Deregulation and therapeutic potential of microRNAs in arthritic diseases

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Abstract | Epigenetic abnormalities are part of the pathogenetic alterations involved in the development of rheumatic disorders. In this context, the main musculoskeletal cell lineages, which are generated from the pool of mesenchymal stromal cells (MSCs), and the immune cells that participate in rheumatic diseases are deregulated. In this Review, we focus on microRNA (miRNA)-mediated regulatory pathways that control cell proliferation, drive the production of proinflammatory mediators and modulate bone remodelling. The main studies that identify miRNAs as regulators of immune cell fate, MSC differentiation and immunomodulatory properties — parameters that are altered in rheumatoid arthritis (RA) and osteoarthritis (OA) — are also discussed, with emphasis on the importance of miRNAs in the regulation of cellular machinery, extracellular matrix remodelling and cytokine release. A deeper understanding of the involvement of miRNAs in rheumatic diseases is needed before these regulatory pathways can be explored as therapeutic approaches for patients with RA or OA.

Rheumatic diseases are chronic inflammatory disorders that involve haematopoietic progenitors and mesenchymal stromal or stem cells (MSCs). Osteoarthritis (OA) and rheumatoid arthritis (RA), as well as osteoporosis, share similar pathophysiological pathways that include increased bone remodelling, cell senescence and accumulation of activated immune cells in the skeletal tissue and joints. More precisely, RA is characterized by chronic synovitis, subchondral bone resorption and osteoclast activation; in OA, degradation of the cartilage extracellular matrix (ECM) is accompanied by subchondral bone remodelling, osteophyte formation and, at late stages of the disease, synovial inflammation; osteoporosis is characterized by bone weakness and fractures due to an imbalance between osteoclast and osteoblast activity, as well as cytokine release.

Multiple genes have been shown to have variants that increase susceptibility to RA, OA or osteoporosis, but the connection between these variants and disease phenotype remains elusive. The link between genotype and the emergence of musculoskeletal diseases depends on both the transcriptional level of critical genes and the type of cells in which these genes are expressed. These tightly regulated biological processes are under the control of epigenetic modifications, including microRNAs (miRNAs). Mature miRNAs are short, single-stranded, noncoding RNAs (18–24 nucleotides) that bind one or more mRNAs, thereby modulating protein expression through either repression of translation or by increasing mRNA turnover and degradation. The molecular mechanisms of regulation of gene transcription and translation by miRNAs have been reviewed extensively[1–3]. These small molecules participate in the regulation of ECM remodelling as well as of a wide range of cellular functions (including proliferation, motility, differentiation and apoptosis), achieved by targeting multiple pathways[4].

Abnormal expression of miRNAs was first reported in rheumatic diseases less than a decade ago. In the past 2 years, miRNAs were suggested to act as regulators of cellular metabolism and, indirectly, to affect the immune cell niche[5]. Although abnormal expression of miRNAs occurs in several rheumatic diseases, this Review focuses on miRNAs deregulated in RA and OA, as the involvement of miRNAs in systemic lupus erythematosus has been reviewed elsewhere[6,7]. Additionally, this Review discusses the therapeutic potential of miRNAs that target key cellular processes.

Role of miRNAs in MSC biology

MSCs originate from the mesoderm and encompass cells of the connective or supporting tissues, the smooth muscle and the vascular endothelium. MSCs

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Key points

- MicroRNA (miRNA) deregulation has a role in the breakdown of cartilage homeostasis and in osteoarticular diseases
- Most miRNAs deregulated in mesenchymal stromal or stem cells (MSC) in the context of rheumatic diseases are involved in cell differentiation or anti-inflammatory mechanisms
- Fibroblast-like synoviocytes (FLS) from patients with rheumatoid arthritis (RA) have altered levels of specific miRNAs that have important roles in the modulation of inflammatory or catabolic functions, or both
- Modulation of miRNAs in MSC or FLS for therapeutic purposes in rheumatic diseases has not been developed yet
- Abnormal expression of miRNAs in immune cells in the context of arthritis has been described, and promising candidates for therapy have been identified
- Targeting miRNA expression in monocytes to silence inflammatory and bone catabolic pathways could be a promising and efficient strategy to treat arthritic conditions

are adherent fibroblastic cells that express CD73 (5′-nucleotidase), CD90 (thy-1 membrane glycoprotein) and CD105 (endoglin), and lack haematopoietic lineage markers such as CD11b (integrin αM), B-lymphocyte antigen CD19, haematopoietic progenitor cell antigen CD34, CD45 (receptor-type tyrosine-protein phosphatase C) and HLA-DR. These cells have the potential to differentiate into a wide range of tissues, including bone, cartilage and fat[8]. MSCs are mainly found in the bone marrow and fat tissue, but are also present in the synovial membrane.

MSC differentiation

Numerous studies have reported the global expression profile of miRNAs in MSCs, generally by comparing steady-state and pathological conditions or differentiated lineages (reviewed elsewhere[9,10]). Most miRNAs described to be deregulated in rheumatic diseases are involved in either MSC differentiation or anti-inflammatory functions.

