAs can occur either before or after treatment. Therefore, the alterations of miRNAs may affect the duration of RA or the therapeutic effect, leading to RRA. However, the mechanisms of miRNAs mediating RRA are still largely unknown. Up to date, the mechanism of miRNA on RRA has been known to be related to the regulation of drug efflux transporters, apoptosis and cell cycle modification. Notably, some somatic genes, such as p53, may also influence RA via miRNAs.

ATP-binding cassette (ABC) transporters are located in cell membrane responsible for transporting endogenous metabolites and xenobiotics across cell membranes in an ATP-dependent manner [78]. The high expression levels of ABC transporters were commonly found in cells from the inflammatory area of refractory RA patients [79]. These abnormal expressions in RA patients subsequently increased drug efflux and caused patients a lower response to treatment. The reduction of therapeutic effect of MTX by increased expression of ABCB1 in RA patients is a distinguishable example [80]. Importantly, the MTX-treated group showed the ABCC1 expression in synovial tissues higher than the untreated group [81]. These studies corroborated that the increasing MTX resistance in RA patients may result from the upregulations of ABC transporters. Hence, declining ABC transporter expression provides a potential solution for reversing drug resistance in RA chemotherapy. One of the reasons for miRNAs being as potential therapeutic targets for chemoresistant cancers is their regulations on the expression of ABC transporter. Research in ovarian cancer demonstrated that miR-522 inhibited ABCB5 in HT29 colon cancer cells to reverse drug resistance to doxorubicin [82]. ABCG2-mediated drug resistance to 5-FU in colon cancer side population cells was overcome by overexpressed miR-34a via suppressing DLL1 expression [83]. Similarly, ABCB1 (P-gp) expression was downregulated by miR-491-3p via directly bound to the 3'-UTR of ABCB1 [84]. MiR-214-3p also acts as a tumor suppressor to inhibit chemoresistance in retinoblastoma by targeting ABCB1 [85]. MiR-1268a regulated ABCC1-mediated drug resistance to temozolomide in glioblastoma [86].

Based on the important role of RAFLS in RA development, most drugs achieve the remission of RA by controlling RAFLS-related activities. MiRNA has been considered as a potential reason for refractory RA owing to its important role in regulating RAFLS mechanisms. On the one side, miRNA promoted the secretion of pro-inflammatory cytokines or MMPs; and increased RAFLS proliferation, invasiveness, survival and anti-apoptosis. On the other side, they can regulate various

intracellular pathways in RAFLS, which commonly include Wnt, NF-κB, JAK/STAT and TLRs signaling pathways. For example, reduced miR-20a expression in RASFs activated the JAK-STAT3-mediated inflammation, and promoted cell proliferation and apoptosis-resistance [87]. The regulation of PI3K/AKT pathway by targeting PIK3R2 with miR-126 promoted RA synovial fibroblasts proliferation and apoptosis-resistance [88]. In a separate study, miR-650 was down-regulated in RA patients compared with patients with joint trauma undergoing joint replacement surgery [89]. Further study confirmed that miR-650 targeted AKT2 to promote FLS proliferation and migration, and reduce apoptosis. In another example, down-regulated miR-375 in an AIA rat model aggravated the inflammation of FLS through Wnt signal pathway [90]. Interestingly, the expression level of the same miRNA varied in different tissues, along with different functions. One example is miR-21, which increased significantly in a rat model of collagen-induced RA with the promotion of FLS proliferation via NF-kB pathway [91]. In contrast, the miR-21 level in RA patients was declined due to the participation in the imbalance of Th17 and Treg cells [92].

Generally, p53 being as a tumor suppressor regulates many signaling pathways like apoptosis, cell cycle, DNA repair, and cellular stress responses by modulating the expressions of miRNAs [93]. Since p53 plays important roles in inflammation, apoptosis, and cell proliferation, the p53 function lost by gain-of-function (GOF) mutation or its low expression influences RA pathogenesis. Similarly, GOF mutation of p53 can confer tumor cell oncogenic properties such as chemoresistance and invasion. According to statistical investigations, the mutation rate of p53 gene in RA patients was about 50% [94]. In particular, a pro-apoptotic molecule, p53-regulated apoptosis-inducing protein 1 (p53AIP1) was suppressed by p53 mutation (from arginine to glutamine at site 248) in RAFLS, leading to an anti-apoptotic effect [95]. However, the mechanisms of p53-mediated apoptosis resistance are yet to elucidate.

Typically, wild-type p53 regulates miRNAs to work. For instance, p53 controlled cell apoptosis through regulating miRNAs expressions (e.g. miR-34a, miR15a, and miR16–1) [93]. In RA patients, miR-15a and miR16–1 initiated anti-apoptosis by inhibiting anti-apoptotic molecule B cell lymphoma 2 (Bcl2) [96]. In addition, miR-34a expression in RA-FLSs was positively related to X-linked inhibitor of apoptosis protein (XIAP) expression which induced RAFLS anti-apoptosis [97]. Since p53 activates all the above-mentioned miRNAs, functionally defective p53 (p53 mutation) may influence RAFLS apoptosis resistance.

