REVIEW

MicroRNA-124: a promising therapeutic agent for various human diseases, including rheumatoid arthritis

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MicroRNAs (miRNAs) are non-coding RNAs, approximately 22 nucleotides in length that act as post-transcriptional regulators. Thousands of miRNAs have been identified in animals, and they are well conserved across species. MicroRNAs play essential regulatory roles in cellular processes, and changes in miRNA expression are associated with human diseases. Originally, miR-124 was identified as a brain-enriched miRNA and shown to be involved in brain and neuronal development. MiR-124 has since been reported to be expressed in other organs and to be involved in various biological phenomena. MiRNA-124 plays roles in various pathologic conditions, including cancers, acute stress, cardiovascular disorders, inflammatory responses, chronic pain, and osteoclast differentiation. MiR-124 has also been shown to suppress various tumor functions, including proliferation, activation, survival, invasion, metastasis, and migration. Rheumatoid arthritis (RA) is a chronic auto-inflammatory disorder of unknown etiology, whose treatment has been significantly improved by the advent of biological drugs. Even so, some RA patients show little or no response to these therapies, suggesting the need for additional treatments. In a study comparing miRNA expression in RA and osteoarthritis (OA) fibroblast-like synoviocytes (FLS), we found that miR-124a was the only miRNA whose expression was lower in RA than in OA FLS. MiR-124a was found to directly downregulate the production of CDK-2 and MCP-1. In the rat adjuvant-induced arthritis (AIA) model, a single injection of pre-miR-124 into one ankle joint suppressed joint swelling in all of the limbs. Histological examination showed that AIA rats treated with pre-miR-124 exhibited reduced synoviocyte proliferation, less leucocyte infiltration into synovial tissue, and less cartilage and bone destruction than untreated AIA rats. The joints of the pre-miR-124-treated AIA rats also showed reduced osteoclast numbers and reduced RANKL, integrin β1 (ITGB1), and NFATc1 expression levels. MiR-124 was shown to directly target the 3'UTRs of the rat NFATc1, ITGB1, SP1, and CEBPα mRNAs. Both miR-124 and miR-124a were also found to directly target human NFATc1 mRNA and to suppress the differentiation of human osteoclasts from monocytes. Taken together, recent studies suggest that MiR-124 may be a promising therapeutic agent for RA and other diseases.

Keywords: rheumatoid arthritis; RANKL; NFATc1; miR-124

MiRNA biogenesis

MicroRNAs (miRNAs) are short, ~22-nucleotide-long non-coding RNAs that act as post-transcriptional regulators [1]. Thousands of miRNAs have been identified in animals, and they are well conserved across species. MiRNAs are first transcribed as pri-miRNAs by RNA polymerase II and are derived from individual miRNA genes or from introns, intergenic regions, or polycistronic transcripts [2, 3]. Pri-miRNAs, generally several thousand bases long, are processed in the nucleus by the RNase III enzyme Drosha into 70-100-nucleotide-long, hairpin-shaped precursors, called pre-miRNAs [4]. Pre-miRNAs are then exported from the nucleus to the cytoplasm, where the RNA endonuclease Dicer mediates their processing to generate double-stranded miRNAs [5]. The miRNA duplex is incorporated into a multicomponent protein complex known as the RNA-induced silencing complex (RISC). During this process, one strand of the miRNA duplex is selected as the mature miRNA, which guides the RISC complex to the 3'-UTR of target mRNAs [6]. MiRNAs usually bind to imperfect complementary sites in their target mRNAs. The 5' portion of a miRNA spanning bases two-eight, termed the seed region, is especially important for target recognition. [7]. MiRNAs silence gene expression via at least three mechanisms: translation inhibition, translation initiation inhibition, and target mRNA destabilization [1]. Each miRNA is predicted to regulate hundreds of different mRNAs, and miRNAs in general are predicted to control the expression of ~50% of the protein-coding genes, and to contribute to the regulation of multiple cellular processes. With the potential to target hundreds of different mRNAs via imperfect binding of target genes, miRNAs act to coordinate or fine-tune the expression of proteins to achieve physiologically optimal levels [8]. In neuroblastomas, for example, there are ~1,250 mRNAs whose expression is inversely correlated with at least one miRNA, and cross-talk between miRNAs and epigenetic regulatory mechanisms have been shown to play a role in neuroblastoma cell differentiation [9]. MiRNAs are expressed in a tissue-specific or developmental/stage-specific manner, at which they can regulate a large number of cellular processes. The changes of expression of miRNAs might influence to the development of many human diseases.