MiRNAs have been shown to regulate the differentiation of the three main MSC lineages. Among the miRNAs whose targets have been validated (mostly in *in vitro* studies), the majority promote commitment to one differentiation pathway while inhibiting differentiation of alternative lineages. This is the case for miRNAs shown to promote adipogenesis and inhibit osteogenesis (FIG. 1). For instance, *in vitro* overexpression of miR-30a, miR-30d, miR-204, miR-211, miR-320 or miR-3077-5p downregulates *RUNX2*, which encodes runt-related transcription factor 2, the master regulator of osteoblast differentiation; this inhibition results in upregulation of master regulator genes of adipogenesis in MSCs, including *PPARG* (encoding peroxisome proliferator-activated receptor γ), *FABP4* (encoding adipocyte protein 2, also known as fatty-acid-binding protein, adipocyte) and *CFD* (encoding complement factor D, also known as adipsin)[11–13]. By silencing the expression of *SP7*, which encodes transcription factor Sp7 (also known as zinc finger protein osterix) — another osteogenic transcription factor — miR-637 can also inhibit osteogenesis and promote adipogenesis[14]. Notably, three miRNAs inhibit both adipogenic and osteogenic pathways: miR-31, which targets *CEBPA* (encoding CCAAT/enhancer-binding protein α) and *SP7* in MSCs; and miR-138 and miR-335, which inhibit the expression of *PTK2* (encoding focal adhesion kinase 1) and *RUNX2*, respectively, and can both also inhibit the expression of *PPARG*[15,16]. Interestingly, miR-335 is expressed at higher levels in MSCs than in fibroblasts, suggesting that this molecule has a possible role in MSC self-renewal and maintenance of an undifferentiated state.

The miRNAs that silence key osteogenic genes such as *RUNX2* are often observed to promote chondrogenesis[17] (FIG. 1). For example, miR-143, a negative regulator of osteogenesis, can downregulate SP7 (REFS 18,19). By contrast, two positive regulators of osteogenesis, miR-29b and miR-200a, can act as negative regulators of chondrogenesis *in vitro*[20,21]: miR-200a targets *DLX5* (encoding homeobox protein DLX-5) and miR-29a binds to *DKK1* (encoding Dickkopf-related protein 1), *KREMEN2* (encoding Kremen protein 2) and *SFRP2* (encoding secreted frizzled-related protein 2) mRNAs, hence establishing a positive regulatory loop that induces osteogenesis[22].

Some miRNAs seem to specifically regulate one particular pathway. The miR-27 family members (miR-27a and miR-27b) and miR-130 strongly inhibit adipogenic differentiation by targeting *PPARG*[23–25]. Other functionally validated miRNAs modulated during adipogenesis include miR-103, miR-107, miR-155, miR-221, miR-222 and miR-369-5p, but their targets are still to be identified[26]. miR-146b was reported to be a positive regulator of adipogenesis *in vivo* by targeting *SIRT1*, which encodes NAD-dependent protein deacetylase sirtuin 1, the transcriptional inhibitor of *PPARG*[27]. Concerning miRNAs that have been shown to solely enhance osteogenesis, enforced expression of miR-196a, miR-210 and miR-2861 (which are upregulated during osteogenic differentiation) in MSCs *in vitro* promotes osteoblast differentiation by inhibiting the expression of *ACVR1B*, *HOXC8* and *HDAC5* (encoding activin receptor type 1B, homeobox protein Hox-C8 and histone deacetylase 5, respectively)[28–30]. Conversely, miR-23a, miR-133, miR-355 and miR-433 inhibit osteogenic differentiation of MSCs by silencing expression of *RUNX2*, whereas miR-100, miR-141 and miR-182 do so by targeting *BMPR2* (which encodes bone morphogenetic protein receptor type-2), *DLX5* and *FOXO1* (which encodes the transcription factor forkhead box protein O1), respectively[9,31,32].

Few miRNAs have been shown to modulate chondrogenesis. This is the case for miR-145, which targets the key master regulator gene *SOX9* (encoding the transcription factor SOX-9), and for miR-194, miR-199a* and miR-574-3p, which silence *SMAD1* (encoding MAD homolog 1), *SOX5* (encoding transcription factor SOX-5) and *RXRA* (encoding retinoic acid receptor RXR-α), respectively[33–36]. Two miRNAs have been reported to specifically enhance chondrogenesis: miR-23b targets *PRKACB*, which encodes cAMP-dependent protein kinase catalytic subunit β, and miR-140 silences *HDAC4*, *ADAMTS5* (which encodes a disintegrin and

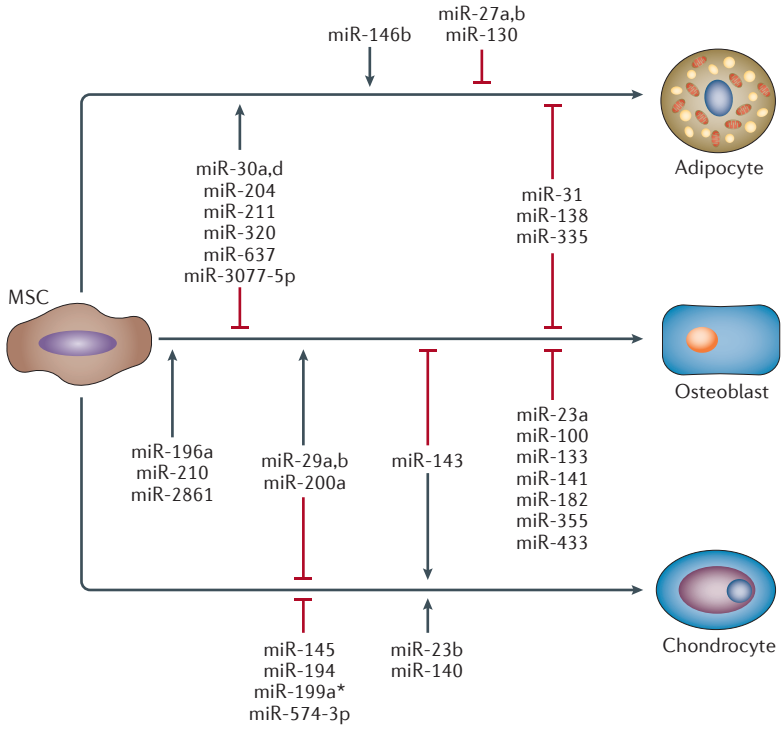

Figure 1 | **miRNAs that control differention of MSCs.** Adipocytes, osteoblasts and chondrocytes are the main three lineages that differentiate from mesenchymal stromal or stem cells (MSCs). The main microRNAs (miRNAs), which are tightly regulated during these differentiation pathways, are represented. Among these, individual miRNAs often promote commitment into one differentiation pathway (black arrows) while simultaneously inhibiting an alternative pathway (red lines). Few miRNAs seem to be specific to one lineage.