Cyr61, which is a secreted and cysteine-rich extracellular matrix (ECM) protein produced by RAFLS, is stimulated by IL-17 for FLS proliferation [98]. Over-expressed Cyr61 is an important mediator in a malicious cycle, where a high level of Cyr61 promotes RAFLS proliferation and Th17 cell differentiation [99]. Generally, wild-type p53 from RA patients promoted expression of miR-22 targeting the 3-UTR of Cyr61, leading to a decrease of Cyr61 expression [100]. However, functional defect of mut-p53 in RA synovial tissue was unable to activate miR-22 expression, causing abnormally high Cyr61 expression and, in turn, promoted RAFLS proliferation and IL-6 production [100]. Thus, a reduced miR-22 level in RA synovial tissue and the resulting RRA attributes to somatic mutations of p53.

MiR-155 is also an important regulator in the pathogenesis of RA. Highly expressed miR-155 in PBMCs of RA patients was positively related to inflammatory cytokine (e.g. TNF-α and IL-1β), RA activity laboratory indicators (CRP, ESR) levels and DAS28 respectively [101]. Recent study indicated that mut-p53 increased miR-155 expression in breast cancer, which accelerated cell proliferation, epithelial-mesenchymal-transition (EMT) and invasion [102]. This implied that p53 mutations may affect the process of RA via miR-155 as similar to breast cancer.

Overall, miRNAs are not only an independent factor that affects the refractory of RA, but also are an intermediate link of certain gene mutations related to RRA. This may provide a new direction for treating refractory RA or reversing miRNA-related apoptosis resistance.

6. Conclusions

MiRNA, a non-coding RNA, widely exists in tissue cells and body fluids. It is worth mentioning that the studies on miRNA in RA are still limited, but the results verify its important role in immune response regulation and sustained inflammatory response to date. SNPs in specific miRNA genes, such as miR-541, are related to the high risk of RA development. Moreover, most miRNAs in synovial tissues can influence the process of RA by regulating RAFLS proliferation, invasion and apoptosis via targeting inflammatory or immune signaling pathways like NF-κB and Wnt pathways. Current efforts have confirmed that the expression level and mechanism of the same miRNA varies in different tissues or cells from RA. For example, miR-21 level in PBMCs was declined to regulate Th-Treg balance by targeting STAT3, STAT5 and Foxp3, but that in RAFLS was overexpressed to promote proliferation of RAFLS through NF-κB signaling pathway. For clinical management, the dynamic change of miRNAs can be a bio-marker for monitoring disease activity and therapeutic response, as exemplified by the association of high miR-223 level with high disease activity and RA relapse. In addition, some miRNAs may play a crucial role in regulating refractory RA or drug-resistance RA.

Finally, an increasing extent of data demonstrates the importance of miRNAs to the regulation of the RA process, along with its potential developments in biomarker discovery and special targets for treatment. Hence, understanding miRNA analysis can be served as a diagnostic and/or prognostic tool in an array of inflammatory disorders, especially RA.

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Conflict of interest

The authors declare no conflict of interest.

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References

[1] Stoll JG, Yasothan U. Rheumatoid arthritis market. Nature Reviews Drug Discovery. 2009;8(9):693-694. doi: 10.1038/nrd2947

[2] Rawla P. Cardiac and vascular complications in rheumatoid arthritis. Reumatologia/Rheumatology. 2019;57(1):27-36. doi: 10.5114/reum.2019.83236

[3] Guo Q, Wang Y, Xu D, Nossent J, Pavlos NJ, Xu J. Rheumatoid arthritis: pathological mechanisms and modern pharmacologic therapies. Bone Research. 2018;6(1):15. doi: 10.1038/s41413-018-0016-9

[4] Schanen BC, Li X. Transcriptional regulation of mammalian miRNA genes. Genomics. 2011;97(1):1-6. doi: 10.1016/j.ygeno.2010.10.005

[5] Sharma AR, Sharma G, Lee S-S, Chakraborty C. miRNA-Regulated Key Components of Cytokine Signaling Pathways and Inflammation in Rheumatoid Arthritis. Medicinal Research Reviews. 2016;36(3):425-439. doi: 10.1002/med.21384

[6] Filková M, Jüngel A, Gay RE, Gay S. MicroRNAs in Rheumatoid Arthritis. BioDrugs. 2012;26(3):131-141. doi: 10.2165/11631480-000000000-00000

[7] Bartok B, Firestein GS. Fibroblast-like synoviocytes: key effector cells in rheumatoid arthritis. Immunological Reviews. 2010;233(1):233-255. doi: 10.1111/j.0105-2896.2009.00859.x

[8] Meng H-Y, Chen L-Q, Chen L-H. The inhibition by human MSCsderived miRNA-124a overexpression exosomes in the proliferation and migration of rheumatoid arthritis-related fibroblast-like synoviocyte cell. BMC Musculoskeletal Disorders. 2020;21(1):150. doi: 10.1186/s12891-020-3159-y