MiR-124

MiR-124 is a highly conserved miRNA that is abundantly expressed in the brain and spinal cord of humans, fish, mice, and flies [10]. Human mature miR-124 is produced from three pre-miRNAs, pre-miR-124-1, pre-miR-124-2, and pre-miR-124-3, which are derived from separate

chromosomal locations, 8p23.1, 8p12.3, and 20q13.33, respectively, and are transcribed at different levels. MiR-124 was initially identified as has-miR-124a, with the sequence UUAAGGCACGCGGUGAAUGCCA, and was registered in miRBase, a public database [11, 12]. The revised sequence is identical to the original one, but is missing the 5' and 3' terminal nucleotides [13], and is referred to as has-miR-124 in miRBase, version 10.

Regulation of miR-124

The regulation of miR-124 expression is only partially understood. Nicotine is reported to upregulate miR-124 expression by activating the α7-nicotinic acetylcholine receptor (α7nAChR) in murine macrophages [14]. In addition, either morphine or the inhibition of acetylcholine receptor signaling significantly promotes miR-124 upregulation in murine bone marrow-derived macrophages or microglia. In these cases, NF-κB p65 binds directly to the promoters of pri-miR-124-1 and pri-miR-124-3 in mice, resulting in miR-124 upregulation [15]. The same study also showed that c-Fos overexpression in BV2 microglial cells significantly inhibits miR-124 expression, while the siRNA-mediated inhibition of cAMP response element binding protein (CREB) induces miR-124 expression [15]. Notably, c-Fos and CREB are transcription factors that also regulate the expression of osteoclast-specific genes [16].

We recently found that miR-124 is not expressed in E11 cells, a cell line established from human rheumatoid synovial fibroblasts by transformation with SV40 large T antigen. In this cell line, the CpG islands in the putative promoters of pre-miR-124-1, pre-miR-124-2, and pre-miR-124-3 are methylated, and miR-124 expression is induced when the cells are treated with histone deacetylase inhibitors (unpublished data). In addition, Lujambio *et al*. found that the CpG islands in the putative promoters of these pre-miR-124s are methylated in human cancer cells [17].

MiR-124 and neuronal development

MiR-124 is a brain-enriched miRNA that contributes to physiological neural development by degrading non-neuronal transcripts [18, 19]. The polypyrimidine tract binding protein 1 (PTBP1) mRNA, which encodes a global repressor of alternative brain-specific pre-mRNA splicing in non-neuronal cells, is a target of miR-124 [20]. The blockage of miR-124 expression in mature neurons leads to a selective increase in non-neuronal transcripts [21], while an increase in miR-124 expression in non-neuronal cells results in a shift toward neuronal cell-specific gene expression [22].