metalloproteinase with thrombospondin motifs 5) and *CXCL12* (encoding CXC chemokine 12, also known as stromal cell-derived factor 1)[37,38]. All these observations show the importance of miRNAs for the lineage commitment of MSC and the maintenance of differentiated phenotypes, as summarized in FIG. 1. The expression of many other miRNAs known to be regulated during MSC differentiation into the three lineages[9] could represent further promising candidates to modulate MSC differentiation.

MSC immunosuppressive function

In addition to their differentiation capacity, MSCs display anti-inflammatory properties[39–41] mainly associated with the secretion of soluble factors, including prostaglandin E_2 (PGE_2), IL-6, indoleamine 2,3-dioxygenase (IDO) and TNF-induced protein 6 (REFS 39–41). Although various hypotheses have been proposed for the immunomodulatory mechanisms mediated by MSCs, these are still only partially understood; nevertheless, several papers highlight the importance of miRNAs in these mechanisms. Most miRNAs described in this context are negative regulators of MSC immunosuppressive functions. For instance, miR-181a was shown to reduce the ability of MSCs to inhibit T-cell proliferation both *in vitro* and *in vivo*[42]. In another study, upregulation of miR-155 attenuated MSC-mediated immunosuppression by targeting *TAB2* (encoding TGF-β-activated kinase 1 and MAP3K7-binding protein 2) and repressing *NOS2* (encoding nitric oxide synthase, inducible)[43]. Furthermore, in an *in vivo* sepsis model, downregulation of miR-143 activated MSC immunosuppressive functions by targeting *MAP3K7* (also known as *TAK1*, which encodes mitogen-activated protein kinase kinase kinase 7), and transfer of MSCs transfected with miR-143 led to a lower survival rate in septic animals when compared with transfer of control MSCs[44].

Conversely, modulation of other miRNAs might enhance the ability of MSCs to suppress T-cell proliferation, opening future avenues of research into innovative MSC-based strategies. Silencing of miR-27b amplified the inhibitory effect on T-cell proliferation induced by adipose-derived MSCs by inducing CXCL12 production[45]. Likewise, miR-146a inhibition reversed the loss of immunoregulatory function of MSCs that had differentiated into neurons, a tendency that might be related to increased PGE_2 production[46]. These emerging data emphasize the role of deregulated miRNAs in MSC function, and this interesting topic should be explored more deeply in the next few years in the context of rheumatic diseases.

Deregulated miRNAs in osteoarthritis

Deregulation of miRNAs is involved in the breakdown of cartilage homeostasis and in osteoarticular diseases. Among these miRNAs, several play a part in cartilage protection by regulating genes that mediate catabolic activity[47,48]. For instance, in human OA cartilage, miR-140 expression is drastically decreased compared to its expression in normal tissue[49], and downregulation or overexpression of miR-140 in mice accelerated or delayed the development of OA lesions, respectively[50–52]. Downregulation of miR-140 was associated with increased expression of *ADAMTS5*, *IGFBP5* (encoding insulin-like growth factor-binding protein 5), *MMP13* (encoding collagenase 3) and *HDAC4* (encoding histone deacetylase 4). Similar findings were observed with miR-27a and miR-27b, whose expression is decreased in OA chondrocytes and coincides with increased expression of *IGFBP5* and *MMP13* (REFS 49,53). Other miRNAs drive cartilage degradation by suppressing the expression of key molecules involved in cartilage formation (reviewed elsewhere[54]); indeed, increased expression of miR-455-3p in OA cartilage contributes to its destruction by targeting *ACVR2B* (encoding activin receptor type-2B), *SMAD2* (encoding MAD homolog 2), *CHRDL1* (encoding chordin-like protein 1) and by suppressing transforming growth factor β signalling[55]. Similarly, the expression of miR-145 is enhanced in OA chondrocytes in response to IL-1β stimulation: targeting the expression of the transcription factor SOX9 and SMAD proteins results in the decreased expression of collagen α1(II) chain and aggrecan core protein[33]. *SOX9* is also targeted by miR-101, but, when overexpressed, miR-101 can inhibit cartilage destruction in a murine model of OA via targeting of *DNMT3B*, the gene encoding DNA (cytosine-5)-methyltransferase 3B,

and inhibiting *ITGA1* (encoding integrin α1) expression[31]. The expression of miR-23b is upregulated in OA chondrocytes and has been shown to induce chondrogenic differentiation; these findings might contribute to the development of new treatments for OA[56]. Other miRNAs, such as miR-25, miR-483-5p and miR-675 are also upregulated in OA cartilage; miR-21, miR-193b and miR-199-3p are increased in aged cartilage, but their role in OA remains unclear[17,54].

