Heliyon 11 (2025) e41983

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Review article

Exosomal miRNAs involvement in pathogenesis, diagnosis, and treatment of rheumatoid arthritis

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ARTICLE INFO

Keywords:
Rheumatoid arthritis
Exosome
miRNA
Exosomal miRNA

ABSTRACT

Rheumatoid arthritis (RA) is the most common chronic autoimmune arthropathy worldwide. The initiation, and progression of RA involves multiple cellular and molecular pathways, and biological interactions. Micro RNAs (miRNAs) are characterized as a class of small non-coding RNAs that influence gene expression at the post-transcriptional level. Exosomes are biological nanovesicles that are secreted by different types of cells. They facilitate communication and signalling between cells by transferring a variety of biological substances, such as proteins, lipids, and nucleic acids like mRNA and miRNA. Exosomal miRNAs were shown to be involved in normal and pathological conditions. In RA, deregulated exosomal miRNA expression was observed to be involved in the intercellular communication between synovial cells, and inflammatory or regulatory immune cells. Furthermore, circulating exosomal miRNAs were introduced as available diagnostic and prognostic biomarkers for RA pathology. The current review categorized and summarized dysregulated pathologically involved and circulating exosomal miRNAs in the context of RA. It highlighted present situation and future perspective of using exosomal miRNAs as biomarkers and a specific gene therapy approach for RA treatment.

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by joint inflammation, and the presence of autoantibodies. Progressive disability and a diminished quality of life may be the outcome of RA in the absence of treatment [1]. MicroRNAs (miRNAs) are implicated in the diagnosis, prognosis, and monitoring of RA, according to a variety of lines of evidence. MiRNAs are a class of small, single-stranded RNAs. They control gene expression by binding to target messenger RNA (mRNA). Besides, these

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https://doi.org/10.1016/j.heliyon.2025.e41983
Received 21 March 2024; Received in revised form 18 October 2024; Accepted 14 January 2025
Available online 15 January 2025

Abbreviations

3′-UTR	3′-untranslated region
ACPA	anti-citrullinated protein antibody
AHR	aryl hydrocarbon receptor
BMP	bone morphogenetic protein
CIA	collagen-induced arthritis
COX2	cyclooxygenase 2
CR	clinical remission
CRP	C-reactive protein
DMARD	disease-modifying anti-rheumatic drugs
DKK2	Dickkopf associated protein 2
ESCRT	endosomal sorting complex required for transport
ESR	erythrocyte sedimentation rate
Esr1	estrogen receptor alpha
EV	extracellular vesicle
FLS	fibroblast-like synoviocyte
FoxP3	forkhead box protein 3
HLA	human leukocyte antigen
HUVEC	human umbilical vein endothelial cells
IL	interleukin
JAK/STAT	Janus kinase/signal transducer and activator of transcription
lncRNA	long non coding RNA
LPS	lipopolysaccharide
mRNA	messenger RNA
miRNA	microRNA
MMP	matrix metallopeptidase
MSC	mesenchymal stem cell
MVB	multi-vesicular bodies
NDRG2	N-myc downstream-regulated gene 2
NFATc1	transcription factor nuclear factor of activated T cell 1
NF-κB	nuclear factor kappa B
NSAID	non-steroidal anti-inflammatory drugs
OPG	osteoprotegerin
PDK4	pyruvate dehydrogenase kinase isozyme 4
PsA	psoriatic arthritis
RA	rheumatoid arthritis
RANKL	receptor activator of nuclear factor kappa-B ligand
RF	rheumatoid factor
RISC	RNA-induced silencing complex
RUNX2	Runt-related transcription factor 2
SLE	systemic lupus erythematosus
SMAD	suppressor of mothers against decapentaplegic
TCF4	transcription factor 4
TGFBR II	transforming growth factor-beta receptor II
Th	helper T cell
TLR	toll-like receptor
TNF-α	tumor necrosis factor-alpha
TOB1	transducer of erb-b2 receptor tyrosine kinase 1
Treg	regulatory T cell
TWEAK	TNF-like weak inducer of apoptosis
VEGF	vascular endothelial growth factor
UC	ulcerative colitis

molecules are involved in various processes. These include growth, differentiation, apoptosis, and response to stress [2,3]. On the other hand, exosomes were implicated in the development and progression of inflammatory diseases, such as RA. Exosomes are nano-sized vesicles derived from endosomes inside cells. Depending on the cell of origin, exosomes contain a diverse array of components, including proteins, lipids, metabolites, DNA, and a variety of ribonucleic acids, including mRNA. Exosomes act as intercellular messengers, and play a key role in local and systemic communication between different cells [4,5]. We aim to discuss the dysregulated

and circulating exosomal miRNAs in RA condition, and present the current possibility and future perspective of using exosomal miRNAs as biomarkers and as specific target for gene therapy in RA treatment.

