

The Role of miRNA in Rheumatoid Arthritis

Seiji Kawano and Yuji Nakamachi
Kobe University
Japan

1. Introduction

MicroRNAs (miRNAs) are a well-established class of small (~22 nucleotides) endogenous non-coding RNAs that influence the stability and translation of messenger RNA (mRNA) (van Rooij et al., 2007). Hundreds of miRNA have been identified in numerous animal species. The miRNA genes are transcribed by RNA polymerase II as primary miRNA (pri-miRNA). The RNase III enzyme Drosha then processes the nuclear pri-miRNA, to precursor miRNA (pre-miRNA), which is exported from the nucleus with the help of exportin. Maturation of the pre-miRNA into miRNA is then mediated by the cytoplasmic enzyme Dicer, after which the single-stranded mature miRNA is loaded into the RNA-induced silencing complex (RISC). Once loaded, the miRNA guides this complex to the 3'-untranslated region (3'-UTR) of target mRNA (Figure 1). The so-called 'seed region' (nucleotides 2-8) of miRNA is most important for target recognition and silencing. miRNA usually binds with imperfect complementarity to its target, which is called the 'seed sequence'. Association of miRNA with its target mRNA silences expression via at least three mechanisms: inhibition of translation, inhibition of the initiation of translation and destabilization of target mRNA.

Recent advances have shown that miRNA expression during development is highly tissue-specific, which suggests that miRNA may be involved in specifying and maintaining tissue identity. Recent studies have shown that a single miRNA can generate a huge impact to the whole profile of protein expression (Selbach et al., 2008; Baek et al., 2008). For most interactions, microRNAs act as 'rheostats' to make fine-scale adjustments to protein output (Baek et al., 2008). Several miRNAs have been shown to regulate the 3'-UTR of mRNA that encode transcription factors, and a circuit that sequentially involves miRNA and transcription factors in a mutual negative feedback loop has been described (Tsang et al., 2008). The role of miRNA on protein expression, therefore, is not one-directional: from miRNA to protein. If we consider, however, a simple setting of miRNA and its direct target mRNA, the miRNA upregulated in a particular cell type from patients with specific disease possibly put the cell in short of a group of proteins which are necessary to maintain the physiological homeostasis by targeting the translation of those proteins. In contrast, the miRNA downregulated in a particular cell type from patients with specific disease might allow the cell to increase translation of a group of proteins that are suppressed at low levels in normal conditions.

In 2009, the direct involvement of single nucleotide polymorphisms of a miRNA in human hereditary disease was reported for the first time (Mencia et al., 2009). They found that two different single nucleotide polymorphisms of miR-96 are related the hearing loss found in two families. Their report was the start of a new era, in which the involvement of genetic

