MicroRNA Regulation in Rheumatoid Arthritis: Improving Tocilizumab-Based Treatment

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Abstract. Rheumatoid arthritis (RA) is a chronic inflammatory disease characterised by persistent joint inflammation, pain, swelling, and progressive joint damage. miRNAs, small non-coding RNAs, play critical roles in immune regulation, and their dysregulation contributes to RA development. miRNAs are promising therapeutic targets in RA due to their broad effects and stage-specific regulatory roles. Tocilizumab (TCZ), a biological DMARD targeting the IL-6 receptor, has shown good clinical outcomes and plays a role in modulating miRNA expression, offering new insights into RA pathogenesis and treatment response. Recent studies suggest that miRNA-based therapies could modulate miRNA profiles to reduce inflammation and tissue damage. This paper explores TCZ's role in miRNA regulation in RA, focusing on the modulation of miR-146a-5p and miR-150-5p and their effects on angiogenesis and inflammation. However, gaps remain in understanding the mechanisms through which miRNAs influence RA outcomes, the optimal delivery methods for miRNA therapies, and the long-term effects of TCZ on miRNA profiles. Further research is needed to optimize miRNA-based therapies, particularly in combination with TCZ and other conventional DMARDs, to enhance efficacy in RA treatment.

Keywords: Rheumatoid Arthritis, miRNA, Tocilizumab.

1. Introduction

Rheumatoid arthritis (RA) is a highly heterogeneous, chronic, and multisystemic inflammatory disease characterized by persistent joint inflammation, leading to pain, swelling, progressive damage, severely impaired physical function, disability, and reduced quality of life. RA is a growing global health issue, with a prevalence rate of 0.46% and an incidence rate ranging from 0.5% to 1% worldwide [1, 2]. Current treatments, including conventional (disease-modifying antirheumatic drugs) DMARDs, biologic DMARDs, and JAK inhibitors, aim to slow disease progression while alleviating symptoms. However, conditions like Difficult-to-Treat Rheumatoid Arthritis (D2T RA) and Late-Onset Rheumatoid Arthritis (LORA) show the variability in patient responses., emphasizing the need for ongoing research to develop more effective and personalized treatment strategies with better-targeted therapeutic approaches.

MicroRNAs (miRNAs) are small endogenous noncoding RNAs that act as posttranscriptional regulators of gene expression. The first miRNA, lin-4, was discovered in Caenorhabditis elegans in 1993, revealing miRNAs as key regulators in posttranscriptional modificatio [3]. The role of miRNA deregulation in human diseases was first recognised in 2002, leading to extensive research on miRNA dysregulation in conditions such as cancer, cardiovascular diseases, and autoimmune disorders [4-6]. Because of their broad effects and stage-specific regulatory roles, miRNAs have been explored as diagnostic biomarkers and therapeutic targets, making them particularly attractive candidates for RA research. During RA pathogenesis, the significantly decreased level of miR-124a promotes the proliferation of fibroblast-like synoviocytes (FLS), leading to increased MCP-1 expression and enhanced leukocyte chemotaxis, which contributes to inflammation [7]. Several regulatory miRNAs play protective roles in RA. For instance, miR-155 helps alleviate tissue damage by downregulating the expression of specific MMPs, while miR-346 indirectly suppresses the expression of the pro-inflammatory cytokine IL-18 [7]. miRNAs not only contribute to RA pathogenesis but also have the potential as biomarkers for disease activity and therapeutic targets.

The development of miRNA-based therapies for RA focuses on regulating miRNAs involved in inflammation and tissue damage. This strategy aims to silence overexpressed pathogenic miRNAs while activating the expression of beneficial, underexpressed miRNAs. Chemically modified miRNA mimics (agomirs) and inhibitors (antagomirs) have been explored, such as miR-708-5p mimics, miR-34a antagomirs, miR-132 antagomirs, and pre-miR-124 [5]. To enhance the effectiveness of miRNA therapy, various delivery methods are being investigated: local administration, lentiviral vectors for in vivo gene transfer, MSC-derived exosomes as miRNA carriers, and synthetic materials for efficient delivery [5]. These approaches aim to improve the stability, targeting, and therapeutic efficacy of miRNA-based treatments.