1.1. RA, immunopathogenesis and therapeutic approaches

RA is defined as an autoimmune disease affecting joints, cartilage and bone. Patients experience joint deterioration, pain, swelling and stiffness that reduces joint function, causes bone deformity and significantly affects their quality of life [6,7]. The generation of autoantibodies and the influx and buildup of immune cells in the joints are the causes of RA's systemic involvement [8]. Although exact pathogenic cause of RA is not clearly understood, the development of RA is strongly associated with genetic, epigenetic, and environmental factors [9,10].

Several cellular and molecular mechanisms were identified that induce the formation of synovitis and the eventual progression of disease to a destructive state [11]. In synovial tissue, interactions between innate and acquired immune cells, vascular endothelial cells, fibroblast-like synoviocytes (FLS) and osteoclasts create an inflammatory microenvironment characterized by increased levels of pro-inflammatory cytokines, including interleukin (IL)-1β, IL-6, IL-8, IL-17 and tumor necrosis factor-alpha (TNF-α). These cytokines are accountable for the occurrence inflammation and the deterioration of joints. This localized inflammatory process also impacts peripheral bone structures [12,13]. The primary contributors to inflammation in the joints are $CD4^+$ T cells and the monocytes/macrophages, which are among the immune cell types that have gathered there [14]. Imbalance in $CD4^+$ T cell subpopulations, particularly in helper T (Th)17/regulatory T cell (Treg) arm; with predominance of Th17 cells and associated inflammatory cytokines such as IL-17A, IL-17F and IL-22, and suppression of Treg differentiation and reduced levels of anti-inflammatory cytokines; is an important pathway in the pathogenesis of RA [15–17]. T cells are effective in the activation, differentiation and stimulation of B cell responses, and consequently the production of rheumatoid factor (RF), anti-citrullinated protein antibodies (ACPA), and other RA-related autoantibodies [18–20]. In other words, the inflammatory T cells disrupt both cellular and humoral immune responses. As a result, the autoimmune condition of RA is initiated [21–23]. A deregulated M1/M2 macrophage ratio with M1 dominance is another factor contributing to the development of synovitis [24,25]. Hyperproliferation of FLS results in an increase in the production of IL-6, CXCL8 and receptor activator of nuclear factor kappa-B ligand (RANKL) pro-inflammatory mediators, which contribute to the accumulation of inflammatory cells in the joints, synovial hyperplasia, and the pathological processes of rheumatoid arthritis [26]. In general, the presence of autoantibodies, inflammatory cytokines and RANKL leads to the synovial inflammation and the differentiation of monocytes into osteoclasts, triggering bone destruction [27].

Advances in understanding molecular and cellular mechanisms underlying RA have led to the development of novel treatments that focus on precise molecular targets [28]. The clinical management of patients with RA evolved from simply reducing symptoms and inflammation to actively suppressing inflammation. The primary therapeutic approach for RA consists of corticosteroids, non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs (DMARDs) and novel biologic agents that target specific biological pathways [29–31].

1.2. miRNAs biogenesis, mechanism of action, and biological importance

MiRNAs are a class of small, single-stranded RNAs that regulate the expression of genes by binding to mRNA [2]. Besides, miRNAs are involved in growth, differentiation, apoptosis and stress responses. Additionally, they are released into fluids outside of cells and transported to other cells by vesicles and exosomes, functioning as messengers between cells [32–34]. The biogenesis of miRNAs happens via two distinct mechanisms, namely the canonical pathway and the non-canonical pathway. The modulation of gene expression by miRNAs occurs via various mechanisms, including mRNA degradation, translational repression, transcriptional activation or inhibition, and chromatin remodeling. For mRNA degradation, miRNAs integrate into RNA-induced silencing complex (RISC), thereby facilitating their role in gene silencing. In addition, miRNAs specifically bind to complementary sequences in 3′-untranslated regions (3′-UTR) of target mRNAs, leading to their destruction to inhibit specific protein production [14,28,35].

MiRNAs are highly conserved across species which are critically involved in human health and disease. Indeed, miRNAs are critical regulators of the immune response, affecting the proliferation and activation of immune cells via a complex regulatory network. As a result, dysregulation or disturbance of miRNAs has been identified in a variety of disorders, including cancer, cardiovascular, inflammatory, and autoimmune diseases, making them promising targets as diagnostic biomarkers and therapeutics [36–40]. Several lines of evidence highlighted the relationship between miRNA expression patterns, and autoimmune diseases [41,42]. For example, in the patients diagnosed with RA, miR-146a and miR-155 are consistently upregulated in various cells and tissues of patients compared to normal individuals and osteoarthritis patients [3,43]. Mechanistically, the involvement of extracellular miRNAs in the pathogenesis of RA was described in several ways. One mechanism is the involvement of Toll-like receptor (TLR) 7/8 in a miRNA-dependent manner. TLR7/8 induces joint inflammation, activates dendritic cells and promotes osteoclast differentiation. In this situation, TLR7/8 inhibitors, which are presently utilized in systemic lupus erythematosus, might be employed as a treatment strategy for RA that involves miRNA-mediated pathways [44–46]. Therefore, miRNAs are present in various body fluids and biological secretions ranging from serum, plasma and urine to saliva and cerebrospinal fluid. These molecules are therefore being introduced as potential biomarkers for the diagnosis, prognosis and monitoring of autoimmune diseases, as well as therapeutic targets or drugs to modulate immune responses and restore tolerance [47–49].