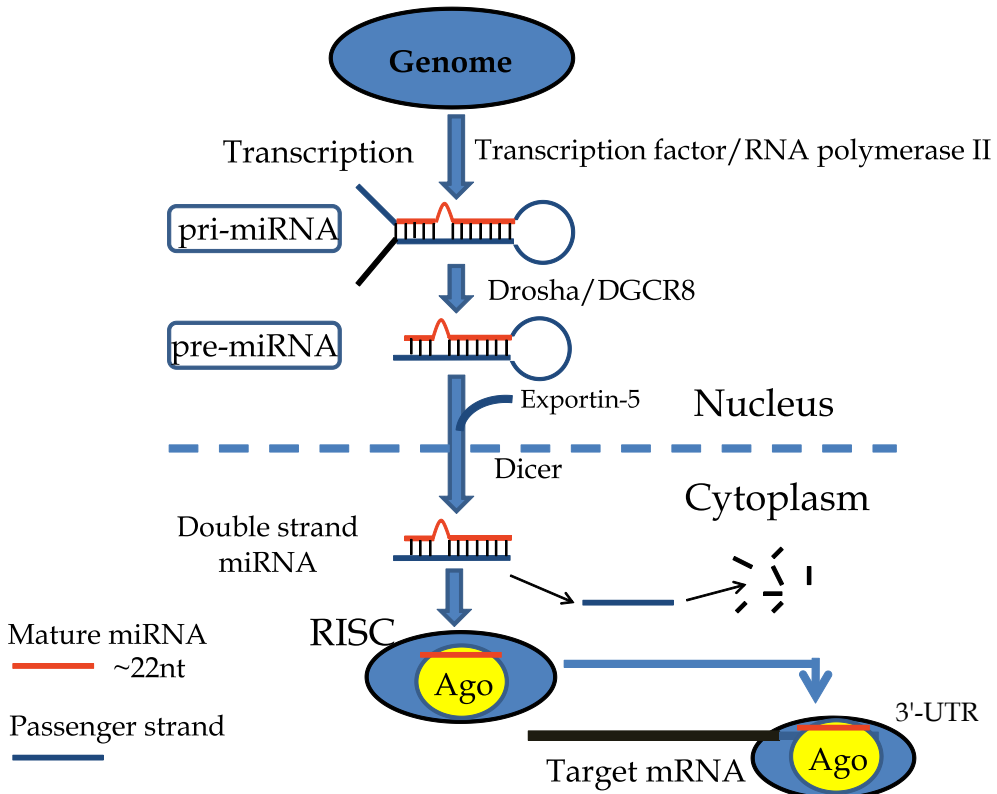


Fig. 1. Biogenesis of miRNA

changes of non-coding RNAs in hereditary diseases and possibly multifactorial disorders such as autoimmune diseases should be considered. As we discuss in this review, our understanding of RA pathogenesis has been enriched by recent miRNA studies, it is now obvious that miRNAs play important roles in the critical aspects of RA pathogenesis such as joint destruction, inflammation, proliferation of synoviocytes, and chemotaxis of inflammatory cells. We summarize the recent advances of RA studies focusing on miRNAs, and provide the current understanding of the role of miRNAs in RA pathogenesis.

2. Immunity and miRNA

More than 100 miRNAs are expressed by cells of the immune system, and they have the potential to broadly influence the molecular pathways that control the development and function of innate and adaptive immune responses (O'Connell et al., 2010). miRNA levels are dynamically regulated during lineage differentiation of haematopoietic stem cells and also during the course of the immune response, including innate immunity and acquired immunity. Animal experiments of the ablating-specific miRNA genes have shown that miRNA expression in haematopoietic cells is critical for mounting an appropriate immune response. In innate immunity, miRNAs have unique effects on granulocytes, monocytes/

macrophages, dendritic cells and natural killer cells. In particular, miR-146, miR-155, miR-147, miR-21 and miR-9 have been reported to regulate the macrophage activation via a Toll-like receptor pathway or cytokine production. miRNAs are also involved in acquired immunity by modulating the differentiation and function of T and B cells. miR-17-92 cluster targeting BIM and PTEN and miR-181a targeting DUSP5, DUSP6, SHP2 and PTPN22 are important for T-cell development in the thymus. For T-cell function, miR-142, miR-146, miR-150, miR-155, and others have been reported to regulate the development of functional T cells such as Th1, Th2, Th17, and Treg.

Recently, mutant mice with a targeted deletion of miR-146a (Boldin et al., 2011) have been created. *Baldin et al* have found that miR-146a is expressed predominantly in immune tissues, and its expression can be induced in immune cells upon cell maturation and/or activation. Lack of miR-146a expression results in hyperresponsiveness of macrophages to bacterial Lipopolysaccharide (LPS) and leads to an exaggerated inflammatory response in endotoxin-challenged mice. In contrast, overexpression of miR-146a in monocytes has the opposite effect. Interestingly, miR-146a-null mice developed a spontaneous autoimmune disorder, characterized by splenomegaly, lymphadenopathy, and multiorgan inflammation, resulting in premature death. Using a combination of gain and loss of function approaches, they confirmed TRAF6 and IRAK1 genes as miR-146a targets, whose derepression in miR-146a-null mice might account for some of the observed immune phenotypes. In addition, they found that miR-146a seems to play a role in the control of immune cell proliferation; aging miR-146a-null mice display an excessive production of myeloid cells and develop frank tumors in their secondary lymphoid organs, suggesting that miR-146a can function as a tumor suppressor in the context of the immune system. Their results showed that miR-146a as an important negative regulator of inflammation, myeloid cell proliferation, and cancer.

2.1 miRNA and Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic disease of unknown cause that presents a characteristic inflammatory features, including synovial hyperplasia, which results in pannus formation and joint destruction. The local production of cytokines and chemokines accounts for many of the pathological and clinical manifestations of RA. In culture, RA fibroblast like synovial cells (FLS) proliferate and secrete a variety of cytokines/chemokines/angiogenic factors, including fibroblast growth factor, granulocyte-macrophage colony stimulating factor, interleukin 6 (IL-6), IL-8, monocyte chemoattractant protein 1 (MCP-1) and macrophage inflammatory proteins 1 α , and they present various adhesion molecules on their surfaces. Since the first report by *Stanczyk et al* in 2008, several reports have described the altered miRNAs in the joint and/or peripheral blood leucocytes of patients with RA, including miR-155, miR-146 and others (Table 1) (*Stanczyk, et al., 2008; Nakasa, et al., 2008; Pauley, et al., 2008; Murata, et al., 2010; Li, et al., 2010; Fulci, et al., 2010; Nakamachi, et al., 2009. Niimoto, et al., 2010*).