[9] Ospelt C, Gay S, Klein K. Epigenetics in the pathogenesis of RA. Seminars in Immunopathology. 2017;39(4):409-419. doi: 10.1007/s00281-017-0621-5

[10] Yang B, Ge Y, Zhou Y, Wang J, Xie X, Li S, et al. miR-124a inhibits the proliferation and inflammation in rheumatoid arthritis fibroblast-like synoviocytes via targeting PIK3/NF-κB pathway. Cell Biochemistry and Function. 2019;37(4):208-215. doi: 10.1002/cbf.3386

[11] Kawano S, Nakamachi Y. miR-124a as a key regulator of proliferation and MCP-1 secretion in synoviocytes from patients with rheumatoid arthritis. Annals of the Rheumatic Diseases. 2011;70(Suppl 1):i88-i91. doi: 10.1136/ard.2010.138669

[12] Stanczyk J, Ospelt C, Karouzakis E, Filer A, Raza K, Kolling C, et al. Altered expression of microRNA-203 in rheumatoid arthritis synovial fibroblasts and its role in fibroblast activation. Arthritis & Rheumatism. 2011;63(2):373-381. doi: 10.1002/art.30115

[13] Gao J, Kong R, Zhou X, Ji L, Zhang J, Zhao D. MiRNA-126 expression inhibits IL-23R mediated TNF-α or IFN-γ production in fibroblast-like synoviocytes in a mice model of collagen-induced rheumatoid arthritis. Apoptosis. 2018;23(11):607-615. doi: 10.1007/s10495-018-1474-7

[14] Guo J, Du J, Fei D, Xing J, Liu J, Lu H. miR152 inhibits rheumatoid arthritis synovial fibroblast proliferation and induces apoptosis by targeting ADAM10. Int J Mol Med. 2018;42(1):643-650. doi: 10.3892/ijmm.2018.3636

[15] Du J, Zhang F, Guo J. miR137 decreases proliferation, migration and invasion in rheumatoid arthritis

fibroblastlike synoviocytes. Mol Med Rep. 2018;17(2):3312-3317. doi: 10.3892/mmr.2017.8225

[16] Wangyang Y, Yi L, Wang T, Feng Y, Liu G, Li D, et al. MiR-199a-3p inhibits proliferation and induces apoptosis in rheumatoid arthritis fibroblast-like synoviocytes via suppressing retinoblastoma 1. Bioscience Reports. 2018;38(6):BSR20180982. doi: 10.1042/bsr20180982

[17] Guo T, Ding H, Jiang H, Bao N, Zhou L, Zhao J. miR-338-5p Regulates the Viability, Proliferation, Apoptosis and Migration of Rheumatoid Arthritis Fibroblast-Like Synoviocytes by Targeting NFAT5. Cellular Physiology and Biochemistry. 2018;49(3):899-910. doi: 10.1159/000493222

[18] Chen J-Q, Papp G, Szodoray P, Zeher M. The role of microRNAs in the pathogenesis of autoimmune diseases. Autoimmun Rev. 2016;15(12):1171-1180. doi: 10.1016/j.autrev.2016.09.003

[19] Wang L, Wang C, Jia X, Yu J. Circulating Exosomal miR-17 Inhibits the Induction of Regulatory T Cells via Suppressing TGFBR II Expression in Rheumatoid Arthritis. Cellular Physiology and Biochemistry. 2018;50(5):1754-1763. doi: 10.1159/000494793

[20] Evangelatos G, Fragoulis GE, Koulouri V, Lambrou GI. MicroRNAs in rheumatoid arthritis: From pathogenesis to clinical impact. Autoimmun Rev. 2019;18(11):102391. doi: 10.1016/j.autrev.2019.102391

[21] Migita K, Iwanaga N, Izumi Y, Kawahara C, Kumagai K, Nakamura T, et al. TNF-α-induced miR-155 regulates IL-6 signaling in rheumatoid synovial fibroblasts. BMC Research Notes. 2017;10(1):403. doi: 10.1186/s13104-017-2715-5

[22] Long L, Yu P, Liu Y, Wang S, Li R, Shi J, et al. Upregulated MicroRNA-155 Expression in Peripheral Blood Mononuclear Cells and Fibroblast-Like Synoviocytes in Rheumatoid Arthritis. Clinical and Developmental Immunology. 2013;2013:296139. doi: 10.1155/2013/296139

[23] Wang Y, Feng T, Duan S, Shi Y, Li S, Zhang X, et al. miR-155 promotes fibroblast-like synoviocyte proliferation and inflammatory cytokine secretion in rheumatoid arthritis by targeting FOXO3a. Exp Ther Med. 2020;19(2):1288-1296. doi: 10.3892/etm.2019.8330

[24] Zhang B, Wang L-S, Zhou Y-H. Elevated microRNA-125b promotes inflammation in rheumatoid arthritis by activation of NF-κB pathway. Biomedicine & Pharmacotherapy. 2017;93:1151-1157. doi: 10.1016/j.biopha.2017.07.042