1.3. Exosome biogenesis and its biological relevance

Extracellular vesicles (EVs) are a family of small vesicles released by cells, including exosomes, microvesicles and apoptotic bodies, which are classified on the basis of their size and biogenesis [50–52]. Exosomes are nano-sized vesicles, typically 40–160 nm in diameter, which are derived from endosomes within cells. Different cell types discharge these nano-sized biological into the extracellular space and biological fluids, where they are encased in a bilayer formed from the cell membrane [53–55]. Exosomes are formed by inward budding of phospholipid bilayer within multivesicular bodies (MVBs), which are derived from endosomes. Once formed, exosomes are then released from the cell by two mechanisms: an endosomal sorting complex required for transport (ESCRT) protein-dependent mechanism or an ESCRT-independent mechanism [56–59]. Exosomes contain a wide range of components, including proteins, lipids, metabolites, DNA and a variety of ribonucleic acids such as mRNA, small non-coding and long non-coding RNAs (lncRNA), which vary depending on the cell of origin. The specific composition of exosomes can be affected by cellular source and various factors such as the physiological or pathological state of the cell and its surrounding environment [60–65].

Exosomes act as intercellular messengers by regulating various biological processes that are important for local and systemic communication held among cells. These entities have undergone thorough examination and have been linked to the emergence and advancement of inflammatory conditions such as rheumatoid arthritis, diabetes, inflammatory bowel disease, neurological illnesses, and many forms of cancer [66–68]. Recent studies provided valuable insights into the importance of dysregulated expression of exosomal miRNAs in disease pathogenesis [69,70]. In the case of RA, a higher abundance of exosomes in the circulation and synovial fluid of RA patients than healthy controls was observed and highlighted in several studies [71]. Although, specific origin of these exosomes is not fully understood, studies suggest that immune cells and local stromal and tissue cells may contribute to the production of exosomes [1,72–74].

2. Exosomal miRNAs and RA

The importance of exosomal miRNAs in RA is considerable in several aspects; biomarker value of circulating exosomal miRNAs for RA diagnosis, prognosis, and treatment response monitoring; interactions among different cells involved in RA pathology such as FLS, chondrocytes, and inflammatory cells via secreted exosomal miRNAs; and finally, delivery of exosomal miRNAs as gene therapy strategies for RA [75]. Exosomal miRNAs are more specialized for intercellular communication, more durable and protected, and more selective and sensitive as biomarkers than free extracellular miRNAs [76]. This review summarized and highlights the pathophysiological, diagnostic and therapeutic roles of exosomal miRNAs in RA. As exosomes are released from various cells and are of great importance in the immune response and pathogenesis of RA, exosomal miRNAs may be involved in the pathogenesis and related biological processes [77]. Therefore, the transfer of biologically active miRNA species between cells by exosomes potentially causes the repression or overexpression of target genes that alter responses involved in maintaining balance and suppressing of inflammatory and pathogenic responses [78].

2.1. Interaction between synovial and immune cells via exosomal miRNAs

FLSs are among the key orchestrators in the pathogenesis of RA. Deregulated activation and proliferation of FLSs leads to the production of inflammatory mediators such as CXCL8, IL-6, and IL-15, adhesion molecules and matrix proteins, such as fibronectin, which potentially exacerbate leukocyte activation and recruitment to synovial regions [79–82]. Exosomal miRNAs released from FLSs under inflammatory and pathological conditions are other mediators actively involved in the inflammatory processes of RA. Disturbances in miRNA expression in the FLS of RA patients are reflected in the secreted exosomes. For instance, the miR-146a-5p, miR-155-5p, miR-323a-5p, and miR-1307-3p are four miRNAs that are recognized as being significant in the development of RA. They were discovered to be upregulated in exosomes derived from FLS and after FLS stimulation with the inflammatory cytokine TNF-α [83,84]. It was predicted that miR-232a-5p targets CD6 and influences T-cell activation signaling, while miR-1307-3p targets N-myc downstream-regulated gene 2 (NDRG2) and inhibits the expression of genes associated with osteoclasts [83,85]. The miR-221-3p is an upregulated miRNA in FLSs-derived exosomes under stimulation with TNF-α *in vitro*, which is involved in bone destruction in RA patients [86]. Similar stimulation by TNF-α under *in vivo* conditions occurs through autocrine effects of membrane-bound TNF-α released by FLSs in the autoimmune microenvironment of RA [87,88]. Overexpressed miR-221-3p targets Wnt and bone morphogenetic protein (BMP) signaling pathways in synovial tissue and suppresses osteoblast differentiation and mineralization. The inhibition of bone production results in the disruption of the normal process of osteoblast development. MiR-221-3p is expected to cause bone degradation and decreased bone ossification in RA erosions by targeting many anabolic genes, including Runt-related transcription factor 2 (RUNX2), transcription factor 4 (TCF4), estrogen receptor alpha (Esr1) and Dickkopf associated protein 2 (DKK2) [88].