2.1.1 Overexpressed miRNAs in RA

Stanczyk et al reported that constitutive expression of miR-155 and miR-146a was higher in RA synovial fibroblasts (RASf) than in those from patients with osteoarthritis (OA), and expression of miR-155 was induced by tumour necrosis factor α (TNF- α), IL-1 β , LPS, polyinosinic/polycytidylic acid. Enforced expression of miR-155 in RASf was found to

repress the levels of matrix metalloproteinase 3 (MMP-3) and reduce the induction of MMP-3 and MMP-1 by Toll-like receptor ligands and cytokines. Moreover, RA synovial fluid monocytes displayed higher levels of miR-155 than peripheral blood monocytes. *Nakasa et al* reported that miR-146 is expressed in RA synovial tissue and its expression is induced by stimulation with TNF- α and IL-1 β . They also showed by in situ hybridisation that CD68-positive macrophages and CD3 T cells strongly express miR-146 (Nakasa et al., 2008). *Pauley et al* reported that peripheral blood mononuclear cells (PBMCs) from patients with RA exhibit significantly increased expression levels of miR-16, miR-132, miR-155 and miR-146a compared with healthy and disease control individuals (Pauley et al., 2008). Furthermore, they demonstrated that high levels of miR-16 and miR-146a expression correlated with active disease. Although miR-146a expression is increased in patients with RA, levels of the two established miR-146a targets, TNF receptor-associated factor 6 (TRAF-6) and IL-1 receptor-associated kinase 1 (IRAK-1), in patients with RA are similar to those in control individuals. Recently, *Murata et al* reported that synovial fluid concentrations of miR-16, miR-146a, miR-155 and miR-223 in patients with RA were significantly higher than those in patients with OA. In addition, plasma miRNAs or ratio of synovial fluid miRNAs to plasma miRNAs, including miR-16 and miR-146a, significantly correlated with tender joint counts and 28-joint Disease Activity Score (DAS-28) (Murata et al., 2010).

Upregulated miRNA	Downregulated miRNA	tissue	Ref.
miR-146, miR-155		SF	Stanczyk et al, 2008
miR-146		ST	Nakasa et al, 2008
miR-16, miR-132, miR-146a, miR-155		PBMC	Pauley et al, 2008
miR-16, miR-146a, miR-155, miR-223		JF	Murata et al, 2010
miR-146a	miR-363, miR-498	CD4T	Li et al, 2010
miR-223		CD4T	Fulci et al, 2010
Let-7a, miR-26, miR-146a/b, miR-150, miR-155		IL-17+CD4 T	Niimoto et al, 2010
miR-133a, miR-146a, miR-142-3p, miR-142-5p, miR-223	miR-124a	SF	Nakamachi et al, 2009

SF: Synovial fibroblast, ST: Synovial tissue, PBMC: Peripheral blood mononuclear cell, JF: Joint fluid, CD4T: peripheral blood CD4+T cells.

Table 1. miRNA expression in Rheumatoid Arthritis

These studies showed that miR-146 is strongly expressed in synovial fibroblasts and mononuclear cells from patients with RA. *Li et al* reported that the level of miR-146a expression was positively correlated with levels of TNF- α , and in vitro studies showed that TNF- α upregulated miR-146a expression in T cells (Li et al., 2010). They also reported that miR-146a overexpression was found to suppress Jurkat T-cell apoptosis. Interestingly,

transcriptome analysis of miR-146a overexpression in T cells identified Fas associated factor 1 as a miR-146a-regulated gene, which was critically involved in modulating T-cell apoptosis. *Nimoto et al* reported that six miRNAs, let-7a, miR-26, miR-146a/b, miR-150, and miR-155 were significantly upregulated in the IL-17 producing CD4⁺ T cells from RA patient (*Nimoto et al*, 2010). miR-146a was intensely expressed in RA synovium in comparison to OA. miR-146a expressed intensely in the synovium with hyperplasia and high expression of IL-17 from the patients with high disease activity. Double staining revealed that miR-146a was expressed in IL-17 expressing cells. These results indicated that miR-146a was associated with IL-17 expression in the PBMC and synovium in RA patients. Recently, *Nasaka et al* reported that the number of TRAP-positive multinucleated cells in human PBMCs was significantly reduced by miR-146a in a dose-dependent manner when isolated PBMCs from healthy volunteers were transfected with double-stranded miR-146a and cultured in the presence of M-CSF and either TNF- α or RANKL (*Nasaka et al.*, 2011). The expression of c-Jun, NF-ATc1, PU.1, and TRAP in PBMCs was significantly down-regulated by miR-146a. Their results suggest that miR-146a might suppress the osteoclastogenesis from human monocytes. Thus, our knowledge about the roles of miR-146a has been expanding to various cell types including not only synoviocytes, monocytes, lymphocytes but also differentiated functional cells such as Th17 cells and osteoclasts. Taken together, several miRNAs have been repeatedly described as upregulated in RA studies. Among those, the most redundant miRNA has been miR-146 and miR-155. Interestingly, miR-146 is downregulated in PBMCs from patients with systemic lupus erythematosus (SLE) (*Tsang et al.*, 2009). This contrast is attractive in illustrating a difference in the cytokine profiles of RA and SLE, with type I interferon playing a dominant role in SLE, whereas TNF- α , IL-1 and IL-6 are the principal cytokines in RA (*Chan et al.*, 2009).