MiRNA-based therapies are gaining attention for their potential to complement RA treatments, including tocilizumab (TCZ) and DMARDs. TCZ, a biological DMARD, targets the interleukin-6 (IL-6) receptor, inhibiting both classical (cis) and trans-signaling pathways [8]. It has demonstrated significantly greater efficacy than conventional DMARDs, making TCZ monotherapy highly effective for RA patients who do not respond to treatments like methotrexate (MTX) [9]. Additionally, TCZ can control disease activity in many patients unresponsive to tumor necrosis factor (TNF) inhibitors [9].

TCZ has been shown to regulate miR-146a-5p expression, as well as reversing the global downregulation of miRNAs in RA neutrophils [7]. This regulation enhances miRNA processing, reduces cytokine and chemokine production, and blocks neutrophil activation while also normalizing C-reactive protein (CRP) levels [7, 9]. However, compared to other biological DMARDs, TCZ has a distinct adverse reaction profile [9]. It can cause elevated liver enzymes (ALT, AST), reduced white blood cell and platelet counts, and increased blood cholesterol levels, with both LDL and HDL rising after administration [9]. Additionally, long-term use of such immunosuppressants may increase the risk of infections. Meanwhile, MTX remains a key component of conventional DMARD therapy, but its unresponsiveness in some patients has been linked to single nucleotide polymorphisms (SNPs) that affect miRNA function [7]. Understanding miRNA regulation in the context of TCZ and MTX resistance may provide valuable insights into new therapeutic strategies aimed at improving treatment outcomes.

2. Mechanisms of TCZ in RA Treatment

Tocilizumab (TCZ) is a monoclonal antibody that plays a crucial role in inhibiting the interleukin-6 (IL-6) signaling pathway. Its therapeutic efficacy in RA is primarily due to its ability to block IL-6 signaling through two distinct pathways: classical (cis) signaling and trans-signaling [8, 10]. In classical (cis) signaling, IL-6 binds to membrane-bound IL-6 receptor (IL-6R) on specific cells such as T cells, B cells and dendritic cells. On the other hand, trans-signaling involves the soluble form of IL-6R (sIL-6R), which circulates in bodily fluids, enabling IL-6 to activate cells that lack membrane-bound IL-6R, such as endothelial cells and synoviocytes [10]. This expands the range of IL-6-responsive cells and significantly contributes to the chronic inflammation observed in RA. SIL-6R can be generated by limited proteolysis of membrane-bound IL-6R or alternative mRNA splicing [10]. Furthermore, the combination of IL-6 and sIL-6R IL-6 and sIL-6R together induce tubule formation in fibroblast-like synovial cells from RA patients—an effect that is not observed with IL-6 alone or with TNF-α [11].

TCZ can inhibit both classical (cis) and trans-signaling pathways by inhibiting IL-6R, resulting in the suppression of downstream inflammatory signaling, particularly through the inhibition of the JAK/STAT3 pathway. This inhibition blocks the activation of pro-inflammatory cytokines, especially IL-6 and IFN-γ, which reduces immune cell recruitment and cytokine production. Additionally, it helps prevent bone erosion by decreasing RANKL expression and osteoclast activity. Moreover, TCZ has been shown to modulate other inflammatory pathways, including the MAPK and PI3K/Akt pathways, which are involved in inflammation, synovial fibroblast activation, and joint destruction in

RA [12]. These combined actions help to mitigate IL-6-driven inflammation, a key feature of RA pathogenesis, leading to improved clinical outcomes in patients.

The extracellular region of the IL-6R consists of three domains: D1, D2, and D3. IL-6 binds to a site between the D2 and D3 domains, facilitating the formation of a hexameric signaling complex composed of IL-6, IL-6R, and the gp130 co-receptor [13]. TCZ functions as a competitive antagonist by blocking IL-6/IL-6R signaling, primarily through competition for the IL-6 binding site located on the D3 domain of IL-6R [13]. Furthermore, the binding of TCZ to the D3 domain induces steric clashes with the D2 domain, disrupting its structural positioning and effectively preventing IL-6 from binding to the receptor complex [13]. Hence, not only blocks the formation of the IL-6/IL-6R/gp130 complex but also inhibits the subsequent inflammatory signaling cascade.