FLS-derived exosomal miR-424 is another overexpressed miRNA that exacerbates the inflammatory state of RA. Treg/Th17 balance is regulated by miR-424. Ding et al. showed that the hypoxic environment of synovium in RA patients increased miR-424 expression in FLSs and secreted exosomes, inhibited Treg differentiation, increased the Th17 population, and disrupted the Th17/Treg balance towards an enhanced inflammatory state. Hence, miR-424 targets forkhead box protein 3 (FoxP3) and exerts the aforementioned effects, and exosomal miR-424 knockdown effectively suppresses RA exacerbation [89].

Pyruvate dehydrogenase kinase isozyme 4 (PDK4) normally regulates RANKL/RANK/osteoprotegerin (OPG) signaling pathway and induces chondrocyte proliferation and migration. The miR-106b could directly target PDK4 and potentially suppress chondrocyte proliferation, and migration and induce apoptosis. The miR-106b overexpressed in FLSs was demonstrated in an animal model of

collagen-induced arthritis (CIA) to be transported to chondrocytes via exosomes and accelerated chondrocyte death via modulating the aforesaid pathway. *In vivo* studies confirmed the role of FLS-derived exosomal miR-106b in RA progression, while miR-106b inhibition ameliorated CIA-induced inflammatory responses [90].

Osteoclast differentiation, proliferation, and mineralization are a mechanism that was shown to be inhibited during RA pathogenesis, leading to exacerbation and disease progression. Investigation of interactions between FLSs and osteoblasts in RA patients and CIA animal models revealed that FLS-derived exosomal miR-485-5p potentially promotes osteoblast differentiation and alleviates inflammatory symptoms, thereby ameliorating disease severity along with accelerating repair. Mechanistically, overexpressed miR-485-5p targets and represses transducer of erb-b2 receptor tyrosine kinase 1 (TOB1), which leads to upregulation of BMP/suppressor of mothers against decapentaplegic (SMAD) pathway. Therefore, it seems that FLS-derived exosomal miR-486-5p could potentially be introduced as a promising target for therapeutic approaches that would be beneficial for RA patients [91].

Studying the interactions between immune cells and synovial cells via exosomal miRNAs is important for elucidating the mechanisms are involved in RA pathogenesis and for designing therapeutic approaches. It was discovered that the intercellular package exosomal miR-204-5p moved from T cells to FLS. The overexpression of miR-204-5p in FLSs targets genes linked to invasion and proliferation of cells. Therefore, miR-204-5p transfer by exosomal cargo could efficiently suppress FLS proliferation, activation, invasion, and inflammatory cytokine production. Therefore, it was shown that lentiviral-mediated delivery of miR-204-5p could effectively attenuate disease progression in the CIA mouse model [92].

On the other hand, exosomal miR-132 released from Th17 cells promotes the differentiation of osteoclast precursors into mature cells, and local miR-132 knockdown could potentially suppress osteoclastogenesis in the arthritic joints as a therapeutic approach. Cyclooxygenase 2 (COX2) is the major downregulated target of miR-132 for the induction of osteoclast differentiation. Besides, cigarette smoke was identified as an inducer of miR-132 overexpression in RA patients via the agonistic AHR signaling pathway [93]. Furthermore, by the exosomal production of miR-506-3p and miR-103a, which block the RANKL/transcription factor nuclear factor of activated T cell 1 (NFATc1) signaling pathway and promote the Janus kinase/signal transducer and activator of transcription 3 (JAK/STAT3) signaling pathway, macrophages play a significant role in regulating the course of RA [94,95]. Fig. 1 and Table .1 provided the summary of exosomal miRNAs involved in interactions between synovial and immune cells in the context of RA.

2.2. *Circulating exosomal miRNAs as diagnostic biomarkers*

Various cell types release exosomal miRNAs to establish intercellular communication and to regulate metabolism and immune responses [96–98]. Circulating exosomal miRNAs are protected from RNase degradation by bilayer lipid membrane of exosomes and circulate stably in body fluids, thus their expression could be easily measured, and introduced as potent specific biomarkers for physiological and various pathological conditions [14,99]. Recent studies showed that a sorting sequence in miRNAs could determine whether a cellular miRNA sorted into exosomes to be engaged in intercellular communications or retained in the cells. In this case, different cells use specific sequences for sorting issues and this could be implicated to define the exosomal miRNAs profile of cells and even differentiate the source of circulating exosomal miRNAs [97]. The significance of early diagnosis in the context of debilitating diseases like RA has been underscored in order to create therapeutic strategies that can reverse the progression of the disease and prevent disability outcomes. Regarding low sensitivity and specificity of RF and ACPA indices, the main diagnostic approaches for RA are limited to history, clinical symptoms and radiological examinations. Therefore, circulating exosomal miRNAs were highlighted as