[25] Tang X, Yin K, Zhu H, Tian J, Shen D, Yi L, et al. Correlation Between the Expression of MicroRNA-301a-3p and the Proportion of Th17 Cells in Patients with Rheumatoid Arthritis. Inflammation. 2016;39(2):759-767. doi: 10.1007/s10753-016-0304-8

[26] Guo J, Du J, Fei D, Xing J, Liu J, Lu H. miR-152 inhibits rheumatoid arthritis synovial fibroblast proliferation and induces apoptosis by targeting ADAM10. Int J Mol Med. 2018;42(1):643-650. doi: 10.3892/ijmm.2018.3636

[27] Du J, Zhang F, Guo J. miR-137 decreases proliferation, migration and invasion in rheumatoid arthritis fibroblast-like synoviocytes. Mol Med Rep. 2018;17(2):3312-3317. doi: 10.3892/mmr.2017.8225

[28] Zhang C, Fang L, Liu X, Nie T, Li R, Cui L, et al. miR-22 inhibits synovial fibroblasts proliferation and proinflammatory cytokine production in RASF via targeting SIRT1. Gene. 2020;724:144144. doi: 10.1016/j.gene.2019.144144

[29] Li S, Jin Z, Lu X. MicroRNA-192 suppresses cell proliferation and induces apoptosis in human rheumatoid arthritis fibroblast-like synoviocytes by downregulating caveolin 1. Molecular and Cellular Biochemistry. 2017;432(1):123-130. doi: 10.1007/s11010-017-3003-3

[30] Jin S, Chen H, Li Y, Zhong H, Sun W, Wang J, et al. Maresin 1 improves the Treg/Th17 imbalance in rheumatoid arthritis through miR-21. Annals of the Rheumatic Diseases. 2018;77(11):1644-1652. doi: 10.1136/annrheumdis-2018-213511

[31] Wang Y, Zheng F, Gao G, Yan S, Zhang L, Wang L, et al. MiR-548a-3p regulates inflammatory response via TLR4/NF-κB signaling pathway in rheumatoid arthritis. Journal of Cellular Biochemistry. 2019;120(2):1133-1140. doi: 10.1002/jcb.26659

[32] Nakamachi Y, Kawano S, Takenokuchi M, Nishimura K, Sakai Y, Chin T, et al. MicroRNA-124a is a key regulator of proliferation and monocyte chemoattractant protein 1 secretion in fibroblast-like synoviocytes from patients with rheumatoid arthritis. Arthritis & Rheumatism. 2009;60(5):1294-1304. doi: 10.1002/art.24475

[33] Peng J-S, Chen S-Y, Wu C-L, Chong H-E, Ding Y-C, Shiau A-L, et al. Amelioration of Experimental Autoimmune Arthritis Through Targeting of Synovial Fibroblasts by Intraarticular Delivery of MicroRNAs 140-3p and 140-5p. Arthritis & Rheumatology. 2016;68(2):370-381. doi: 10.1002/art.39446

[34] Li Y-T, Chen S-Y, Wang C-R, Liu M-F, Lin C-C, Jou I-M, et al. Brief Report: Amelioration of collagen-induced arthritis in mice by lentivirusmediated silencing of microRNA-223. Arthritis & Rheumatism. 2012;64(10):3240-3245. doi: 10.1002/art.34550

[35] Nakasa T, Shibuya H, Nagata Y, Niimoto T, Ochi M. The inhibitory effect of microRNA-146a expression on bone destruction in collageninduced arthritis. Arthritis & Rheumatism. 2011;63(6):1582-1590. doi: 10.1002/art.30321

[36] Wu J, Fan W, Ma L, Geng X. miR-708-5p promotes fibroblast-like synoviocytes' cell apoptosis and ameliorates rheumatoid arthritis by the inhibition of Wnt3a/β-catenin pathway. Drug Des Devel Ther. 2018;12:3439-3447. doi: 10.2147/DDDT.S177128

[37] Gavrilă BI, Ciofu C, Stoica V. Biomarkers in Rheumatoid Arthritis, what is new? J Med Life. 2016;9(2):144-148

[38] Szekanecz Z, Soós L, Szabó Z, Fekete A, Kapitány A, Végvári A, et al. Anti-Citrullinated Protein Antibodies in Rheumatoid Arthritis: As Good as it Gets? Clinical Reviews in Allergy & Immunology. 2008;34(1):26-31. doi: 10.1007/s12016-007-8022-5

[39] Aletaha D, Smolen JS. Diagnosis and Management of Rheumatoid Arthritis: A Review. JAMA. 2018;320(13):1360-1372. doi: 10.1001/jama.2018.13103

[40] Taheri M, Eghtedarian R, Dinger ME, Ghafouri-Fard S. Dysregulation of non-coding RNAs in Rheumatoid arthritis. Biomed Pharmacother. 2020;130:110617. doi: 10.1016/j.biopha.2020.110617

[41] Glinsky GV. SNP-guided microRNA maps (MirMaps) of 16 common human disorders identify a clinically accessible therapy reversing transcriptional aberrations of nuclear import and inflammasome pathways. Cell Cycle. 2008;7(22):3564-3576. doi: 10.4161/cc.7.22.7073