2.1.2 Manipulation of overexpressed miRNAs in arthritis models

Several groups have been focused on particular miRNAs upregulated in human RA tissues. They downregulated those upregulated miRNAs in human RA by targeting miRNA expression in mouse models of inflammatory arthritis and examined if the suppression of those miRNAs could regulate the arthritis development. *Nasaka et al* reported that administration of miR-146a prevented joint destruction in mice with collagen-induced arthritis (CIA) when double-stranded miR-146a or nonspecific double-stranded RNA was administered twice by intravenous injection, although it did not completely ameliorate inflammation (*Nasaka et al.*, 2011). Their results indicate that expression of miR-146a inhibits osteoclastogenesis and that administration of double-stranded miR-146a prevents joint destruction in arthritic mice. *Blüml et al* examined the role of miRNA in the pathogenesis of autoimmune arthritis, using CIA and K/BxN serum-transfer arthritis in wild-type (WT) and miR-155-null mice (*Blüml et al*, 2011). They found that the miR-155-null mice did not develop CIA. Deficiency in miR-155 prevented the generation of pathogenic autoreactive B and T cells, since anti-collagen antibodies and the expression levels of antigen-specific T cells were strongly reduced in miR-155-null mice. Moreover, Th17 polarization of miR-155-null mouse T cells was impaired, as shown by a significant decrease in the levels of IL-17 and IL-22. In the K/BxN serum-transfer arthritis model, which only depends on innate effector mechanisms, miR-155-null mice showed significantly reduced local bone destruction, attributed to reduced generation of osteoclasts, although the severity of joint inflammation was similar to that in WT mice. They concluded that miR-155 is

essentially involved in the adaptive and innate immune reactions leading to autoimmune arthritis. These two reports implicate that the manipulation of overexpressed miRNAs in PBMCs or synoviocytes such as miR-146a and miR-155, may provide a novel target for the treatment of patients with RA.

2.1.3 Suppressed miRNA in RA

There have been two reports regarding the suppressed miRNAs in RA. *Li et al* reported that miR-146a expression was significantly upregulated while miR-363 and miR-498 were downregulated in CD4⁺ T cells of RA patients (Li et al., 2010). However, their analyses were focused on miR-146a but not on miR-363 and miR-498. We compared synovial fibroblast derived from patients with RA (RASF) with those from patients with OA (OASF) for their expression of a panel of 156 miRNAs with quantitative stem-loop RT-RPCR (Nakamachi et al., 2009). We found that the miR-124a level significantly decreased in RASF. Five other miRNAs (miR-146a, miR-223, miR-142-3p, miR-142-5p and miR-133a) were, on the other hand, expressed more strongly in RA than in OA. In published reports, the expression of miR-124a has been restricted to the brain and nerve tissues of animals and insects (Kloosterman et al., 2006). In those tissues, miR-124a contributes to the differentiation of neural progenitors into mature neurons through degradation of non-neuronal transcripts. In addition to neural tissue development, miR-124a also appears to be involved in carcinogenesis (Agirre et al., 2009).

To investigate the function of miR-124a in RA, we started with the transfection of precursor miR-124a (pre-miR-124a) into RASF and it significantly suppressed their proliferation and arrested the cell cycle at the G1 phase. Interestingly, transfection with pre-miR-124a suppressed proliferation of RASF, but did not induce cell death. These results suggest that the low expression of miR-124a in RASF might protect them from cell-cycle arrest, thereby promoting cell proliferation. For comparison, we transfected pre-miR-146a, pre-miR-223, premiR-142-3p and pre-miR-133a, all of which were elevated in RASF, into OASF, but transfection of these pre-miRNAs did not promote the proliferation of OASF.

