Published in final edited form as:

Autoimmun Rev. 2010 July ; 9(9): 618–621. doi:10.1016/j.autrev.2010.05.009.

MicroRNAs in Sjögren's syndrome as a prototypic autoimmune disease

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Abstract

MicroRNAs are endogenous non-coding RNAs, approximately 22 nucleotides in length. They regulate gene expression and are important in a wide range of physiological and pathological processes. MicroRNA expression is tightly regulated during hematopoiesis and lymphoid cell differentiation and disruption of the entire microRNA network or selected microRNAs may lead to dysregulated immune responses. Abnormalities in microRNA expression related to inflammatory cytokines, Th-17 and regulatory T cells as well as B cells have been described in several autoimmune diseases. Sjögren's syndrome is characterized by features of systemic autoimmunity and chronic inflammation and dysfunction in exocrine organs. Its clinical characteristics along with the relatively easy access to the target tissue and its product makes Sjögren's syndrome appealing to study many aspects of microRNAs in a systemic autoimmune disease, such as their potential as diagnostic or prognostic biomarkers and their role in pathogenesis of autoimmunity, inflammation or organ dysfunction. Encouraging preliminary data from pilot studies in Sjögren's syndrome demonstrate the potential of microRNAs as putative diagnostic and prognostic biomarker candidates which should be tested in larger more definite studies. Combining the comparison of microRNA expression profiles between various clinical subsets of Sjögren's syndrome with bioinformatic modeling tools may predict formerly unsuspected pathways which may contribute to the disease process and lead to the generation of testable novel hypothesis of pathogenesis.

Keywords

biomarker; autoimmunity; epigenetics; exocrine dysfunction; pathogenesis

Introduction

Mature microRNAs are endogenous non-coding RNAs, approximately 22 nucleotides in length that regulate gene expression and are important in a wide range of physiological and pathological processes [1]. MicroRNAs are generated in the nucleus as primary microRNAs (pri-miRNAs) [2] and are cleaved [3] into pre-microRNAs in the nucleus by an RNase III enzyme called Drosha. Pre-microRNAs are then transported to the cytoplasm for further processing by the cytoplasmic endonuclease Dicer, which in collaboration with RNA binding proteins cleaves both strands of the pre-microRNA hairpin duplex generating a double stranded RNA, 19–24 nucleotides long, with one strand being loaded in a protein complex called RISC[1]. RISC is a ribonucleoprotein complex consisting of the RNA

helicase A and proteins such as argonaute 2 and TRBP[4] which facilitate the binding of miRNA to its mRNA target.

Most microRNAs exert their effect on the 3'UTR of the targeted mRNAs, through a perfect or imperfect complementarity to sequences in the 3'UTR. The imperfect complementarity requires perfect target matching of the 2nd through the 7th nucleotides (the so called seed sequence) starting from the 5' end of the microRNA [1]. Recently it was realized that microRNAs can also target sequences within the 5'UTR and the coding sequences of the mRNAs [5]. There is also some evidence that microRNAs might be targeting splice variants of specific genes, as exon-exon junctions have been shown to be specific microRNA targets[6] adding another layer of complexity to the regulation of expression of specific isoforms within a system. The binding of microRNAs to their targets leads to either mRNA degradation or repression of translation[7]. It is important to note that the microRNA regulation of gene expression is promiscuous and redundant at the same time: most microRNAs have multiple mRNA targets and vice versa the expression of most genes can be regulated by a multitude of microRNAs. This leads to a high degree of complexity and makes microRNAs central to the fine tuning of various biologic processes. Since first showing a role in cellular development microRNAs have been implicated in most physiological functions and many disease processes.

microRNAs in autoimmune diseases

As master regulators of gene expression, microRNAs are instrumental in regulating immune development, normal immune function and autoimmunity . MicroRNA expression is tightly regulated during hematopoiesis and lymphoid cell differentiation and lineage specific disruption of the entire microRNA network, by disabling Dicer or RISC, leads to dysregulated lineage differentiation and/or activation and may lead to autoimmunity [8].

There are several recent reviews on the role of microRNAs in immunity and animal models of autoimmune diseases [8–12]. WE summarized the microRNAs implicated in human autoimmune diseases in Table 1 and will focus on some recent developments in selected autoimmune diseases in humans.