[42] Anaparti V, Smolik I, Meng X, Spicer V, Mookherjee N, El-Gabalawy H. Whole blood microRNA expression pattern differentiates patients with rheumatoid arthritis, their seropositive first-degree relatives, and healthy unrelated control subjects. Arthritis Research & Therapy. 2017;19(1):249. doi: 10.1186/s13075-017-1459-x

[43] Lenert A, Fardo DW. Detecting novel micro RNAs in rheumatoid arthritis with gene-based association testing. Clin Exp Rheumatol. 2017;35(4):586-592. doi:

[44] Romo-García MF, Bastian Y, Zapata-Zuñiga M, Macías-Segura N, Castillo-Ortiz JD, Lara-Ramírez EE, et al. Identification of putative miRNA biomarkers in early rheumatoid arthritis by genome-wide microarray profiling: A pilot study. Gene. 2019;720:144081. doi: 10.1016/j.gene.2019.144081

[45] Dunaeva M, Blom J, Thurlings R, Pruijn GJM. Circulating serum miR-223-3p and miR-16-5p as possible biomarkers of early rheumatoid arthritis. Clinical & Experimental Immunology. 2018;193(3):376-385. doi: 10.1111/cei.13156

[46] Zhu J, Huang X, Su G, Wang L, Wu F, Zhang T, et al. High expression levels of microRNA-629, microRNA-525-5p and microRNA-516a-3p in paediatric systemic lupus erythematosus. Clinical Rheumatology. 2014;33(6):807-815. doi: 10.1007/s10067-014-2583-5

[47] Zeng L, Wu J-l, Liu L-m, Jiang J-q, Wu H-j, Zhao M, et al. Serum miRNA-371b-5p and miRNA-5100 act as biomarkers for systemic lupus erythematosus. Clinical Immunology. 2018;196:103-109. doi: 10.1016/j.clim.2018.10.004

[48] Alsaleh G, Suffert G, Semaan N, Juncker T, Frenzel L, Gottenberg JE, et al. Bruton's Tyrosine Kinase Is Involved in miR-346-Related Regulation of IL-18 Release by Lipopolysaccharide-Activated Rheumatoid Fibroblast-Like Synoviocytes. The Journal of Immunology. 2009;182(8):5088-5097. doi:10.4049/jimmunol.0801613

[49] Ogando J, Tardáguila M, Díaz-Alderete A, Usategui A, Miranda-Ramos V, Martínez-Herrera DJ, et al. Notch-regulated miR-223 targets the aryl hydrocarbon receptor pathway and increases cytokine production in macrophages from rheumatoid arthritis patients. Scientific Reports. 2016;6(1):20223. doi: 10.1038/srep20223

[50] Luo S, Ding S, Liao J, Zhang P, Liu Y, Zhao M, et al. Excessive miR-152-3p Results in Increased BAFF Expression in SLE B-Cells by Inhibiting the KLF5 Expression. Frontiers in Immunology. 2019;10:1127. doi: 10.3389/fimmu.2019.01127

[51] Smolen JS, Breedveld FC, Burmester GR, Bykerk V, Dougados M, Emery P, et al. Treating rheumatoid arthritis to target: 2014 update of the recommendations of an international task force. Annals of the Rheumatic Diseases. 2016;75(1):3-15. doi: 10.1136/annrheumdis-2015-207524

[52] Tamhane A, Redden DT, McGwin G, Brown EE, Westfall AO, Reynolds RJ, et al. Comparison of the Disease Activity Score Using Erythrocyte Sedimentation Rate and C-reactive Protein in African Americans with Rheumatoid Arthritis. The Journal of Rheumatology. 2013;40(11):1812-1822. doi: 10.3899/jrheum.121225

[53] Xun C, Li H. Correlation between microRNA and rheumatoid arthritis activity. Chin J Rheumatol. 2019;23(6). doi: 10.3760/cma.j.issn.1007-7480.2019.06.010

[54] Hruskova V, Jandova R, Vernerova L, Mann H, Pecha O, Prajzlerova K, et al. MicroRNA-125b: association with disease activity and the treatment response of patients with early rheumatoid arthritis. Arthritis Research & Therapy. 2016;18(1):124. doi: 10.1186/s13075-016-1023-0

[55] Murata K, Furu M, Yoshitomi H, Ishikawa M, Shibuya H, Hashimoto M, et al. Comprehensive microRNA Analysis Identifies miR-24 and miR-125a-5p as Plasma Biomarkers for Rheumatoid Arthritis. PLOS ONE. 2013;8(7):e69118. doi: 10.1371/journal.pone.0069118

[56] Liu C, Pan A, Chen X, Tu J, Xia X, Sun L. MiR-5571-3p and miR-135b-5p, derived from analyses of microRNA profile sequencing, correlate with increased disease risk and activity of rheumatoid arthritis. Clinical Rheumatology. 2019;38(6):1753-1765. doi: 10.1007/s10067-018-04417-w