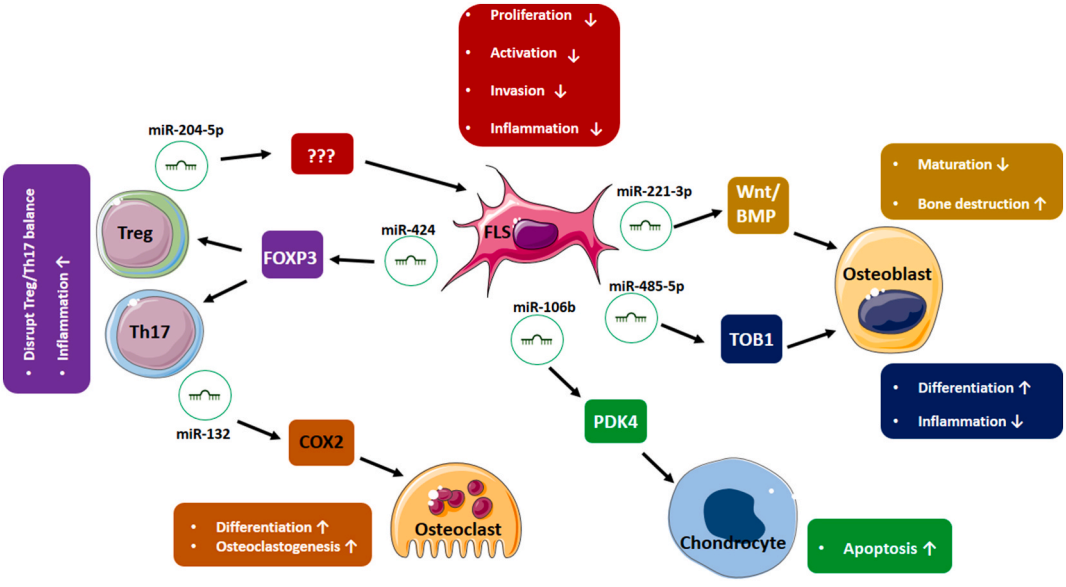

Fig. 1. Exosomal miRNAs are mediators of the interactions between synovial and immune cells in RA.

Table 1
Summary of exosomal miRNAs involved in synovial-immune cells interactions in RA.

Type of exosomal miRNA	Source of exosomes	Targets	Importance and role	Ref.
miR-221-3p	FLS	Wnt/BMP, RUNX2, TCF4, Esr1, and DKK2	Osteoblast maturation ↓ Bone destruction and erosion ↑	[86–88]
miR-424	FLS	FoxP3	Disrupt Treg/Th17 balance Inflammation ↑	[89]
miR-106b	FLS	PDK4	Chondrocyte apoptosis ↑	[90]
miR-485-5p	FLS	TOB1	Osteoblast differentiation ↑ Inflammatory responses ↓	[91]
miR-204-5p	Treg	–	FLS proliferation, activation, invasion, and inflammatory cytokine production ↓	[92,93]
miR-132	Th17	COX2	Osteoclast differentiation ↑ Osteoclastogenesis ↑	[93]
miR-506-3p	Macrophage	RANKL/NFATc	RA progression ↑	[94,95]

Abbreviations; **BMP,** bone morphogenetic protein; **COX2,** cyclooxygenase 2; **DKK2,** Dickkopf associated protein 2; **Esr1,** estrogen receptor alpha; **FLS,** fibroblast-like synoviocyte; **FoxP3,** forkhead box protein 3; **miRNA,** microRNA; **NFATc1,** transcription factor nuclear factor of activated T cell 1; **PDK4,** pyruvate dehydrogenase kinase isozyme 4; **RA,** rheumatoid arthritis; **RANKL,** receptor activator of nuclear factor kappa-B ligand; **RUNX2,** Runt-related transcription factor 2; **TCF4,** transcription factor 4; **Th,** helper T cell; **TOB1,** transducer of erb-b2 receptor tyrosine kinase 1; **Treg,** regulatory T cell.

promising importance as indicators and biomarkers for prediction, early diagnosis, monitoring and management of RA patients [14] (Table .2).