Multiple Sclerosis

In patients with multiple sclerosis microRNA-326 expression was significantly higher in peripheral blood of patients with the relapsing remitting form of the disease compared to healthy controls, and patients with neuromyelitis optica, another demyelinating disease with overlapping clinical features but different etiology. The expression was high in patients in the relapsing but not in the remitting phase of MS and was due to an increased expression of microRNA-326 [13]. Interestingly, microRNA 326 was identified as a microRNA associated with Th-17 cells.

Rheumatoid Arthritis

Several reports described altered microRNA expression in the rheumatoid synovium. Lipopolysaccharide-activated rheumatoid arthritis fibroblast-like synoviocytes (FLS) overexpressed miR-346, which negatively regulated IL-18 response of the FLS[14]. Compared to osteoarthritis, RA FLS showed a significant decrease of miR-124a which was identified as a key player in the regulation of proliferation and chemokine production of RA FLS[15]. MicroRNA-146a was highly expressed in human rheumatoid arthritis (RA) synovial tissue and its expression was induced by the proinflammatory cytokines TNF α and IL-1 β [16]. The cells with the highest expression of microRNA-146a were CD68+ macrophages, CD3+ T cells and CD79a+ B cells in the superficial and sublining layers of the synovium[16].

miR-146 was also upregulated in the peripheral blood of RA patients along with miR-155 and miR-16. When comparing patients with active or inactive disease miR-146 and miR-16 levels were higher in active (n=8) than inactive (n=3) patients[17] suggesting that these two microRNAs may be potential markers of disease activity, if confirmed in a larger number of patients.

Systemic Lupus Erythematosus

In a study comparing microRNA expression profiles in peripheral blood mononuclear cell (PBMC) of patients with SLE and ITP 16 of the 331 human miRNAs tested were differentially expressed (seven were downregulated and nine upregulated) in SLE compared to healthy controls[18], whereas 19 miRNAs were related to ITP (14 downregulated and five upregulated). From the differentially expressed miRNAs, 13 had the same expression pattern in SLE and ITP, six were downregulated in ITP only and three (miR184, miR198 and miR21) were up or downregulated in lupus with no change in ITP. When comparing SLE patients with active (SLEDAI 12) and inactive (SLEDAI 15) lupus eight miRNAs were downregulated in the more active group. Using a cutoff of 2-fold change, the same group identified thirty six microRNAs which were upregulated and thirty which were downregulated in the kidneys of patients with WHO Class II lupus nephritis compared to normal controls[19].

A more recent study found that from 156 miRNAs analyzed, 42 were differentially expressed between controls and lupus patients with seven being more than 6-fold lower in SLE. Since type I Interferon (IFN) is known to play a role in the etiology of SLE, the authors explored the association between the activation of type I IFN pathway with the altered expression of miR-146a, which had been reported to negatively regulate the innate immune system. Overexpression of miR-146a in primary PBMCs resulted in reduction of TLR-7 mediated IFN α and IFN β production. In addition, transfection with synthetic miRNA-146a hairpin inhibitor to decrease the endogenous microRNA expression increased IFN production. The study also suggested that miR-146a expression negatively correlated with SLEDAI and renal SLEDAI scores[20], however the correlation was rather weak (r values -0.28 and -0.38, respectively).

Sjögren's Syndrome

Sjögren's syndrome is characterized by systemic autoimmunity and inflammation and dysfunction in the exocrine organs, primarily the salivary and lachrymal glands [21]. The exact cause of exocrine dysfunction in SS is not known but it is likely that both immunologically mediated and non-immune mechanisms contribute significantly [22]. Most Sjögren's syndrome patients have a stable or slowly progressive course and many do not require intensive immunosuppression. Therefore, Sjögren's syndrome is an appealing target to study many aspects of microRNAs in a systemic autoimmune disease, such as their potential as diagnostic or prognostic biomarkers and their role in pathogenesis of autoimmunity, inflammation or organ dysfunction.