Inflammation is also a characteristic of OA, and several miRNAs involved in the inflammatory response are deregulated in OA. miR-9 and miR-98 were identified as upregulated, and miR-146 as downregulated, in OA cartilage[47], and overexpression of these three miRNAs reduced IL-1β-induced TNF production in OA chondrocytes. Another study showed that miR-146 expression is enhanced in early OA and decreases as OA progresses[57]. In 2015, transfection of pre-miR-146a into IL-1β-treated chondrocytes (obtained from the nucleus pulposus of intervertebral discs) was shown to reduce the production of proinflammatory cytokines, indicating that miR-146a could protect against IL-1β-induced inflammation[58]. Other miRNAs, such as miR-140, miR-199a and miR-558, are also downregulated by IL-1β, whereas miR-145 is upregulated[50,59–61]. In rats with surgically induced OA, expression of both miR-146a and miR-183 was increased; interestingly, downregulation of these two miRNAs was closely associated with the upregulation of inflammatory pain mediators[62]. By contrast, miR-149 expression is decreased in human OA cartilage and this alteration is associated with increased expression of the proinflammatory cytokines TNF, IL-1β and IL-6, suggesting that miR-149 can have a protective effect on OA progression[63]. Finally, miR-203 and miR-483* have been proposed to induce the production of nitric oxide and IL-1β, respectively[54]. These miRNAs might be potential targets for developing new therapeutic strategies for OA (FIG. 2).

Deregulated miRNAs in RA

Disturbed fibroblast-like synoviocyte function

The fraction of FLSs (fibroblast-like synoviocytes) resident in the joints has been suggested to display properties associated with MSCs[64]. In RA, FLSs are crucial protagonists in joint damage and inflammation because they express inflammatory and catabolic molecules that lead to their deregulated proliferation, invasiveness and destructive potency. This activated phenotype of FLSs also has a deleterious effect on the joint environment, supporting the activation of immune cells present locally.

RA-FLSs express a tumour-like phenotype that plays a major part in the invasive joint destruction observed in this disease. Epigenetic abnormalities, including deregulation of DNA methylation, histone modifications and miRNA expression, have been reported and are clearly responsible for the sustained activation and aggressiveness of these cells[65,66]. Whether epigenetic modifications of FLSs can be inherited or are induced by environmental factors remains to be clarified[67]. The current literature on the association of miRNAs with RA involves >10 miRNAs that modulate the inflammatory or catabolic functions of FLSs, or both, thereby contributing to the aggressive phenotype of RA-FLS (FIG. 3).

The first miRNAs found to be abnormally expressed in RA-FLSs were miR-146a and miR-155, both previously known as important regulators of inflammation[68,69]. miR-155 is constitutively upregulated in RA-FLS and its expression is increased by TNF[69,70]. In addition to its proinflammatory action, miR-155 might also have an important regulatory role in the destructive behaviour of RA-FLSs given that it reduces the expression of interstitial collagenase (also known as matrix metalloproteinase 1) and stromelysin-1 (also known as matrix metalloproteinase 3 (MMP3)), which inhibit FLS proliferation and invasiveness[69,70]. Even though mice deficient in miR-155 are protected from inflammation, as expected, no effect on tissue destruction was noted[71–73]. Intriguingly, levels of miR-146a were also found to be augmented in RA-FLSs (in which expression of this miRNA can be induced by lipopolysaccharide and IL-1β, but not TNF)[69]; however, miR-146 has a well-described negative regulatory effect on inflammation in immune cells[74]. This apparent contradiction can be explained by the lack of data describing the role of miR-146a in FLS and, as miRNA-mediated control of biologic pathways is cell-context-dependent, its negative role in immune cells might not be transposable to fibroblasts. Furthermore, miR-146a might target genes and pathways in FLSs that have not yet been identified.

Increased expression in RA-FLSs was also reported for miR-203, miR-221, miR-222 and miR-323-3p[75,76]. These observations are in agreement with the known roles of miR-203 in tumour progression[77] and in regulation of the invasiveness and proliferative abilities of melanoma cells[78]. In addition, miR-221 and miR-222 increase the migration capacity of cancer cells during metastasis[79,80]. Downregulation of miR-221 was shown to induce cell apoptosis and to inhibit the expression of proinflammatory cytokines, as well as reducing FLS cell migration and invasion[81], supporting the existence of a miRNA-regulated mechanism controlling the deleterious functions of RA-FLSs.

The expression of miR-22, miR-34a*, miR-124a, miR-152 and miR-375 is decreased in RA-FLSs. Interestingly, wild-type cellular tumour antigen p53 binds to the promoter region of the *MIR22* gene, inducing its transcription and repressing the expression of *CYR61*, which encodes the extracellular matrix protein Cyr61 (REF. 82). Cyr61 upregulates the expression of a number of genes involved in angiogenesis, inflammation and matrix remodelling. As Cyr61 is overexpressed in arthritic joints, this protein might promote RA-FLS proliferation and contribute to pannus hyperplasia via an autocrine or paracrine loop involving miR-22 repression[83]. Increased RA-FLS proliferation was also sustained by upregulation of the *XIAP* gene (encoding the anti-apoptotic molecule E3 ubiquitin-protein ligase XIAP) as well as of *CDK2* (cyclin-dependent kinase 2) and *CCL2* (encoding CC motif chemokine 2, also known as monocyte chemoattractant protein 1 (MCP-1)), which are targeted by