Thus, a number of exosomal miRNAs are those that are decreased in the context of RA. Rodriguez-Muguruza et al. developed a plasma exosomal miRNA-based biomarker panel for untreated primary RA patients, including miR-25-3p, miR-451a, and soluble TNF-like weak inducer of apoptosis (TWEAK) as an inflammatory mediator associated with RA pathophysiology, as a much more valid diagnostic method than positive ACPA [100]. Several lines of evidence showed an altered expression of plasma exosomal miRNAs in RA patients compared to healthy individuals. For instance, Chen et al. discovered 36 exosomal miRNAs and identified five of them as miRNAs linked to the pathophysiology of RA: miR-151a-3p, miR-199a-5p, miR-370-3p, miR-589-5p and miR-769-5p [101]. Fourteen dysregulated exosomal miRNAs were found in another extensive investigation, most of which included RA patients. Reduced exosomal miR-204a-5p was further confirmed in the mouse CIA model, as well as in replication and validation groups with large and independent sample numbers. Further studies showed that plasma exosomal miR-204a-5p expression was negatively correlated with RA-associated inflammatory parameters, including RF, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) [92]. The miR-548a-3p and miR-6089 are two downregulated exosomal miRNAs in the plasma of RA patients. Mechanistically, miR-548a-3p and miR-6089 have been shown to target TLR4 and regulate TLR4/NF-κB inflammatory signaling pathway. TLR4 is a critical component of the immune response and was implicated in inflammatory and autoimmune diseases such as RA [102,103]. In this case, it appears that exosomal miR-6089 could potentially suppress the production of lipopolysaccharide (LPS)-induced inflammatory mediators including, IL-6, IL-29, and TNF-α in the THP-1 cell line via regulation of TLR4 signaling [103,104]. Moreover, in addition to reduced levels of exosomal miR-548a-3p and miR-6089, a negative correlation between the expression of these exosomal miRNAs and levels of serum

Table 2
Summary of circulating exosomal miRNAs involved in RA pathogenesis or important as biomarker.

Type of circulatory exosomal miRNA	Importance and role	Ref.
miR-204a-5p	- Reduced expression in serum of RA and CIA model - Negative association with ESR, CRP, and RF parameters	[92]
miR-548a-3p miR-6089	- Regulating the TLR4/NF-κB inflammatory signaling pathway - Inhibiting the THP-1 macrophage activation and proliferation - Reduced expression in serum of patients - Negative association with ESR, CRP, and RF parameters	[102,103]
miR-150-5p	- Reduced levels in serum of RA patients - Targeting MMP14 and VEGF secretion and inhibiting angiogenesis	[105]
miR-10a-5p miR-19b miRNA-103a-3p miR-204-3p miR-330-3p	- Downregulated in RA patients - Negative association with oxidative stress and histone deacetylation	[1]
miR-17	- Increased in the plasma of RA patients - Suppressing TGFBR II and inhibiting Treg development	[106]
miR-1915–3p miR-6511b-5p	- Upregulated levels in CR-classified Korean RA patients - Negative correlation with CRP	[108]

Abbreviations; **CIA,** collagen-induced arthritis; **CR,** clinical remission; **CRP,** C-reactive protein; **ESR,** erythrocyte sedimentation rate; **MMP,** matrix metallopeptidase; **NF-κB,** nuclear factor kappa B; **RA,** rheumatoid arthritis; **RF,** rheumatoid factor; **TGFBR II,** transforming growth factor-beta receptor II; **TLR,** toll-like receptor; **VEGF,** vascular endothelial growth factor; **Treg,** regulatory T cell.

CRP, RF and ESR was shown in RA patients, suggesting circulating exosomal miR-548a-3p and miR-6089 as predictive biomarkers for disease development and severity [102,103]. Another miRNA that is downregulated in RA patients is circulating exosomal miR-150-5p. The miR-150-5p is physiologically implicated in the development and proliferation of T lymphocytes, as well as the suppression of matrix metallopeptidase 14 (MMP14) and vascular endothelial growth factor (VEGF) production in FLS. Therefore, decreased serum exosomal miR-150-5p in RA patients is associated with significantly higher MMP14 and VEGF secretion and angiogenesis rates and with a much more aggressive property of RA pathogenesis [105]. A recent study investigated the association between exosomal miRNAs and disease pathogenesis in RA patients. The results showed that miR-10a-5p, miR-19b, miRNA-103a-3p, miR-204-3p, and miR-330-3p are five downregulated circulating exosomal miRNAs in RA patients compared to healthy individuals. They observed a negative correlation between deregulated miRNAs and oxidative stress and histone deacetylation processes, which may be involved in RA initiation and progression [1].

The overexpressed exosomal miRNAs, which are linked to the severity of illness, are the opposite side of the circulating miRNAs. When comparing RA patients to healthy persons, it was shown that circulating blood miR-17 was one of the exosomal miRNAs that was elevated. Further investigation showed that increased circulating exosomal miR-17 is associated with a decrease in the peripheral Treg population. Mechanistically, miR-17 targets Treg differentiation by suppressing transforming growth factor-beta receptor II (TGFBR II) expression [106,107]. A recent study in Korea identified two plasma exosomal miRNA, miR-1915-3p and miR-6511b-5p, as important biomarkers of disease severity in RA patients. They studied the levels of circulating exosomal miRNA in the serum of clinical remission (CR) and non-CR patients and found that the expression of miR-1915-3p and miR-6511b-5p were differentially increased in CR group compared to non-CR patients. Furthermore, there is a negative correlation between elevated levels of miR-1915-3p and miR-6511b-5p with CRP. These two circulating exosomal miRNAs seem to have the potential to serve as distinguishing biomarkers between patients with CR and non-CR RA [108].

