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