[57] Fernández-Ruiz JC, Ramos-Remus C, Sánchez-Corona J, Castillo-Ortiz JD, Castañeda-Sánchez JJ, Bastian Y, et al. Analysis of miRNA expression in patients with rheumatoid arthritis during remission and relapse after a 5-year trial of tofacitinib treatment. International Immunopharmacology. 2018;63:35-42. doi: 10.1016/j.intimp.2018.07.028

[58] Ormseth MJ, Solus JF, Sheng Q, Chen S-C, Ye F, Wu Q, et al. Plasma miRNAs improve the prediction of coronary atherosclerosis in patients with rheumatoid arthritis. Clinical Rheumatology. 2021;40(6):2211-2219. doi: 10.1007/s10067-020-05573-8

[59] Jiang Z, Tao JH, Zuo T, Li XM, Wang GS, Fang X, et al. The correlation between miR-200c and the severity of interstitial lung disease associated with different connective tissue diseases. Scandinavian Journal of Rheumatology. 2017;46(2):122-129. doi: 10.3109/03009742.2016.1167950

[60] Singh A, Patro PS, Aggarwal A. MicroRNA-132, miR-146a, and miR-155 as potential biomarkers of methotrexate response in patients with rheumatoid arthritis. Clinical Rheumatology. 2019;38(3):877-884. doi: 10.1007/s10067-018-4380-z

[61] Yang S, Jiang S, Wang Y, Tu S, Wang Z, Chen Z. Interleukin 34 Upregulation Contributes to the Increment of MicroRNA 21 Expression through STAT3 Activation Associated with Disease Activity in Rheumatoid Arthritis. The Journal of Rheumatology. 2016;43(7):1312-1319. doi: 10.3899/jrheum.151253

[62] Filková M, Aradi B, Šenolt L, Ospelt C, Vettori S, Mann H, et al. Association of circulating miR-223 and miR-16 with disease activity in patients with early rheumatoid arthritis. Annals of the Rheumatic Diseases. 2014;73(10):1898-1904. doi: 10.1136/annrheumdis-2012-202815

[63] ElAtta AA, Ali Y, Bassyouni I, Talaat R. Correlation of myomir-206 and proinflammatory cytokines (IL-16 and IL-17) in patients with rheumatoid arthritis. Reumatologia/Rheumatology. 2019;57(2):72-77. doi: 10.5114/reum.2019.84811

[64] Zhu W, Yu J, Qiu S, Liu H, Wang Y, Xu X, et al. MiR-let-7a regulates anti-citrullinated protein antibody-induced macrophage activation and correlates with the development of experimental rheumatoid arthritis. International Immunopharmacology. 2017;51:40-46. doi: 10.1016/j.intimp.2017.08.001

[65] Lau CS, Chia F, Dans L, Harrison A, Hsieh TY, Jain R, et al. 2018 update of the APLAR recommendations for treatment of rheumatoid arthritis. International Journal of Rheumatic

Diseases. 2019;22(3):357-375. doi: 10.1111/1756-185X.13513

[66] Kaczyński T, Wroński J, Głuszko P, Kryczka T, Miskiewicz A, Górski B, et al. Salivary interleukin 6, interleukin 8, interleukin 17A, and tumour necrosis factor α levels in patients with periodontitis and rheumatoid arthritis. Central European Journal of Immunology. 2019;44(3):269-276. doi: 10.5114/ceji.2019.89601

[67] Thwaites RS, Unterberger S, Chamberlain G, Gray H, Jordan K, Davies KA, et al. Expression of sterile-α and armadillo motif in rheumatoid arthritis monocytes correlates with TLR2 induced IL-1β and disease activity. Rheumatology (Oxford). 2021. doi: 10.1093/rheumatology/keab162

[68] Volkov M, van Schie KA, van der Woude D. Autoantibodies and B Cells: The ABC of rheumatoid arthritis pathophysiology. Immunological Reviews. 2020;294(1):148-163. doi: 10.1111/imr.12829

[69] Hu X-X, Wu Y-J, Zhang J, Wei W. T-cells interact with B cells, dendritic cells, and fibroblast-like synoviocytes as hub-like key cells in rheumatoid arthritis. International immunopharmacology. 2019;70:428-434. doi: 10.1016/j.intimp.2019.03.008

[70] Gonczi L, Bessissow T, Lakatos PL. Disease monitoring strategies in inflammatory bowel diseases: What do we mean by "tight control"? World J Gastroenterol. 2019;25(41):6172-6189. doi: 10.3748/wjg.v25.i41.6172

[71] El-Sayyad SM, Ali MA, kandil LS, Ragab GM, Abdelhamid Ibrahim SS. Metformin and omega-3 fish oil elicit anti-inflammatory effects via modulation of some dysregulated micro RNAs expression and signaling pathways in experimental induced arthritis. International Immunopharmacology. 2021;92:107362. doi: 10.1016/j.intimp.2020.107362

[72] Honghai H, Haihui Y, Yong X. Circulating miR-10a as Predictor of Therapy Response in Rheumatoid Arthritis Patients Treated with Methotrexate. Current Pharmaceutical Biotechnology. 2018;19(1):79-86. doi: 10.2174/1389201019666180417155140