An important concern in using circulating exosomal miRNAs for RA diagnosis or monitoring is their specificity and discriminative value. For example, it was reported that the levels of circulating exosomal miR-22-3p, miR-24-3p, miR-96-5p, miR-134-5p, miR-140-3p, and miR-627-5p are upregulated in RA patients compared to healthy controls, but similar characteristics of RA and other autoimmune and inflammatory diseases, including systemic lupus erythematosus (SLE), lead to failure in discriminating RA from SLE [109]. Besides, Jin et al. introduced exosomal miR-124, miR-448, and miR-551b as specific biomarkers for RA diagnosis, but as expected, comparable levels were reported in the serum of SLE, Sjögren's syndrome, and ulcerative colitis (UC) patients [110]. Notably, hopeful results were reported. For example, recent studies reported higher expression of exosomal miR-16, miR-19b, miR-23a, miR-27a, miR-92a, and miR-223 in RA patients compared to SLE cases [111] or another valuable study reported the importance of serum exosomal miR-140 in differentiating RA from psoriatic arthritis (PsA) [112]. Collectively, using circulating exosomal miRNAs as diagnostic or pathology-differentiating biomarkers is affected by several factors. The identification of cellular source of exosomes and the heterogeneity of miRNAs and exosomal miRNA isolation techniques are two significant issues that should be carefully studies, in addition to the necessity for more thorough and repeatable research to optimize particular and reliable panels.

2.3. A novel mechanism in RA pathogenesis; TLR7/8 engagement by exosomal-miRNAs

TLRs are membrane-expressed pattern recognition innate immunity-associated receptors classified into two groups including; cell surface TLRs [1,2,4,5,and6]] and endosomal TLRs [3,7,8,and9]]. The contribution of TLR2/4 in RA pathogenesis was strongly emphasized in various clinical and experimental studies; however, the role of endosomal TLRs in RA is not well defined [113–115]. Recent research has demonstrated that exosomal miRNAs, which are released by cells into extracellular fluids, can bind to TLR7/8 without sequence specificity, thereby activating downstream signaling pathways that result in the production and secretion of pro-inflammatory mediators. This is due to the fact that endosomal TLR ligands are typically nucleic acid sequences [116,117]. In the context of RA, several guanosine/uridine (GU)-rich exosomal miRNAs in synovial fluid were implicated in TLR7/8 activation. Studies showed that local expression of miR-let-7b is involved in arthritic inflammation via the induction of M1 macrophage polarization. The mechanism is that exosomal GUUGUGU-rich miR-let-7b released into synovial fluid potentially binds to TLR7 on myeloid cells and activates downstream signaling pathway toward M1 macrophage polarization [46].

The other identified exosomal miRNA involved in RA pathogenesis via TLR7/8 ligation is miR-574-5p. ACPA + RA patients exhibited elevated levels of miR-574-5p in synovial fluid-derived exosomes, which were linked to a more severe and aggressive form of the disease. Mechanistic studies showed that exosomal miR-574-5p interaction with TLR7/8 mediates osteoclast maturation and increases bone destruction in terms of increased osteoclast maturation. Therefore, targeting miR-574-5p is proposed as a therapeutic strategy for RA patients, potentially alleviating the progressive destruction of RA by controlling osteoclastogenesis [45].

Another study investigated the role of exosomal miRNAs mediated TLR7/8 activation in RA pathogenesis via the activation of DCs. They stimulated human monocyte-derived DCs with a mixture of GU-rich miRNAs, including let7b, miR-574, miR-21, and miR-203, which are upregulated miRNAs in RA patients and found at high levels in synovial fluids. The results suggested that aforementioned miRNAs engaged TLR7/8, which in turn induced the production of TNF-α pro-inflammatory cytokine and the activation of NF-κB. Furthermore, they demonstrated a robust inhibition of these inflammatory responses through the use of Enpatoran, a TLR7/8 inhibitor, and CU-CPT9a, a specific TLR8 inhibitor [44].

2.4. Exosomal miRNA-based gene therapy approaches for RA

The main physiological function of miRNAs is mRNA silencing. The miRNA-based gene therapy strategies for RA involve using artificial miRNA mimics or miRNA inhibitors to control and regulate overexpressed pathological pathways resulting from deregulated