However, despite these detailed observations of TCZ's effects on IL-6R, limitations remain in the current models. Existing Fab/antigen structural data reveal that a significant portion of IL-6R is missing, limiting a full understanding of the receptor's role in TCZ's therapeutic action [13]. Since TCZ specifically targets a defined surface on the D3 domain, further exploration of other potential IL-6R epitopes is necessary to evaluate their contribution to its overall efficacy. This may provide clearer insights into the full mechanisms of TCZ's interaction with IL-6R and inform improvements in its structural applications.

3. TCZ Treatment and miRNA

TCZ influences RA pathogenesis by modulating miRNA expression and inflammatory signaling pathways, thus reducing inflammation in RA patients. TCZ plays a role in regulating miRNA expression and reducing inflammation in RA neutrophils. Inflammatory mediators, such as IL-6, will lead to miRNA downregulation, as evidenced by the reduced levels of miR-126, let-7b, and miR-17, along with AGO1 and AGO2 [14]. AGO1 and AGO2 are key components of the RNA-induced silencing complex (RISC), which regulates gene expression through miRNA-mediated post-transcriptional repression. In rheumatoid arthritis (RA), their dysfunction affects several downstream processes, including immune cell activation, synovial fibroblast proliferation, cytokine production, and osteoclast differentiation, contributing to the progression of inflammation and joint destruction. TCZ treatment in RA patients reduces this inflammatory effect by upregulating miR-148a and decreasing the expression of inflammatory genes such as IL-6R and STAT3 [14]. Additionally, TCZ reduces TNF-α, IL-8, and IL-1β release in RA neutrophils, further decreasing inflammation and highlighting the therapeutic potential of TCZ in limiting the inflammatory processes of RA [14]. However, while TNF-α inhibition by Infliximab (IFX) restores global miRNA expression and enhances miRNA processing, TCZ's impact is more selective, primarily affecting miR-148a [14].

TCZ influenced the serum expression of miR-146a-5p and miR-150-5p, both of which are linked to angiogenesis in RA. Angiogenesis is a critical process that supports inflammation by providing nutrients and oxygen to immune cells and proliferating synoviocytes, although its regulation remains incompletely understood [15]. TCZ treatment affects miR-146a-5p expression differently in serum and in vitro models, indicating a nuanced effect. In a study that examined nine angiogenesis-associated miRNAs (miR-146a-5p, miR-150-5p, miR-155-5p, etc.) in RA patients before and after TCZ treatment, miR-146a-5p and miR-150-5p were the only ones significantly increased after four months of treatment, although their levels did not differ between treatment responders and non-responders [15]. Further analysis revealed that upregulated miR-146a-5p leads to the downregulation of EMMPRIN (a pro-angiogenic factor) expression in the serum of TCZ-treated RA patients, which in turn modulates key angiogenic factors like VEGF and MMP-9 [15].

Conversely, TCZ treatment downregulates miR-146a-5p levels and upregulates EMMPRIN expression in fibroblast-monocyte co-culture models, suggesting a cell-type-dependent effect. In this context, TCZ treatment led to increased secretion of EMMPRIN, VEGF, and MMP-9 while reducing Tsp-1 levels, thus shifting the balance toward a pro-angiogenic environment [15]. TCZ administration at 500 mg/ml suppressed miR-146a-5p expression specifically in HT1080 cells but not in U937 cells

or primary monocytes [15]. This reduction was further analysed by NF-κB and JAK/STAT inhibitors, leading to increased EMMPRIN secretion, suggesting that these pathways are directly involved in miR-146a-5p regulation during TCZ treatment [15]. Stimulation with TNF-α or IL-6 enhanced EMMPRIN secretion and further reduced miR-146a-5p levels in HT1080 cells, with TCZ amplifying this effect [15]. Functional assays confirmed that miR-146a-5p directly modulated EMMPRIN, as the inhibition of miR-146a-5p increased EMMPRIN secretion, while its overexpression had the opposite effect [15].

The inconsistent results between the serum of RA patients and the in vitro co-culture cell model may be explained by the fact that serum reflects the state of synovial joints, where interactions among multiple cell types influence miR-146a-5p levels and subsequently affect EMMPRIN expression [5]. In conclusion, TCZ has been confirmed to regulate miR-146a-5p expression, hence modulating EMMPRIN expression and contributing to the restriction of angiogenic potential in RA patients.