Regulation of acute stress and inflammation by miR-124

Several studies indicate that miR-124 suppresses acute stress and inflammatory responses. The p38-alpha to -delta mitogen-activated protein kinases (MAPKs) are central regulatory molecules that coordinate acute stress and inflammatory responses. The expression of p38-alpha protein is directly suppressed by miR-124 in the brain [23]. In experimental autoimmune encephalomyelitis (EAE), miR-124 is downregulated in the activated microglia. The overexpression of miR-124 in macrophages directly inhibits expression of the transcription factor, CCAAT/enhancer-binding protein-alpha (CEBPα), resulting in the downregulation of its downstream target PU.1 and reduced macrophage activity. The peripheral administration of miR-124 into EAE mice causes systemic macrophage deactivation and reduced myelin-specific T cell activation, resulting in marked disease suppression [24]. In a recent study, morphine was shown to suppress innate immune responses in microglia and macrophages through the differential regulation of Toll-like receptors (TLRs) and acetylcholinesterase. Morphine induces the expression of MiR-124, which directly inhibits expression of the NF-κB subunit p65 and of TNFR-associated factor 6 (TRAF6) [16]. The vagal nerve has been shown to control the inflammatory response through a "cholinergic anti-inflammatory pathway" that is mediated by the α7-nicotinic acetylcholine receptor (α7nAChR) on macrophages. The treatment of LPS-exposed cells or mice with cholinergic agonists results in the upregulation of MiR-124, which in turn modulates lipopolysaccharide (LPS)-induced cytokine production, by directly targeting Signal transducer and activator of transcription 3 (STAT3) to decrease IL-6 production and TNF-α converting enzyme (TACE) to reduce TNF-α release [15]. These findings suggest that miR-124 is an important anti-inflammatory mediator.

Role of miR-124 in regulating chronic pain

MiR-124 is also known to regulate chronic pain. Microglia/macrophages in the spinal cord play key roles in the development of chronic pain. The microglial levels of G protein-coupled receptor kinase 2 (GRK2) are reduced in models of chronic pain. In LysM-GRK2+/− mice, which exhibit an ~50% reduction in GRK2 levels in the microglia/macrophages, prolonged inflammatory hyperalgesia develops concomitantly with ongoing spinal microglial activation. In these mice, IL-1β induces a transition from acute to persistent hyperalgesia, which is associated with reduced spinal cord microglia miR-124 levels and a switch toward a pro-inflammatory M1 microglia/macrophage phenotype, together with increased pro-inflammatory cytokine production. The treatment of these mice with miR-124 prevents the IL-1β-induced transition to persistent hyperalgesia and normalizes the expression of spinal M1/M2 markers [25].

MiR-124 and bone metabolism

Lee *et al*. recently showed that miR-124 also regulates the osteoclastogenesis of murine bone marrow macrophages by directly suppressing the nuclear factor of activated T cell cytoplasmic 1 (NFATc1) expression [26]. They also found that the overexpression of constitutively active NFATc1 prevents the miR-124-mediated inhibition of osteoclastogenesis. Moreover, miR-124 suppresses the proliferation and motility of osteoclast precursors, and the reduced motility of the osteoclast precursors is associated with reduced RhoA and Rac1 expression. In addition, Okamoto *et al*. reported that miR-124 is an important regulatory factor involved in the osteoblastic differentiation of murine iPS cells [27]. Thus, miR-124 is emerging as a key regulator of bone formation, with roles in both osteoclastic and osteoblastic differentiation.

MiR-124 and cancer regulation

MiR-124 is a potential tumor suppressor and an independent prognostic marker in many cancers, including colorectal [28] and prostate cancer [29]. The loss of miR-124 expression is a common event in prostate cancer, and the restoration of miR-124 expression inhibits prostate cancer cell growth by directly targeting the androgen receptor and inducing p53 upregulation [29]. In addition, miR-124 directly suppresses STAT3 expression, resulting in the increased apoptosis and reduced growth of colorectal cancer cells [30]. The mechanism of hepatocellular carcinoma (HCC) was recently shown to involve a positive feedback loop consisting of the IL-6 receptor, STAT3, miR-24, miR-629, hepatocyte nuclear factor 4-alpha (HNF4-α), and miR-124. HNF4-α is a member of the nuclear receptor family of ligand-dependent transcription factors, and its downregulation promotes HCC initiation. HCC cells and tumors exhibit increased levels of miR-24 and miR-629, each of which directly inhibits HNF4-α expression, which leads to reduced miR-124 expression. Since the IL-6 receptor is a direct target of miR-124, the reduced HNF4-α expression results in increased IL-6 receptor expression, which promotes STAT3 phosphorylation. The presence of STAT3-binding sites in the promoters of miR-24 and miR-629 complete the positive feedback loop, to maintain hepatocyte transformation and induce HCC progression. Notably, the systemic administration of miR-124 suppresses HCC in an animal model, suggesting that this agent may have the potential for treating human liver cancer [31]. MiR-124 also directly reduces the expression of phosphoinositide 3-kinase catalytic