miRNA expression. In this instance, it is critical to comprehend the many roles that distinct miRNAs play in either pathogenic or preventive aspects of RA pathogenesis [118,119]. The inhibitors of miRNAs are single-stranded RNA molecules that complementarily bind and repress endogenous miRNAs, while miRNA mimics are synthetic miRNA sequences that may have chemical modifications to achieve the optimal stability, affinity, and cellular uptake [120]. When designing miRNA-based gene therapy approaches, two major challenges should be carefully considered: first, the selection of the best miRNA candidate whose function is clearly understood, and second, design of a vehicle for optimal and targeted delivery of miRNAs. The value of miRNA delivery systems lies in their ability to resist degradation while still reaching the target regions precisely [120]. Exosomes are biological nano-sized vesicles that could effectively carry miRNA cargo and, in addition to preventing miRNA degradation, facilitate passage through biological barriers and plasma membranes [118]. Both *in vitro* and *in vivo* models of RA showed the beneficial effects of delivering exosomal miRNA-based strategies. Tavasolian et al. indicated that miR-146a/miR-155-overexpressed mesenchymal stem cell (MSC)-derived exosomes increased FoxP3, TGF-β, and IL-10 production by regulatory T cells after *in vitro* treatment of CIA mouse model-derived cultured splenocytes and *in vivo* administration in an animal model of RA [121]. Additionally, Chen et al. found that exosome-delivered miR-150-5p substantially reduced the migration and invasion of FLSs from RA patient and targeted MMP14 and VEGF to down-regulate tube formation in human umbilical vein endothelial cells (HUVEC). In addition, exosomal miR-150-5p administration in the murine CIA model ameliorated clinical and inflammatory symptoms by suppressing synoviocyte hyperplasia and angiogenesis and reducing joint destruction [105]. Exosome therapy has numerous advantages over both cell-based therapeutic approaches and synthetic nanoparticle-based strategies due to its low toxicity, immunogenicity, and better tolerability [67,122,123].

3. Concluding remarks and future prospective

Several theories were proposed to explain the pathogenesis of RA, a progressive autoimmune disease with an increasing prevalence. Nevertheless, the precise mechanism is still not fully understood, which present significant challenges for the development of early diagnosis, treatment, and management strategies. Recent research has illustrated the significance of exosomes in a variety of autoimmune and inflammatory diseases, including RA. Exosomes are secreted by a wide variety of cells and are present in different biological fluids, and their use for therapeutic purposes showed promising potential in RA and other inflammatory diseases. Exosomes mediate intercellular communication by carrying biological components, including proteins, lipids, and nucleic acids as genetic components, which can potentially transfer activation or suppression of signaling pathways from the source to the target cells. This demonstrates the active involvement of exosomes in the pathogenesis of RA, and highlights their potential for delivery as biomarkers and therapeutic tools. The miRNAs are a critical component of exosomal cargo that is implicated in the pathogenesis of RA, as they reprogram a variety immune cell function and signaling pathways. Differential expression of exosomal miRNAs in different autoimmune diseases and even in different phases of inflammatory conditions could potentially provide a rational option for exosomal miRNAs application as biomarkers to facilitate early diagnosis in RA patients. As this field is still in its early stages, further large-scale research is needed to establish the superiority and clinical application of miRNAs as prognostic and diagnostic biomarkers for RA. Therefore, the ability of miRNAs to target multiple genes and signaling pathways makes them promising therapeutic approaches. Unfortunately, this property also carries a significant risk of off-target effects and adverse side effects in terms of the systemic administration of a specific miRNA in most preclinical studies. However, simultaneously regulating the activity and expression of several miRNAs may result in good results in RA patients; miRNAs' wide potential on various targets in diverse tissues has the power to block whole pathways. Limited knowledge of the tissue distribution of miRNA, and systemic toxicity suggests that further studies are needed to evaluate the therapeutic capacity of miRNA and to establish optimized protocols for using miRNAs in clinical applications. In addition, mimicking or suppressing the function of deregulated miRNAs using exosomes as a delivery system is being investigated for exosomal miRNA-based gene therapy approaches for RA. For future clinical trials, however, methodological gaps in the reproducibility of exosomal miRNA isolation, screening and measurement need to be addressed. Furthermore, the pathway and patterns for specific miRNA-based gene therapy strategies could be elucidated by a more comprehensive understanding of the exact molecular, and cellular mechanisms of exosomal miRNAs. Because the function and properties of exosomes determined by their biological source, the kind of cells from which exosomes are isolated is critical for using exosomes as a delivery mechanism for miRNA mimics. Hence, methods of exosome isolation and purification that exclude other factors and components that interfere with the desired results, a precise understanding of the cellular targets, and the mechanisms for specific delivery of the cargo using the required modifications, and the evaluation of *in vivo* safety and efficacy of the therapeutic strategy. Hence, it is difficult and distant to apply exosomal miRNAs as valid, accurate and reproducible biomarkers for RA and as therapeutic tools for miRNA-based gene therapy approaches.

CRediT authorship contribution statement

Mahvash Sadeghi: Writing – original draft, Conceptualization. **Jalil Tavakol Afshari:** Writing – original draft, Methodology. **Afsane Fadaee:** Writing – original draft. **Mohammadreza Dashti:** Writing – original draft. **Fatemeh Kheradmand:** Writing – original draft. **Sajad Dehnavi:** Writing – review & editing, Conceptualization. **Mojgan Mohammadi:** Writing – review & editing, Methodology.

Data availability statement

No data was used for the research described in the article.

Funding

'Not applicable.'

Declaration of competing interest

All authors declare no potential conflict of interest.

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