[73] Rezaeepoor M, Pourjafar M, Tahamoli-Roudsari A, Basiri Z, Hajilooi M, Solgi G. Altered expression of microRNAs may predict therapeutic response in rheumatoid arthritis patients. International Immunopharmacology. 2020;83:106404. doi: 10.1016/j.intimp.2020.106404

[74] Krintel SB, Dehlendorff C, Hetland ML, Hørslev-Petersen K, Andersen KK, Junker P, et al. Prediction of treatment response to adalimumab: a double-blind placebo-controlled study of circulating microRNA in patients with early rheumatoid arthritis. The Pharmacogenomics Journal. 2016;16(2):141-146. doi: 10.1038/tpj.2015.30

[75] Ciechomska M, Bonek K, Merdas M, Zarecki P, Swierkot J, Gluszko P, et al. Changes in MiRNA-5196 Expression as a Potential Biomarker of Anti-TNF-α Therapy in Rheumatoid Arthritis and Ankylosing Spondylitis Patients. Archivum Immunologiae et Therapiae Experimentalis. 2018;66(5):389-397. doi: 10.1007/s00005-018-0513-y

[76] Paoletti A, Rohmer J, Ly B, Pascaud J, Rivière E, Seror R, et al. Monocyte/Macrophage Abnormalities Specific to Rheumatoid Arthritis Are Linked to miR-155 and Are Differentially Modulated by Different TNF Inhibitors. The Journal of Immunology. 2019;203(7):1766-1775. doi: 10.4049/jimmunol.1900386

[77] de la Rosa IA, Perez-Sanchez C, Ruiz-Limon P, Patiño-Trives A, Torres-Granados C, Jimenez-Gomez Y, et al. Impaired microRNA processing in neutrophils from rheumatoid arthritis patients confers their pathogenic profile. Modulation by biological therapies. Haematologica. 2020;105(9):2250-2261. doi: 10.3324/haematol.2018.205047

[78] Liu YM, Chen JW, Chen LX, Xie X, Mao N. Overexpression of Pglycoprotein on fibroblast-like synoviocytes in refractory rheumatoid arthritis patients: a potential mechanism for multidrug resistance in rheumatoid arthritis treatment. Genet Mol Res. 2016;15(2):gmr7927. doi: 10.4238/gmr.15027927

[79] van de Ven R, Oerlemans R, van der Heijden JW, Scheffer GL, de ruijl TD, Jansen G, et al. ABC drug transporters and immunity: novel therapeutic targets in autoimmunity and cancer. Journal of Leukocyte Biology. 2009;86(5):1075-1087. doi: 10.1189/jlb.0309147

[80] Qin K, Chen K, Zhao W, Zhao X, Luo J, Wang Q, et al. Methotrexate Combined with 4-Hydroperoxycyclophosphamide Downregulates Multidrug-Resistance P-Glycoprotein Expression Induced by Methotrexate in Rheumatoid Arthritis Fibroblast-Like Synoviocytes via the JAK2/STAT3 Pathway. Journal of Immunology Research. 2018;2018:3619320. doi: 10.1155/2018/3619320

[81] Stamp LK, Hazlett J, Highton J, Hessian PA. Expression of Methotrexate Transporters and Metabolizing Enzymes in Rheumatoid Synovial Tissue. The Journal of Rheumatology. 2013;40(9):1519-1522. doi: 10.3899/jrheum.130066

[82] Yang G, Jiang O, Ling D, Jiang X, Yuan P, Zeng G, et al. MicroRNA-522 reverses drug resistance of doxorubicin-induced HT29 colon cancer cell by targeting ABCB5. Mol Med Rep. 2015;12(3):3930-3936. doi: 10.3892/mmr.2015.3890

[83] Xie Z-Y, Wang F-F, Xiao Z-H, Liu S-F, Tang S-L, Lai Y-L. Overexpressing microRNA-34a overcomes ABCG2-mediated drug resistance to 5-FU in side population cells from colon cancer via suppressing DLL1. The Journal of Biochemistry. 2020;167(6):557-564. doi: 10.1093/jb/mvaa012

[84] Zhao Y, Qi X, Chen J, Wei W, Yu C, Yan H, et al. The miR-491-3p/Sp3/ABCB1 axis attenuates multidrug resistance of hepatocellular carcinoma. Cancer Letters. 2017;408:102-111. doi: 10.1016/j.canlet.2017.08.027

[85] Yang L, Zhang L, Lu L, Wang Y. miR-214-3p Regulates Multi-Drug Resistance and Apoptosis in Retinoblastoma Cells by Targeting ABCB1 and XIAP. Onco Targets Ther. 2020;13:803-811. doi: 10.2147/ott.S235862

[86] Li Y, Liu Y, Ren J, Deng S, Yi G, Guo M, et al. miR-1268a regulates ABCC1 expression to mediate temozolomide resistance in glioblastoma. Journal of Neuro-Oncology. 2018;138(3):499-508. doi: 10.1007/s11060-018-2835-3

[87] Wei XJ, Li XW, Lu JL, Long ZX, Liang JQ, Wei SB, et al. MiR-20a regulates fibroblast-like synoviocyte proliferation and apoptosis in rheumatoid arthritis. Eur Rev Med Pharmacol Sci. 2020;24(14):7578. doi: 10.26355/eurrev_202007_22253

[88] Gao J, Zhou X-L, Kong R-N, Ji L-M, He L-L, Zhao D-B. microRNA-126 targeting PIK3R2 promotes rheumatoid arthritis synovial fibro-blasts proliferation and resistance to apoptosis by regulating PI3K/AKT pathway. Experimental and Molecular Pathology.