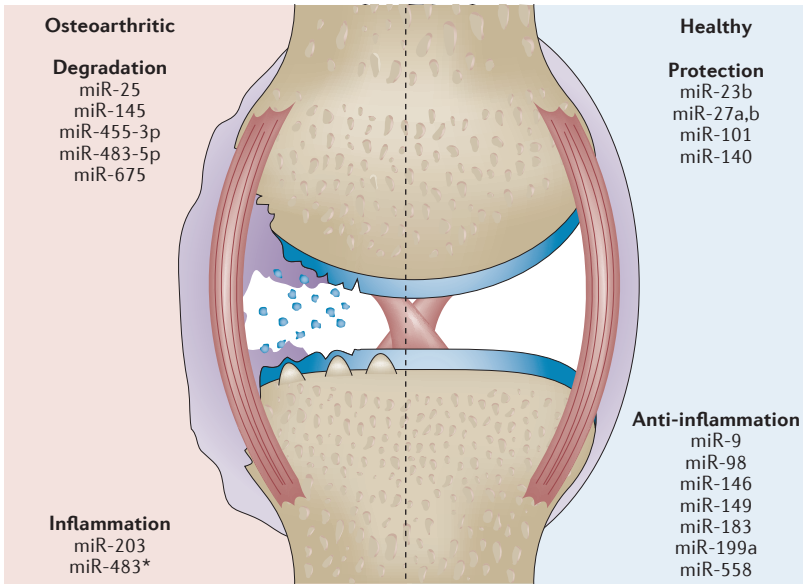

Figure 2 | **miRNAs that are deregulated in OA.** Cartilage degradation and (to a lesser extent) inflammation are important characteristics of osteoarthritis (OA). Deregulation of microRNA (miRNA) expression is associated with both cartilage degradation and protection, as well as inflammation, in patients with OA. The miRNAs associated with inflammation and cartilage degradation, as well as those associated with protection against these processes in OA, are depicted, in contrast to the miRNAs linked to anti-inflammatory and cartilage-protecting effects in healthy controls.

miR-34a* and miR-124a, respectively[84,85]. Similarly to what was reported in tumour cells, the *MIR124A* gene promoter was methylated in the majority of RA samples, being nonmethylated in samples from healthy controls[86]. In animal models, decreased expression of miR-152 and miR-375 was reported in FLSs from arthritic rats, leading to activation of the Wnt signalling pathway through direct targeting of *DNMT1* (encoding DNA (cytosine-5)-methyltransferase 1)[87] and *FZD8* (encoding Frizzled-8)[88], respectively.

As the miR-17-92 cluster is a global regulator of apoptosis, it is not surprising that several research groups found deregulated expression of miRNAs within this cluster in the context of RA[89]. However, these clustered miRNAs do not seem to display coordinated regulation, at least in RA-FLSs, as miR-18a is upregulated in these cells whereas the miR-19a/b, miR-20a and miR-30a-3p are downregulated. Inflammation induces expression of miR-18a, which contributes to NFκB-mediated cartilage destruction and chronic inflammation in the joint through a positive feedback loop involving silencing of the NFκB inhibitor TNF-induced protein 3 (REF. 90). Conversely, inflammation reduces the expression of miR-19a/b in RA-FLSs through Toll-like receptor (TLR)2 and TLR4 signalling, alleviating the miR-19a/b negative control of TLR2 expression and leading to increased IL-6 and MMP3 secretion[91]. The decreased expression of miR-20a and miR-30a-3p in RA-FLSs enables the upregulation of their targets *MAP3K5* and *TNFSF13B* (also known as BAFF), respectively, creating an environment that favours T-cell and B-cell survival[92,93].

Disturbed immune cell function

Several miRNAs involved in innate or adaptive immunity (or both) have been identified. Most studies focusing on miRNAs in autoimmune diseases, including RA, have first monitored their expression levels in tissues, identified their cellular origin, and investigated their pathogenetic roles. The two most studied miRNAs in the context of RA are miR-146a and miR-155. These multifunctional miRNAs are involved in the development of innate and adaptive immune cells and are overexpressed in several immune-mediated inflammatory disorders. They are also upregulated after immune cell maturation, in inflammatory conditions, and in response to a variety of microbial components. Without exception, these miRNAs were overexpressed in all tissues or cells isolated from patients with RA investigated to date when compared to the corresponding tissues or cells from patients with OA or healthy donors[69,94–96]. miR-146a and miR-155 are considered the 'yin and yang' of inflammation as these two miRNAs are negative and positive regulators of inflammatory responses, respectively.

The regulatory functions of miR-146a have been mainly investigated in cells of the myeloid lineage, in which this miRNA is induced by TLR or cytokine receptor stimulation and negatively controls the NFκB signalling pathway[74]. Thus, miR-146a has an inhibitory effect on several functions characteristic of this cell lineage, including proinflammatory cytokine production, osteoclast differentiation, dendritic cell (DC) maturation and antigen presentation. Its overall action within these negative feedback loops is to fine-tune the immune response to avoid deleterious effects owing to excessive immune cell activation[97,98]. Conversely, miR-155 has a clear proinflammatory role. Under inflammatory conditions, miR-155 expression is upregulated, promoting monocyte differentiation into macrophages and DCs by targeting *SOCS1* (encoding suppressor of cytokine signaling 1), *CSF1R* (encoding macrophage colony-stimulating factor 1 receptor) and *RNF123* (encoding E3 ubiquitin-protein ligase RNF123)[99], and repressing their osteoclastic progenitor potential by inhibiting *MITF* (encoding microphthalmia-associated transcription factor) and *SPI1* (encoding transcription factor PU.1)[100]. miR-155 is upregulated in RA synovial macrophages and is associated with low production of phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase 1, an inhibitor of inflammation, which ultimately leads to increased TNF production[72]. Mice deficient in miR-155 display defective B-cell and T-cell immunity and impaired DC antigen-presenting function[101]. Also of relevance to B-cell involvement and the presence of autoantibodies in RA, miR-155 is critical for the activation and regulation of B-cell function, in particular for germinal centre reactions to produce optimal T-cell-dependent antibody responses[102,103].