A computer search with miRanda 3.0 database (<http://microrna.sanger.ac.uk/>) to find possible target mRNAs for miR-124a binding in the 3'-UTR, identified a putative consensus site in cyclin-dependent kinase 2 (CDK-2) mRNA. The induction of precursor miR-124a (pre-miR-124a) in RA synoviocytes significantly suppressed the production of the CDK-2 proteins. A luciferase reporter assay demonstrated that miR-124a specifically suppressed the reporter activity driven by the 3'-UTR of CDK-2 mRNA but not reporters driven by the mutant of 3'-UTR of CDK-2 mRNA. We also found that CDK-6 protein, another CDK that regulates the G1-S phase, was suppressed by miR-124a. These results suggest that miR-124a has a key role in regulating the proliferation of RASF. Based on these results, we assumed that the suppressed expression of miR-124a resulted in high expression of a group of proteins, especially cell-cycle-related proteins. We next measured the levels of cytokines/chemokines in culture medium conditioned with RASF after overexpression of miR-124a, and we detected changes in the levels of three of them: MCP-1, angiogenin and vascular endothelial growth factor (VEGF). While VEGF levels were increased compared with those in medium conditioned with control cells, MCP-1 and angiogenin levels were significantly decreased in the presence of miR-124a overexpression. We also transfected precursors of miR-133a, miR-142-3p, miR-146a and miR-223 into OASF and analysed the culture media for cytokines/chemokines.

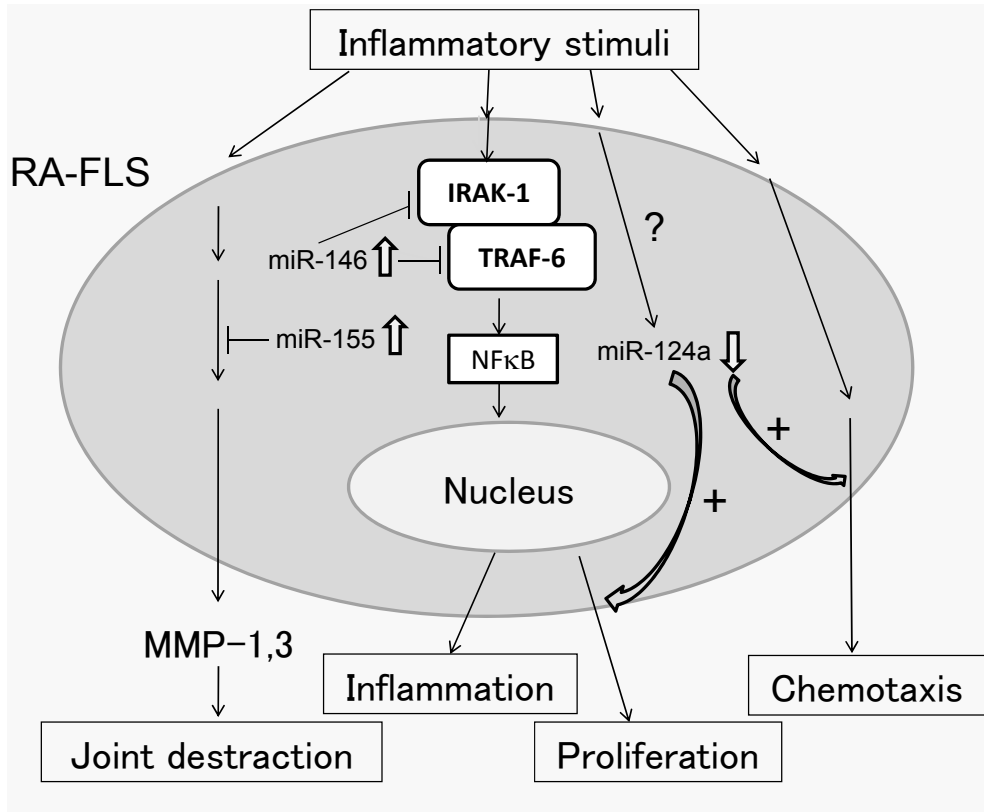


Fig. 2. The role of miRNAs in RASF

In contrast to the findings with pre-miR-124a, the overexpression of these pre-miRNAs did not stimulate any cytokine/chemokine secretion from OASF. We then used the miRanda 3.0 database to search for the 3'-UTR sequences of the mRNA encoding MCP-1, angiogenin and VEGF, and found that only MCP-1 mRNA contained a seed sequence for miR-124a. Subsequent luciferase assays showed that miR-124a specifically suppressed the luciferase activity driven by the 3'-UTR of MCP-1 mRNA. To elucidate the question what kind of stimulants depress the expression of miR-124a, we treated RASF and OASF with TNF- α , IL-1 β , IL-6, IL-12, IL-17, IL-18, interferon α or lipopolysaccharide. However, none of these mediators elicited a change in miR-124a level. Possibly this unresponsiveness is the result of the silencing by hypermethylation of miR-124a loci, since the loci in cancer cell lines have been reported to be hypermethylated (Lujambio et al., 2007). Demethylation treatment with 5-aza-2'-cytidine (5-azaC) did not suppress the proliferation of RASF, and methylation-sensitive PCR revealed that the CpG islands of miR-124a loci in RASF were not methylated (Nakamachi Y, unpublished data, 2009). The hypomethylated status of miR-124a loci reflects the suggestion of Karouzakis *et al* that genes are frequently hypomethylated in RA (Karouzakis et al., 2009). At present, the search for factors or epigenetic events that can induce the suppression of miR-124a in RA pathogenesis is underway.