2016;100(1):192-198. doi: 10.1016/j.yexmp.2015.12.015

[89] Xu X, Chen H, Zhang Q, Xu J, Shi Q, Wang M. MiR-650 inhibits proliferation, migration and invasion of rheumatoid arthritis synovial fibroblasts by targeting AKT2. Biomedicine & Pharmacotherapy. 2017;88:535-541. doi: 10.1016/j.biopha.2017.01.063

[90] Miao C-g, Shi W-j, Xiong Y-y, Yu H, Zhang X-l, Qin M-s, et al. miR-375 regulates the canonical Wnt pathway through FZD8 silencing in arthritis synovial fibroblasts. Immunology Letters. 2015;164(1):1-10. doi: 10.1016/j.imlet.2015.01.003

[91] Chen Y, Xian P-F, Yang L, Wang S-X. MicroRNA-21 Promotes Proliferation of Fibroblast-Like Synoviocytes through Mediation of NF-κB Nuclear Translocation in a Rat Model of Collagen-Induced Rheumatoid Arthritis. BioMed Research International. 2016;2016:9279078. doi: 10.1155/2016/9279078

[92] Dong L, Wang X, Tan J, Li H, Qian W, Chen J, et al. Decreased expression of microRNA-21 correlates with the imbalance of Th17 and Treg cells in patients with rheumatoid arthritis. Journal of Cellular and Molecular Medicine. 2014;18(11):2213-2224. doi: 10.1111/jcmm.12353

[93] Taghadosi M, Adib M, Jamshidi A, Mahmoudi M, Farhadi E. The p53 status in rheumatoid arthritis with focus on fibroblast-like synoviocytes. Immunologic Research. 2021. doi: 10.1007/s12026-021-09202-7

[94] Yamanishi Y, Boyle DL, Rosengren S, Green DR, Zvaifler NJ, Firestein GS. Regional analysis of p53 mutations in rheumatoid arthritis synovium. Proceedings of the National Academy of Sciences. 2002;99(15):10025. doi: 10.1073/pnas.152333199

[95] Hoshida Y, Hongyo T, Xu JX, Sasaki T, Tomita Y, Nomura T, et al. TP53 Gene Mutation, an Unfavorable Prognostic Factor for Malignant Lymphomas in Autoimmune Diseases. Oncology. 2005;69(2):175-183. doi: 10.1159/000087980

[96] Moran-Moguel MC, Petarra-del Rio S, Mayorquin-Galvan EE, Zavala-Cerna MG. Rheumatoid Arthritis and miRNAs: A Critical Review through a Functional View. Journal of Immunology Research. 2018;2018:2474529. doi: 10.1155/2018/2474529

[97] Niederer F, Trenkmann M, Ospelt C, Karouzakis E, Neidhart M, Stanczyk J, et al. Down-regulation of microRNA-34a* in rheumatoid arthritis synovial fibroblasts promotes apoptosis resistance. Arthritis & Rheumatism. 2012;64(6):1771-1779. doi: 10.1002/art.34334

[98] Zhang Q, Wu J, Cao Q, Xiao L, Wang L, He D, et al. A critical role of Cyr61 in interleukin-17–dependent proliferation of fibroblast-like synoviocytes in rheumatoid arthritis. Arthritis & Rheumatism. 2009;60(12):3602-3612. doi: 10.1002/art.24999

[99] Lin J, Zhou Z, Huo R, Xiao L, Ouyang G, Wang L, et al. Cyr61 Induces IL-6 Production by Fibroblast-like Synoviocytes Promoting Th17 Differentiation in Rheumatoid Arthritis. The Journal of Immunology. 2012;188(11):5776-5784. doi: 10.4049/jimmunol.1103201

[100] Lin J, Huo R, Xiao L, Zhu X, Xie J, Sun S, et al. A Novel p53/microRNA-22/Cyr61 Axis in Synovial Cells Regulates Inflammation in Rheumatoid Arthritis. Arthritis & Rheumatology. 2014;66(1):49-59. doi: 10.1002/art.38142

[101] Su L-C, Huang A-F, Jia H, Liu Y, Xu W-D. Role of microRNA-155 in rheumatoid arthritis. International Journal of Rheumatic Diseases. 2017;20(11):1631-1637. doi: 10.1111/1756-185X.13202

[102] Neilsen PM, Noll JE, Mattiske S, Bracken CP, Gregory PA, Schulz RB, et al. Mutant p53 drives invasion in breast tumors through up-regulation of miR-155. Oncogene. 2013;32(24):2992-3000. doi: 10.1038/onc.2012.305