4. miRNA Regulation of Other RA Treatment

DMARDs, including TCZ mentioned earlier, play a significant role both as biomarkers and potential therapeutic agents, having a broad regulatory effect on miRNA in RA treatment. For instance, TNF inhibitors have been found to upregulate miR-27a and miR-886 while downregulating miR-22, miR-23, and miR-223 [16].

Methotrexate (MTX), a folic acid antagonist, serves as an "anchor drug" in conventional DMARD therapy for RA [16]. MTX treatment has been shown to alter the global miRNA expression profile in rheumatoid arthritis fibroblast-like synovial cells (RA-FLSs), with consistent upregulation of miR-877-3p [5]. In RA-FLSs, MTX treatment leads to significant changes in miRNA expression, including an increase in miR-877-3p, as demonstrated by quantitative RT-PCR and miRNA microarray analysis [17]. Among the differentially expressed miRNAs, miR-877-3p was consistently upregulated by MTX, with a 1.784 ± 1.209-fold increase ($p < 0.05$) [17]. This upregulation suggests that MTX's therapeutic effects may be mediated by suppressing key cytokines and chemokines involved in RA pathogenesis [17]. Notably, the production of Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) and CCL3 (MIP-1α, Macrophage Inflammatory Protein-1 alpha) was significantly reduced in pre-miR-877-3p-transfected RA-FLSs, both at the protein and mRNA levels, indicating that miR-877-3p modulates inflammatory responses in RA [17]. The downregulation of GM-CSF and CCL3, which are known to exacerbate RA, supports the potential of miR-877-3p as a therapeutic target for influencing RA-FLS behavior and improving treatment outcomes [17]. Additionally, overexpression of miR-877-3p reduced RA-FLS migration and decreased MKI67 expression, a marker of cell proliferation [17]. In conclusion, miR-877-3p plays a key role in mediating MTX's anti-inflammatory and anti-migratory effects, likely by targeting multiple signaling pathways involved in immune regulation and cellular responses. These findings suggest that miR-877-3p may act as a downstream effector of MTX in RA therapy.

Furthermore, analyzing miRNA regulation across different RA treatments can provide valuable insights into their mechanisms, contributing to the development of miRNA-based therapies and helping to predict therapeutic outcomes, such as in MTX treatment. SNPs affecting miRNA function can alter drug responsiveness. For example, the SNP (829C→T) in the miR-24 binding site of dehydrofolate reductase (DHFR) disrupts miR-24, causing DHFR overexpression and leading to MTX resistance [7]. Identifying such genetic variations could help predict MTX unresponsiveness, guiding more personalized treatment approaches.

5. miRNA Deliveries of miRNA-Based Therapies

The involvement of miRNAs in RA shows their potential in related drug therapies. Many studies suggest miRNAs are expressed differently in RA, but miRNA-based treatments are still in the early stages of discovery. Turning these findings into practical clinical use for RA is still difficult.

Alongside the major difficulties of the need for research on more miRNA functions and downstream pathways related to many symptoms of RA, another main criterion that needs to be considered is miRNA delivery methods. Offering bare miRNA is difficult since, in body fluids, RNases can quickly degrade, preventing them from reaching target sites [5]. MiRNAs are also hard to pass through cell membranes due to their large molecular size and strong anionic charge [5]. Additionally, using miRNAs in treatments can cause off-target effects. A single miRNA can affect RA differently depending on where it is expressed.

MiRNAs control gene expression by binding and silencing specific mRNAs, resulting in the regulation of cellular functions. MiRNA-based therapies aim to regulate gene expression related to RA by silencing downstream pro-inflammatory miRNAs and increasing downstream anti-inflammatory ones. Clinical trials mostly use miRNA inhibitors and mimics. Inhibitors are single-stranded RNAs that bind to miRNAs, while mimics are synthetic double-stranded RNAs that boost miRNA expression. Modified miRNAs are often used in studies to improve their stability and uptake. For example, by injecting modified miR-708-5p mimics into rats with CIA, the arthritis scores are improved as well as reduced ankle swelling [18]. However, injecting miR-145-5p mimics led to worse joint damage in CIA mice [19].