2.2 Polymorphism of miRNA and its target mRNA in RA

RA is clinically heterogeneous, but two disease subgroups of RA can now be clearly defined according to the presence or absence of auto-antibodies against citrullinated proteins (ACPA) (Klareskog et al., 2009). Recently, thanks to genome-wide association (GWA) studies to analyze the genetic predisposition to RA, many susceptibility loci such as PTPN22, TRAF1-C5, OLIG3-TNFAIP3, CD40, CCL21 and STAT4 have been identified in addition to the well established HLA-DRB1 shared epitope alleles (Stahl et al., 2010). Now, evidence emerges of the existence of different genetic backgrounds and immune-response pathways contributing to the development of RA in ACPA-positive and ACPA-negative subgroups of patients. The interruption of miRNAs in the arena of gene regulatory networks has triggered interest from geneticists for looking at naturally occurring miRSNP, a SNP located at or near a miRNA binding site in 3'-UTR of the target gene or in a miRNA (Ryan et al., 2010). *Martin et al* demonstrated that the +1166 A/C polymorphism occurs in the 3'-UTR of the human AT1R(angiotensin II type 1 receptor) gene that has been associated with cardiovascular disease, possibly as a result of enhanced AT1R activity, for unknown reason (Martin et al., 2007). They assume that the +1166 A/C polymorphism disrupts the basepairing complementarity and the ability of miR-155 to interact, thus alleviating miR-155-mediated repression of AT1R translation, and leading to AT1R over-expression, and possibly to cardiovascular disease. Several other examples of miRSNP are now reported.

There is an only one report analyzing the polymorphism of miRNA and its associated gene in RA. *Chatzikyriakidou et al* investigated the potential association of the miR-146a variant rs2910164 and the two polymorphisms located in the 3'-UTR of IRAK-1 gene (rs3027898 and rs1059703), a known target for miR-146a, with RA susceptibility (Chatzikyriakidou et al., 2010). Using cohorts of 136 RA patients and of 147 healthy donors, the authors found a significant difference in the distribution of IRAK-1 rs3027898 A>C genotypes between RA patients and controls. The same association was recently reported by the authors for psoriatic arthritis and ankylosing spondylitis (PsA) (Chatzikyriakidou et al., 2009) and by others for atherothrombotic cerebral infraction (Yamada et al., 2008). However, no difference was observed in the distribution of IRAK-1 rs1059703 and miR-146a rs2910164 variants between RA and control individuals. Thus, no miRSNP has yet been found functionally associated with RA. Therefore, it would be of real interest to assess whether the observed polymorphism has potential consequences on IRAK-1 expression levels and to link a mutation in the 3'-UTR of IRAK-1 with putative binding site for other miRNAs than miR-146a. Another track would be to search for 3'-UTR motif(s) located in the vicinity of the miRNA target sequence, which may be coupled to regulation by miRNA, as it is suggested by a recent publication (Jacobsen et al., 2010).

Finally, although five groups have published since 2008 that miR-146a is over-expressed in RA tissues; it is not a RA-specific miRNA, as it has been found deregulated in many other human disorders. There is thus much room for studies identifying at RA-specific miRNAs and investigating the impact of miRSNPs on RA in particular and on human pathologies in general for the next decade.

2.3 Epigenetics of miRNA in RA

Another important and emerging field of study for autoimmune diseases, including RA, is represented by epigenetics. The three main mechanisms of epigenetic control (DNA methylation, histone modifications, and miRNA regulation) interact in the development of the RA-synovial fibroblast phenotype (Karouzakis et al., 2009a). RA synoviocytes show

epigenetic abnormalities, mainly represented by DNA hypomethylation and histone hyperacetylation, thus leading to synovial proliferation (Brooks et al., 2010). In RA synovial cells overexpression of histone deacetylase has been demonstrated at the transcriptional level, and the use of histone deacetylase small-interfering RNA has revealed that this enzyme plays an important role in the synoviocyte proliferation and apoptosis (Horiuchi et al., 2009). Another study showed that synoviocyte hyperacetylation was associated with an increase of histone acetyl transferases, without variation of the histone deacetylases (Huber et al., 2007). DNA hypomethylation is another epigenetic modification that can occur in RA, leading to the up-regulation of genes coding for growth factors, receptors, adhesion molecules, and other components responsible for the inflammatory milieu and active phenotype of RASFs (Karouzakis et al., 2009b).