In adaptive immunity, whereas miR-146 modulates the T regulatory (T_{REG}) cell phenotype, miR-155 influences the T-cell memory phenotype[104]. In steady-state conditions, miR-146a controls T cell receptor (TCR) signalling via changes in NFκB activity and IL-2 expression,

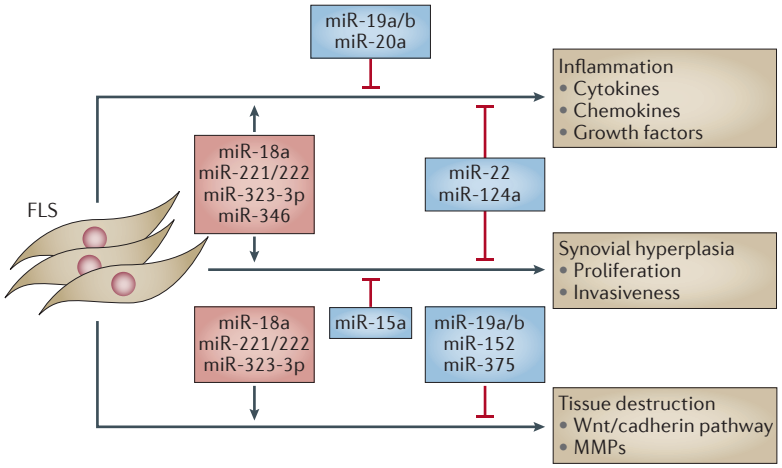

Figure 3 | **miRNAs that are deregulated in RA.** Fibroblast-like synoviocytes (FLSs) are key effector cells in rheumatoid arthritis (RA), as they produce proinflammatory cytokines and chemokines that perpetuate local joint inflammation. RA-FLSs also produce proteases and growth factors that contribute to their own proliferation and aggressive phenotype, increasing their invasiveness into the extracellular matrix and further exacerbating joint damage. The expression of several microRNAs (miRNAs) that negatively (red line) or positively (black arrow) control inflammatory cascades, apoptosis and/or tissue remodelling is downregulated (blue boxes) or upregulated (red boxes) in RA-FLS, respectively, thereby contributing to the pathogenetic role of these cells in RA.

and protects T cells from antigen-induced cell death. In response to TCR stimulation, miR-146a expression is decreased, whereas miR-155 levels are augmented[105]. In arthritic conditions, T_{REG} cells from patients with RA express lower levels of miR-146a and miR-155 upon TCR stimulation than do T_{REG} cells from healthy controls[105]. Interestingly, low disease activity correlates with high expression of miR-146a in T_{REG} cells, but not in $CD4^+CD25^-$ T cells. Thus, in all cell types investigated, miR-146a acts as a general molecular brake on biological processes associated with inflammation, autoimmunity, proliferation and cell activation[106]. Whether high levels of miR-146a contribute to RA pathogenesis — and if so, in what way — remains to be established. Deletion of miR-146a in mice is associated with several immune defects and with the development of autoimmunity owing to hyperresponsiveness of macrophages to pathogens, sustained activation of the NFκB signalling pathway, and loss of peripheral T-cell tolerance[106,107]. Mice transgenic for miR-146a also develop spontaneous immune disorders that mimic human autoimmune lymphoproliferative syndromes, supporting a role for miR-146a in germinal centre formation[108]. Additionally, absence of miR-146a aggravates the severity of Lyme arthritis[109]; in this context, a mirror study investigating arthritis progression in miR-146a transgenic mice would be most informative.

All these studies suggest that miR-146a and miR-155 are essential for the maintenance of immune homeostasis, and that alterations in their expression in patients with RA lead to changes in the cytokine secretion pattern of T cells, B cells, myeloid cells and FLSs, as well as to altered maturation and function of these cell types. However, an apparent contradiction between the observed high levels of miR-146a in RA and its negative regulatory role on inflammation remains. One possibility is that the current view of its function is too global. By focusing on cell subsets instead of whole populations, we might find that the subsets with important functions in RA are not the ones that produce high levels of miR-146a. Of note, monocytes are a phenotypically and functionally heterogeneous population of cells with two main subsets that display different properties *in vivo* in terms of their capacity to differentiate into polarized macrophages, DC or osteoclasts, and that produce different sets of molecules upon pathogen detection. Our group has observed that expression of miR-146a is decreased in blood monocytes isolated from patients with RA compared with those from healthy controls (Apparailly *et al.*, unpublished data). An additional observation is provided by another research group who showed that miR-146 is involved in sensing suboptimal inflammatory signals, whereas miR-155 acts as a modulator of the full-blown inflammatory response[110]. This model fits with the described role of miR-146 as the guardian of haematopoietic stem cell homeostasis, dysregulation of which is observed in miR-146-deficient mice[111]. However, although this guardian function might be adequate under normal conditions, it seems to be overwhelmed in chronic inflammatory conditions, such as RA — in which miR-146a expression reaches its maximal level and can no longer counteract a saturated system.

Many other miRNAs have been identified to be involved in innate or adaptive immunity (or both), but few have been investigated in the context of RA. Among these, miR-21 was reported to promote T helper (T_H)2 cell and T_{REG} cell differentiation[112,113] and to be characteristic of memory phenotypes[104]. This miRNA was found to be underexpressed in circulating T_{REG} cells from patients with RA compared with healthy controls[114]. In addition, as low levels of miR-21 were correlated with upregulation of *STAT3* and increased IL-17 levels, as well as downregulation of *STAT5*, the authors of this report proposed that miR-21 might have a role in the imbalance between T_H17 and T_{REG} cells that is characteristic of patients with RA[114]. T_{REG} cells with a memory phenotype are nevertheless abundant in the synovial fluid of patients with RA, and express higher levels of miR-21 than conventional T cells or T_{REG} cells found in the periphery[115]. A 'miRnome' of $CD3^+$ T cells from patients with RA performed in 2014 revealed that levels of miR-34b and miR-223 are increased in T cells from patients with RA compared to levels in T cells from healthy donors[116]. As the levels of miR-34b and miR-223 were inversely correlated with expression of their known target genes *SCD5* and *IGF1R*, respectively, the authors of this report suggested that these changes implied an abnormal response to T cell activation in patients with RA. In a different set of experiments, in-depth sorting and analysis of T cells showed that miR-223 was expressed at high levels in naive $CD4^+$ lymphocytes, whereas it was barely detectable in T_H17 cells[117]. $CD14^+$ monocytes from RA synovia also overexpress miR-223, which is known to modulate differentiation of myeloid precursors into osteoclasts[118,119]. Thus, in addition to its involvement in abnormal T cell functions