Systemic delivery of miRNAs is unlikely to be effective because miRNAs tend to accumulate in the liver and are then cleared by the kidneys. Local delivery works better for RA treatment since it maintains higher miRNA concentrations in the inflamed joints, reducing the need for frequent injections. For example, injecting miR-132 antagomir into AIA mice reduced osteoclast numbers in the joints [20]. Similarly, injecting miR-141-3p agomir into CIA rats reduced inflammation and cartilage damage [21].

Lentiviruses are RNA viruses that integrate into the host genome, making them effective vectors for gene transfer [5]. Lentiviruses can deliver miRNAs to different tissues, making them a possible approach for RA therapy [5]. Studies have shown that injecting lentiviral vectors carrying miR-204-5p or miR-140 into CIA (collagen-induced arthritis) mice through in vivo gene transfer can reduce inflammation and synovial damage [22, 23]. However, this method still faces challenges, including off-target effects, innate immune responses, and disruptions to endogenous miRNA signaling cascades [5]. Further research is needed to optimize it for clinical application.

Exosomes can naturally deliver miRNAs to target cells, with those derived from mesenchymal stem cells (MSCs) being good targets for RA therapy [5]. Exosomes from BMSCs carrying miR-192-5p, miR-21, and miR-223 have been shown to reduce inflammation and osteoclast activity in RA models [24-26]. MSC-derived exosomes can also be engineered to enhance their targeting, stability, and therapeutic effects. Modifying exosomal surfaces to target macrophages in RA has improved miRNA delivery and resulting in disease remission [5]. However, challenges remain, including safety concerns, efficacy issues, poor biodistribution, dosing difficulties, and administration methods [5]. Further research is also needed to improve the isolation and purification of exosomes.

Another promising route for miRNA delivery in RA treatment involves synthetic materials, such as liposomes and nanoparticles. Liposomes perform well in vitro due to their low immunogenicity but have shown toxicity in vivo [5, 27]. However, cationic lipid-based vectors like PEGylated polyethylenimine (PEI) have demonstrated greater potential for delivering miRNAs to inflamed tissues in RA models, leading to reduced disease progression [28]. MiRNA-loaded nanoparticles, including poly(lactide-co-glycolide) (PLGA) NPs and poly (cyclohexane-1, 4-diylacetone dimethylene ketal) (PCADK), have also been used to deliver miR-124 and other treatments, significantly improving therapeutic efficacy [29]. The use of cell membrane-penetrating peptides (CADY) and silica nanoparticles (MSN) allows for more precise targeting of RA-FLSs [30]. While these approaches show promise for miRNA delivery and have potential for miRNA-based RA treatment, further research is needed to overcome the remaining challenges.

6. Conclusion

TCZ, as a biologic DMARD targeting the IL-6 receptor, influences inflammatory pathways in RA progression. This study explores the role of miRNAs in RA regulation and their potential to improve TCZ-based treatments, such as miR-146a-5p and miR-150-5p, both of which are the most important miRNAs influenced by TCZ therapy. These miRNAs, in turn, impact cytokine production, immune cell activation, and angiogenesis. This study also presents miRNAs as a promising approach to improving RA treatment outcomes, particularly for patients resistant to conventional therapies. As a result, modulating the global miRNA expression profile could complement current RA treatments, including TCZ and other RA treatments such as MTX.

However, challenges remain in turning these findings into clinical practice, particularly the delivery method for miRNA-based therapy and the complex regulation and interaction mechanisms of miRNAs across different RA treatments. Further investigation is necessary to understand how miRNA modulation affects RA pathogenesis at the cellular and molecular levels, particularly within various immune cell types and their interactions during each stage of RA progression. While this study focuses on TCZ, other biologics and conventional DMARDs remain underexplored in terms of their impact on miRNA regulation. There is also a lack of longitudinal data that limits understanding of the long-term effects of TCZ on global miRNA influences and downstream pathways, as well as the long-term effects of using miRNA-based treatments in RA. Future research should expand the miRNA expression profile in RA, taking into account other treatments and genetic variations affecting miRNA function, such as the MTX unresponsiveness mentioned earlier.

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