PCR-based screening of 260 individual miRNAs which were differentially expressed miRNAs in RASF versus OASF revealed that expression of miR-203 was higher in RASF than in OASF or fibroblasts from healthy donors (Stanczyk et al., 2011). Levels of miR-203 did not change upon stimulation with IL-1 β , TNF- α , or LPS; however, DNA demethylation with 5-azaC increased the expression of miR-203. Enforced expression of miR-203 led to significantly increased levels of MMP-1 and IL-6. Induction of IL-6 by miR-203 overexpression was inhibited by blocking of the NF- κ B pathway. Basal expression levels of IL-6 correlated with basal expression levels of miR-203. These results showed that the production of miR-223 exerts its role as a pro-inflammatory miRNA in response to the signal via NF κ B pathway and its level is influenced by methylation/ demethylation status. These observations show that epigenetic control is deficient in RA joint cells, suggesting that broader analysis is required to better understand the role of these mechanisms in the pathogenesis of RA, the identification of epigenetic biomarkers, and the development of specific therapies targeting key molecules of the epigenetic process.

3. Biomarkers for RA

A biomarker is a physical sign or cellular, biochemical, molecular or genetic alteration by which a normal or abnormal biologic process can be recognized or monitored, or both, and that might have diagnostic or prognostic utility (Illei et al., 2004). Biomarkers have several potential applications in rheumatic diseases. Genetic markers can predict or quantify the risk or the severity of diseases in populations or individuals. Classical examples include the increased risk of ankylosing spondylitis in carriers of the HLA-B27 allele (Thomas and Brown, 2010), and the association between the 'shared epitope' and RA (de Vries et al., 2005). An increasing number of genetic polymorphisms have been identified as risk factors for autoimmune diseases in general (Gregersen and Olsson, 2009; Mackay, 2009) as well as for specific diseases (Scofield, 2009). Autoantibodies are frequently used to establish or confirm a diagnosis. Some, such as anti-cyclic citrullinated peptide (CCP) antibodies in RA, are fairly specific for a particular disease, whereas others, such as antinuclear antibodies and anti-Ro (SS-A) antibody are present, albeit at different frequencies and levels, in a number of conditions (Schulte-Pelkum et al., 2009).

Once a diagnosis is established, some biomarkers provide prognostic information regarding disease progression and severity. For example, the combination of anti-CCP antibody positivity and the presence of the shared epitope defines a subset of patients with a severe form of RA (Kaltenhauser et al., 2007; Sanmarti et al., 2007), whereas the presence of specific autoantibodies define distinct subsets of inflammatory myopathies (Gunawardena et al.,

2009). Other biomarkers are used to monitor the degree of immunologic activity or inflammation. Measures of levels of complement activation and of anti-double stranded DNA autoantibodies are commonly used to monitor disease activity in lupus nephritis, and nonspecific markers of inflammation, such as the erythrocyte sedimentation rate or CRP levels, are measured in many diseases. Some of the biggest challenges, especially in the late stages of most chronic rheumatic diseases, include distinguishing between ongoing inflammation and irreversible organ damage, and assessing the impact of comorbidities or the adverse effects of treatments. Probably most needed are biomarkers that predict response to a particular therapy. Such markers could be used to optimize the risk to benefit ratio of a treatment in individual patients. If a strong correlation between a biomarker and a change in clinical activity can be established, a biomarker could act as a surrogate marker of a clinically important end point. Monitoring that surrogate end point could permit the use of targeted preemptive therapy if the measurement predicts relapse, or could be used as a guide to discontinue therapy if it denotes remission.

3.1 Detection of miRNAs in body fluids

Since 2008, several studies have evidenced the possibility of detecting miRNAs in body fluids including serum, plasma, urine, saliva, tears, amniotic and placental fluids, thus opening up major opportunities for a novel type of diagnostic molecules (Chen et al., 2008; Gilad et al., 2008; Mitchell et al., 2008). Since RA is a systemic chronic inflammatory disorder for which peripheral blood gene expression signature has been reported and molecular biomarkers are of great interest, identification of miRNA-based signatures is indeed a major issue. Most of the studies supporting the clinical utility of miRNAs as biomarkers in body fluids or diseased tissues have been conducted in cancer. High concentrations of cell-free miRNAs originating from the primary tumour have been found in the plasma of cancer patients, and several lines of evidence indicate that circulating miRNAs represent a promising source of cancer biomarkers (White et al., 2010). Indeed, correlations between miRNA expression levels and the development of malignancies, disease severity and aggressiveness, metastatic potential, therapeutic response and survival are reported in various cancer types. Interestingly, tumour-associated miRNomes appear highly tissue-specific.