subunit alpha (PIK3CA), and the treatment of HCC cells with miR-124 was found to inhibit the PI3K/Akt pathway, resulting in reduced cell survival and proliferation [32]. In a separate study, miR-124 was shown to directly suppress the expression of two oncogenes, Rho-associated protein kinase 2 (ROCK2) and Enhancer of zeste homolog 2 (EZH2). The expression level of miR-124 is frequently reduced in HCC cells and tissues, and a low miR-124 expression level is significantly associated with a more aggressive and/or poor prognostic phenotype of patients with HCC [33]. In breast cancer, miR-124 directly targets the epithelial mesenchymal transition (EMT) regulator Slug, which is an E-cadherin transcription repressor. MiR-124 levels are frequently reduced in breast cancer cells, and the restoration of miR-124 expression in these cells increases the expression of E-cadherin, a repressor of cell invasion and metastasis [34]. MiR-124 directly regulates the cell cycle dependent kinase 6 (CDK6) expression in medulloblastoma, and the treatment of medulloblastoma cells with miR-124 results in significantly decreased cell growth and reduced CDK6 expression [35]. MiR-124 also downregulates integrin β1 (ITGB1) in oral squamous cell carcinoma cells, and the introduction of MiR-124 into these cells reduces their adherence and motility [36]. In B-cell lymphomas, miR-124 directly targets NF-κB p65, and the introduction of miR-124 into these cells suppresses MYC and BCL2, and inhibits cell proliferation and survival [37].

Mir-124 and pulmonary arterial smooth muscle

The abnormal proliferation and differentiation of pulmonary arterial smooth muscle cells (PASMCs) contributes to the pathogenesis of cardiovascular disorders. Hypoxia results in the down-regulation of miR-124 in human PASMCs, consistent with the activation of NFAT during this process. In human PASMC, miR-124 directly regulates multiple target genes, including a component of the NFAT pathway, NFATc1, and two regulators of NFAT signaling, calmodulin-binding transcription activator 1 (CAMTA1) and PTBP1. MiR-124 overexpression inhibits PASMC proliferation and prevents phenotypic changes [38]. The miR-124 expression is also reduced in fibroblasts isolated from patients with severe pulmonary hypertension. The overexpression of miR-124 significantly attenuates the proliferation, migration, and expression of monocyte chemotactic protein-1 (MCP-1) in the activated fibroblasts. In addition to targeting MCP-1 in these cells, MiR-124 targets the alternative splicing factor, PTBP1, and MiR-124 overexpression results in reduced PTBP1 expression and the subsequent regulation of Notch1/phosphatase, tensin homolog/FOXO3/p21Cip1, and p27Kip1 signaling. Furthermore, miR-124 expression is suppressed by histone deacetylases, and the treatment of activated fibroblasts with histone deacetylase inhibitors increases the miR-124 expression [39].