in the context of RA, miR-223 also plays a part in abnormal bone erosion. Other studies demonstrated the existence of a vicious cycle involving miR-148a in T_H1 cells[120]: the chronic activation of T_H1 cells observed in inflamed arthritic joints induces the expression of *TWIST1*, encoding transcription factor Twist-related protein 1 — which, together with the T_H1 master regulator T-box transcription factor TBX21 (also known as T-bet), increases the expression of miR-148a, in turn targeting and inhibiting the proapoptotic gene *BCL2L11*, and ultimately leading to the local persistence of activated T_H1 cells.

Neutrophils are also important players in RA. Not surprisingly, miR-451, which negatively regulates the migration of neutrophils by silencing *CPNE3* (encoding copine-3) and *RAB5A* (encoding Ras-related protein Rab-5A), is downregulated in neutrophils from patients with RA[121]. miR-451, one of the miRNAs most conserved in vertebrates, regulates cell proliferation, invasion and apoptosis, and might have a function in other cell types in the context of RA. High expression of miR-451 in T cells of patients with RA was positively correlated with 28-joint disease activity scores, erythrocyte sedimentation rate and serum IL-6 levels[104]; no functional study has been performed yet to further characterize this miR-451–IL-6 loop in T cells. Finally, the abnormal TLR3 overexpression described in RA was linked to decreased expression of miR-26a and aberrant macrophage differentiation from myeloid precursors[122]. This deregulated loop might promote RA development by participating in the increased expression of type I interferon and downstream proinflammatory cytokines.

These findings reinforce the importance of the cellular context when studying the functions of miRNAs, not only in terms of the cell type itself, but also of the environment of a given cell type. Future studies to be launched in experimental models of arthritis should take advantage of conditional gene knockout and knock-in mice to address the role of specific miRNAs in specific cell subsets.

Therapeutic potential of miRNAs

MSC-based strategies

Tissue engineering and miRNA regulation. Current musculoskeletal tissue engineering applications generally propose a combination of cells, scaffolds and growth factors that are important components for effective tissue regeneration[123]. Novel strategies also involve the administration of miRNAs to enhance the repair of damaged tissue[124]. Although several miRNAs are implicated in diseases that affect cartilage or have been identified as essential in cartilage homeostasis or repair, no data on the use of miRNAs in cartilage engineering have been reported to date. To our knowledge, a single study has evaluated the effect of miRNA expression in a bone engineering approach. In this study[125], the authors evaluated the effect of miR-26a on bone formation, as it positively regulates the coupling of angiogenesis and osteogenesis. Overexpression of miR-26a in MSCs incorporated into a heparin-containing hyaluronan and gelatin hydrogels, which was implanted into a calvarial defect in mice, significantly improved vascularization and bone regeneration, leading to complete repair of the defect[125]. The effect of miR-210, which is important for vascular endothelial growth factor (VEGF)-driven endothelial-cell migration and capillary formation, has also been investigated in ligament engineering[126]. Injection of miR-210 along with atelocollagen into the knee joints of rats with partial transection of the anterior cruciate ligament led to enhanced healing of the transected areas compared with their counterparts in a control group. Both repair and biomechanical properties were improved by the enhancement of angiogenesis and upregulation of VEGF and fibroblast growth factor 2 (REF. 126). Although studies of miRNA-based tissue engineering strategies are rare, we can expect that these therapies will be developed in the near future for clinical orthopaedics.

Cell therapy. The potency of paracrine factors produced by MSCs has raised interest in their exploration as potential therapies. Increasing MSC therapeutic efficiency with miRNA modulation might represent an interesting new strategy. However, to our knowledge, only one paper has provided emerging data on this topic: overexpression of miR-23b induced differentiation of synovial fluid MSCs from patients with OA into chondrocytes[56]. This procedure still needs to be assessed in a preclinical model, but might become a possible treatment for OA[56]. Importantly, a 2013 study showed upregulation of immune genes, mediated by the cytosolic viral sensor retinoic acid inducible-gene 1 (RIG-I), after liposomal transfection of miR-145 in MSCs; these findings suggest the potential for off-target immunological effects of transfection techniques and need to be taken into account when developing miRNA delivery approaches[127].

Targeting miRNAs in myeloid cells

Monocytes can differentiate into macrophages, DCs or osteoclasts upon entry in the tissues, depending on the surrounding conditions. Their high numbers and activity in inflamed joints is a hallmark of RA, and thus monocytes have emerged as the key myeloid subset to target in RA for maximum influence on inflammatory responses and bone erosion[128]. However, only a limited number of groups have explored the therapeutic potential of miRNAs in RA, and their strategies are not designed to target the myeloid lineage directly. An in-depth investigation of the cellular mechanisms involved in miRNA-mediated control of myeloid cells is needed to elaborate future strategies targeting these cells.