The detection of miRNAs in serum was quite unexpected as RNA molecules are unstable in the circulation. Studies (Chen et al., 2008; Mitchell et al., 2008; Mraz et al., 2009) showed that miRNAs exhibit high stability in the serum and plasma as they circulate within membrane vesicles such as exosomes or microparticles which protect them from endogenous RNase activity (Hunter et al., 2008). These microvesicles are resistant to drastic conditions and express tissue-specific markers. Although it is easily accessible and of great interest for new biomarker discovery, very few studies report the optimisation of extraction and detection of miRNAs in plasma or serum (Gilad et al., 2008; Mitchell et al., 2008). Several technical challenges in miRNA extraction, detection and quantification in serum or plasma are still underinvestigated. Moreover, all the miRNAs detected in total blood samples are not found in serum or plasma, and/or the low concentration of most of the miRNAs in serum or plasma precludes their detection, thus limiting the panel of miRNAs for solid profiling. Indeed, it is currently estimated that 20 miRNAs are needed for solid definition of biomarker signatures. For all these reasons, and mainly technical reasons, whole blood profiling is to be definitively the main focus in future studies on biomarker discovery, as opposed to plasma- or serum-based signatures. Although it seems to be very close, many

more studies are needed before the use of miRNAs can fulfil criteria for their use as reliable tools in diagnostic and prognostic settings.

3.2 miRNAs as biomarkers for RA management

Although the potential value of miRNAs as molecular biomarkers for diagnosis, prognosis, and prediction of therapeutic response is widely documented in cancer, it is still largely unexplored in RA. The identification of abnormal miRNA expression in the circulation or inflamed joints of RA patients is still in its beginning. Until now there has been only one publication in which the concentrations of 5 miRNAs in RA patients' body fluids were measured, bringing the first proof for the use of miRNA biomarker potential in RA (Murata et al., 2010). In the report, synovial fluid miRNAs were present and as stable as plasma miRNAs for storage at -20°C and freeze-thawing from -20°C to 4°C. In RA and OA, synovial fluid concentrations of miR-16, miR-132, miR-146a, and miR-223 were significantly lower than their plasma concentrations, and there were no correlation between plasma and synovial fluid miRNAs. Interestingly, synovial tissues, fibroblast-like synoviocytes, and mononuclear cells secreted miRNAs in distinct patterns. The expression patterns of miRNAs in synovial fluid of OA were similar to miRNAs produced by synovial tissues. Plasma miR-132 of healthy controls (HC) was significantly higher than that of RA or OA. Synovial fluid concentrations of miR-16, miR-146a miR-155 and miR-223 of RA were significantly higher than those of OA. Plasma miRNAs or ratio of synovial fluid miRNAs to plasma miRNAs, including miR-16 and miR-146a, significantly correlated with tender joint counts and DAS-28. Therefore, plasma miRNAs had distinct patterns from synovial fluid miRNAs, which appeared to originate from synovial tissue. Plasma miR-132 well differentiated HC from patients with RA or OA, while synovial fluid miRNAs differentiated RA and OA. Furthermore, plasma miRNAs correlated with the disease activities of RA. Thus, synovial fluid and plasma miRNAs have potential as diagnostic biomarkers for RA and OA and as a tool for the analysis of their pathogenesis. However, this study shows that only miR-132 plasma concentrations were significantly lower in RA than in HC, but certainly not useful as diagnostic biomarkers since miR-132 plasma concentrations were not correlated with DAS28 and not disease-specific, since miR-132 plasma concentrations were also significantly lower in OA than in HC. Further investigations are required to find more suitable miRNAs for utilizing as biomarkers for diagnosis, predicting drug efficacy in order to plan optimal management of RA patients.

4. Conclusion

Our understanding of RA pathogenesis has been enriched by recent miRNA studies. We modified the schematic diagram described by *Furer et al* (Furer et al., 2010) to explain the mechanism of miRNA in RASF in Figure 2. It is obvious that miRNAs have important roles in the critical aspects of RA pathogenesis such as joint destruction, inflammation, proliferation of synoviocytes, and chemotaxis of inflammatory cells. Taking the involvement of miRNAs in leucocyte functions into account, miRNA has become one of necessary tools to address many unanswered questions as well as to envision the whole story of RA pathogenesis. It has been proposed that a gene transfer system which provides a direct delivery of nucleic acid into an affected joint may be useful for RNA therapy (Adriaansen et al., 2006). Therefore, developing a method for direct and selective transfer of specific

miRNA or inhibitor of miRNA into lymphocyte, synovial fibroblast, or DC may be a possible candidate for future treatment of RA rather than a systemic administration of miRNA which may cause off-target or harmful effects to different tissues and cell types.

In the meantime, miR-124a is unique in that it is downregulated in RASF and directly associated with cell-cycle regulation. When you consider the proliferative nature of RASF, miR-124a may have a good candidacy for a novel drug for RA treatment. Therefore, developing a drug delivery system for direct and selective transfer of miR-124a into RASF, such as intra-articular injection, is an attractive idea, since systemic administration of miR-124a may possibly cause 'off-target effects' or harmful side effects on different tissues and cell types. Besides the miRNAs described in this article, it is expected that new biomarkers and therapeutic tools will be developed in the near future based on the research generated from miRNAs.

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6. References

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