RA Pathogenesis

RA is a chronic inflammatory disorder of unknown etiology that is associated with progressive disability, systemic complications, early death, and high socioeconomic costs. RA occurs in 0.5-1.0% of the adult population worldwide [40]. RA patients present with joint swelling and pain, associated with synoviocyte hyperplasia, which if left untreated, leads to pannus formation and the destruction of bone and cartilage [41]. T cells, B cells, dendritic cells, and other leucocytes infiltrate the rheumatoid synovial tissue, where they produce cytokines and chemokines that cause many of RA's pathological and clinical manifestations [41]. Three prominent cytokines associated with RA, interleukin (IL)-17, IL-6, and TNF-alpha, enhance local inflammation, which further increases the production of inflammatory mediators. IL-6, in particular, activates the JAK-STAT3 pathway [40, 42]. Various cytokines induce the expression of receptor activator for nuclear factor kB ligand (RANKL) on osteocytes and other mesenchymal cells that support osteoclastogenesis [40, 43]. RANKL specifically and strongly induces the expression of NFATc1, which is the master regulator of osteoclast differentiation [44]. NFATc1 cooperates with other transcription factors to regulate a number of osteoclast-specific genes that have roles in osteoclastogenesis and bone loss [42].

RA treatment strategies

The current recommendations for RA treatment involve the early initiation and long-term administration of conventional, synthetic disease modifying anti-rheumatic drugs (csDMARDs), including the commonly used csDMARD, methotrexate (MTX) [45, 46]. For patients with inadequate responses to csDMARDs or who initially demonstrate high disease activities, biologic DMARDs (bDMARDs), also called "biologics," which target TNF-alpha, IL-6, and T cells, are recommended as either add-on or independent treatments to achieve better disease control [45, 46]. In the current "biologics era" of RA treatment "treat to target" (T2T) is a new treatment strategy, in which patients are treated to achieve either disease remission or low-disease activity. Although this strategy has greatly improved the outcomes for many RA patients, some patients remain non- or only partially responsive to current treatments. Because biologics or combination treatments do not work for all RA patients, and yet are very costly, additional therapies that are more efficacious for this group of patients are needed [47].
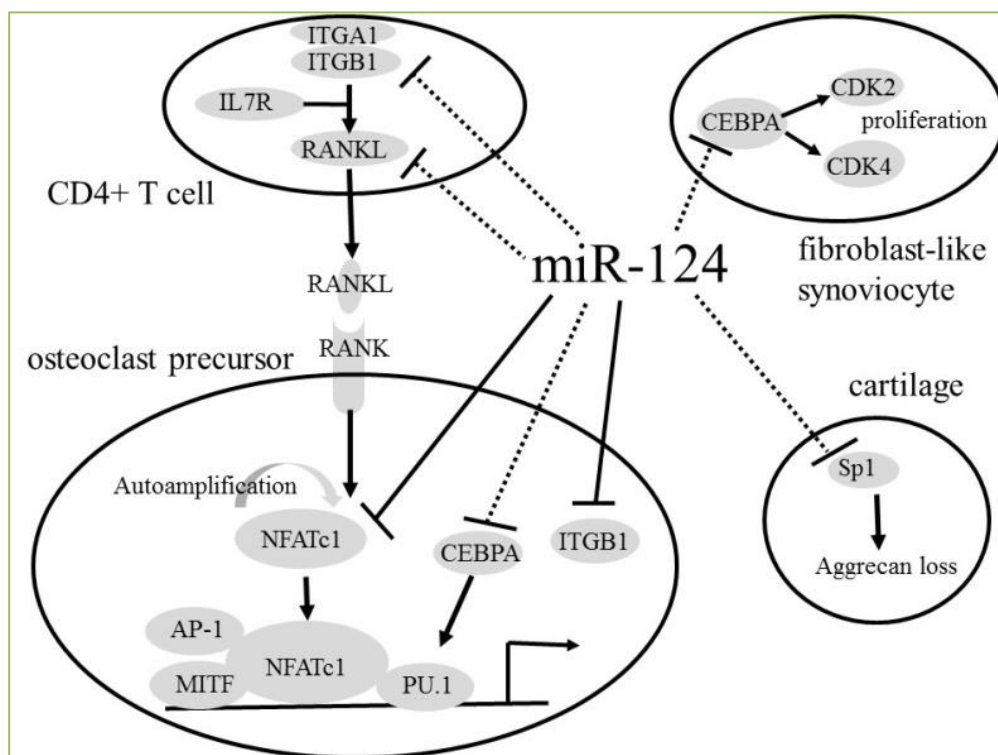
Figure 1. Target molecules of miR-124 in rat adjuvant-induced arthritis. ITGA1: integrin α1, ITGB1: integrin β1, IL7R: interleukin-7 receptor, RANKL: receptor activator of nuclear factor kappa-B ligand, NFTAc1: nuclear factor of activated T-cells cytoplasmic 1, CEBPA: CCAAT/enhancer-binding protein alpha, AP-1: activator protein 1, MITF: microphthalmia-associated transcription factor, PU.1: spleen focus forming virus proviral integration oncogene, SPI1, CDK2: cyclin-dependent kinase 2, SP1: Specificity protein 1.