The expression of miR-26a was shown to be reduced in splenic cells isolated from rats with pristane-induced arthritis and to be inversely correlated with TLR3 levels[122]. Furthermore, treatment with methotrexate restored the expression of TLR3 and miR-26a to levels comparable to those of nonarthritic controls[122]. Repeated intraperitoneal injections of miR-26a mimics stabilized disease severity 2 weeks after treatment initiation and reduced synovitis, but had no effect on joint destruction[122]. Another group used a systemic strategy to increase the expression level of miR-451 in SKG mice; this approach led to an efficient reduction in the number of cells infiltrating the synovium and to decreased arthritis severity[121]. miR-124

was also found to be underexpressed in the joints of rats with adjuvant-induced arthritis (AIA) as compared with nonimmunized rats[129]. Consistent with previous findings showing that miR-124 negatively regulates osteoclastogenesis of mouse bone-marrow macrophages by suppressing the expression of *NFATC1* (REF. 130), injection of miR-124 into rat AIA joints resulted in reduced disease severity[129]. This effect was in part due to miR-124-mediated targeting of *NFATC1*, a master transcription factor of osteoclast differentiation, suggesting a key role for monocytes in the amelioration of AIA. Other approaches have been suggested by which intra-articular miRNA injection might be used to target FLSs for molecular synoviectomy. Indeed, building on the evidence that miR-15a has a proapoptotic effect in the joints of mice with collagen-induced arthritis (CIA), other researchers showed that intra-articular injection of a double-stranded miR-15a–atelocollagen complex leads to an increase in local expression of miR-15a, which is associated with reduced expression of the apoptosis regulator *BCL2* and a consequent increase in FLS apoptosis in the CIA mouse model[131].

Owing to the negative regulatory role of miR-146a on the inflammatory response of monocytes and DCs, as well as on osteoclast differentiation, this molecule seems to be, theoretically, an optimal candidate for RA gene therapy. This fact should have prompted the rheumatology community to investigate strategies aiming at increasing miR-146a expression in cells of the myeloid lineage to impair inflammation or bone erosion (or both) in experimental models of RA. Injection of miR-146a mimics into CIA mice resulted in a marked reduction of tartrate-resistant acid phosphatase staining (which stains osteoclasts and activated macrophages, among other cell types) and bone erosion in the joints of treated mice compared with those of controls[97]. Interestingly, this treatment had only a marginal effect on the synovial inflammatory response[97]. The absence of a positive effect of enforced expression of miR-146a on joint inflammation in this model is coherent with the high expression of this molecule observed in all RA tissues not being sufficient to counteract the overwhelmingly proinflammatory environment. These results reinforce the concept that miR-146a seems to be implicated in fine-tuning the inflammatory response, and acts as a sensor to control inflammatory cascades under homeostatic conditions only, not under arthritic conditions.

Although inhibition of miR-155 in RA monocytes was shown to reduce TNF secretion[72], it also negatively regulates osteoclasts, and thus might produce unwanted adverse effects on bone erosion during its beneficial blocking of aberrant inflammatory cytokine expression. However, *in vivo* studies suggest that this might not be the case, as miR-155-deficient mice develop less severe disease than wild-type controls[72]. Depending on the RA experimental model selected (more specifically on whether it is driven solely by innate immunity or by both innate and adaptive immunity) miR-155-deficient mice display either reduced bone destruction with no effect on joint inflammation[74], or reductions in both joint inflammation and bone erosion[72].

Although these preclinical studies validate the efficacy of delivering therapeutic miRNAs, we are still lacking evidence of similar miRNA deregulation in human RA, and of the *ex vivo* correction of disease phenotypes in patient-derived cell or tissue samples. Furthermore, the cellular mechanisms that could explain observed clinical benefits of miRNA therapies still need to be characterized in depth.

Perspectives

A number of miRNAs expressed in MSCs or FLSs have been shown to regulate many aspects of cartilage development and homeostasis, and their deregulated expression has been associated with pathogenesis in both RA and OA. Although modulation of expression of tens of miRNAs has showed promising results *in vitro*, there is currently no demonstration that changing the expression of one of these in MSCs or FLSs might be of therapeutic relevance for rheumatic diseases. Further study of miRNAs will help to advance understanding of their effects at the pathophysiological level, and enable the development of strategies to modulate their expression in MSCs.

Studies showing abnormal expression of miRNAs in immune cells under arthritic conditions are increasing, and genetic models point at a few promising candidate miRNAs. Targeting of monocytes — the key players in inflammation and bone homeostasis — seems to be an ideal approach to treat the major features of RA. In this context, the delivery of one or more anti-inflammatory and anti-osteoclastogenic RNA-interfering molecules could represent an innovative and efficient strategy to reduce both synovitis and bone loss in arthritis. Among all approaches that have been evaluated *in vivo* to date, and considering the dogma that one miRNA can control several genes and pathways, the inhibition of miR-155 represents the most promising miRNA-based therapeutic strategy today. As has been suggested previously[132], miRNA-based therapies have advantages over current drug design strategies, allowing two birds to be killed with one stone: one miRNA can target several genes and affect multiple pathways simultaneously. Deregulated miRNAs could also be used as biomarkers of key pathological features such as inflammation or skeletal damage to predict disease severity and response to treatment[133–136].

Before these techniques reach clinical development, much work remains to be done and several hurdles need to be overcome. The design of vehicles for targeted delivery of miRNAs to specific cell subsets, with few adverse effects and optimal safety profiles, are issues that the gene therapy field has been facing for several years. A deeper knowledge of the functions of new or already identified miRNAs is needed before we can envisage the development of miRNA-based therapeutics for the treatment of RA. With regard to the possibility of targeting monocytes, an in-depth evaluation that takes into account the functional heterogeneity of these cells is also required. The need for a multidisciplinary approach is clear — the successful development of miRNA-based therapies will have to bring together chemists, biologists and rheumatologists.

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All authors contributed equally to all aspects of the manuscript (researching data for the article, discussions of its content, writing, review and editing of the manuscript before submission).

Competing interests statement

The authors declare no competing interests.

ERRATUM

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In Figures 1 and 3 of the above article, arrows described as red in the figure legends were shown as black. Additionally, there was a typographical error on page 217 of the above article. These have now been corrected in the online pdf.