MiR-124 and RA

We previously reported that five miRNAs (miR-146a, miR-223, miR-142-3p, miR-142-5p, and miR-133a) are expressed more strongly in RA than in OA FLS. In contrast, MiR-124a is the only miRNA whose expression is reduced in RA compared with OA FLS. The transfection of precursor (pre)-miR-124a into RA FLS significantly suppresses FLS proliferation by arresting the cell cycle in the G1 phase and suppressing the secretion of MCP-1. Furthermore, miR-124a directly affects the production of both CDK-2 and MCP-1 by regulating the translation of their mRNAs [48]. We investigated the in vivo effects of miR-124 on inflammation using the rat adjuvant induced arthritis (AIA) model [49]. The endogenous expression of miR-124 in the ankle joints of AIA rats was found to be significantly lower than that in the ankle joints of non-AIA rats. Pre-miR-124 injection into the right ankle significantly elevated the miR-124 in that ankle and slightly elevated it in the left ankle of AIA rats. Notably, a single injection of pre-miR-124 into one ankle joint was found to suppress arthritis in all four paws. A comparison of body weights in the treated and non-treated mice indicated that the pre-miR-124 treatments were not associated with any apparent systemic toxicity. Histopathological analysis of the ankles revealed that the synoviocyte proliferation, leucocyte infiltration into synovial tissue, and the destruction of cartilage and bone were all improved in the AIA rats treated with pre-miR-124. Furthermore, micro-CT analysis revealed that miR124 attenuates the bone destruction in AIA rats, and tartrate-resistant acid phosphatase (TRAP) staining of the ankle joints revealed that miR-124 reduces osteoclast differentiation. RANKL, which is essential for osteoclast differentiation and activation, was found to be significantly suppressed in the ankles of AIA rats treated with pre-miR-124. Next, we analyzed the target genes of rat miR-124 using luciferase assays, and found that NFATc1, ITGB1, specificity protein 1 (Sp1), and CEBPα are all regulated by miR-124. The NFATc1 and ITGB1 protein levels were found to be reduced in the ankle tissues of the pre-miR-124-treated AIA rats. Notably, the ITGB1-expressing cells were found to be TRAP-positive osteoclasts. Thus, our studies revealed that treating rats with pre-miR-124 suppresses the development of AIA and directly reduces osteoclast differentiation (Figure 1).

Conclusions

Recent studies indicate that miR-124 is involved in the

pathogenesis of cancers, acute stress, cardiovascular disorders, inflammatory responses, chronic pain, and osteoclast differentiation. MiR-124 levels are suppressed in RA tissue, and the treatment of AIA rats with pre-miR-124 suppresses the progression of joint damage. Thus, miR-124 is a potential therapeutic target for RA and other diseases.

Conflicting interests

The authors have declared that no conflict of interests exist.

Abbreviations

RANKL: receptor activator for nuclear factor kB ligand; NFATc1: nuclear factor of activated T cell cytoplasmic 1; miR-124: microRNA-124; CDK-2: cell cycle dependent kinase 2; MCP-1: monocyte chemotactic protein-1; SP1: specificity protein 1; CEBPα: CCAAT/enhancer-binding protein-alpha.

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