To explore some of these questions we have conducted a pilot study to test the potential of salivary gland microRNAs as biomarkers of Sjögren's syndrome. Preliminary results were presented during the 2009 Annual Meeting of the American College of Rheumatology. We used Agilent microRNA microarrays to profile miRNAs isolated from minor salivary glands (MSGs) of healthy volunteers (n=8) and Sjögren's patients with high (focus score of 12; n=8) and low focus scores (focus score of 1 or 2, n=8). We showed that miRNA expression profiles can separate glands of Sjögren's patients from controls and can distinguish subsets of SS patients with low or high grade inflammation [23]. Interestingly, in half of the patients with a focus score of 12 the mir-17-92 cluster, which has been associated with specific types

of lymphocytes and lymphocytic pathologies, was downregulated. It had been previously reported that a decrease of the mir-17-92 cluster was associated with accumulation of pro-B cells with a marked reduction of pre-B and more mature B cells, and overexpression of it has been linked to lymphoproliferative disease and autoimmunity [24, 25]. Next, we identified two microRNAs which had an opposite relationship to inflammation: one increased the other decreased between the low and high focus score groups. We found that the relative expression levels of those two microRNAs correlated well with the inflammatory status of the MSGs in an independent cohort of 15 samples, including biopsies with an intermediate focus scores of 4–7 [23]. Larger studies are currently underway to validate SG microRNAs as diagnostic markers in SS but the ultimate goal is to replace biopsies with less invasive methods. Saliva is an obvious source for a non-invasive biomarker in Sjögren's syndrome since it is a direct product of the affected target organ. Therefore, we explored the presence of microRNAs in exosomes isolated from parotid and submandibular saliva [26]. Exosomes are small cell-secreted vesicles of about 30–100 nm, derived from fusion of multivesicular bodies to plasma membranes [27]. They are derived from a wide range of cells, including hematopoietic cells [28] and various epithelial cells [29]. Exosomes feature a wide range of surface and internal proteins specific to their source [27], and recent studies found that they can also transport mRNA and microRNA [29]. Saliva samples ranging from 200ul up to 5mL volume yielded an adequate amount of exosomal RNA for quantitative PCR. To confirm the presence of microRNAs within the exosomes, we performed TaqMan microRNA quantitative PCR amplification for three microRNAs (hsa-mir-203, hsa-mir-768-3p and has-mir-574-3p) that we have previously identified in minor salivary glands, as well as whole saliva. PCR reactions with appropriate negative and positive controls demonstrated the presence of salivary gland derived microRNAs within the exosomes. As a proof of concept we showed that the microRNAs present within the exosomes from parotid saliva from a normal volunteer is different from a Sjögren's syndrome patient's parotid saliva sample. Although preliminary, these results suggest that microRNAs can be identified in saliva raising the potential of obtaining information from the targeted organ in this autoimmune disease by a non-invasive method[26].

MicroRNA expression profiling may also be a useful approach to address questions related to the pathogenesis of diseases. Among others they may help understanding if specific miRNAs are involved in controlling saliva production, or regulation of the peripheral inflammatory response in the salivary gland. To test his potential we have identified two distinct patterns of microRNA expressions: one which was related to inflammation (inflammatory pattern) and one which was independent from it (non-inflammatory pattern). Using a bioinformatics approach to predict the targeted pathways we identified some biologic processes and pathways which overlapped between the two groups and some which were distinct to one or the other [30]. It was reassuring that most of the pathways predicted to be targeted by the inflammatory group were directly related to inflammation or autoimmunity whereas the non-inflammatory group targeted pathways involved in cellular processes or neurologic regulation. Any prediction of course has to be confirmed by more direct methods but these data suggest that the analysis of microRNA expression may lead to novel hypotheses about pathogenesis and may identify targets of research and hopefully even therapies.

Acknowledgments

This research was supported by the Intramural Research Program of National Institute of Dental and Craniofacial Research

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Key messages

- microRNAs are small RNAs playing an important regulatory role in gene expression
- microRNAs play a role in normal and abnormal immune responses
- microRNA abnormalities have been described in several autoimmune diseases
- in Sjögren's syndrome microRNAs are promising biomarker candidates and may provide clues to the pathogenesis of autoimmunity, inflammation and end-organ damage

microRNAs in Sjögren's syndrome

Potential Applications

Diagnosis

- MicroRNA profiling of salivary and lachrymal glands, PBMC, exosomes from salivary, serum or urine origin
- Might prove good adjunct to the broad current diagnostic criteria

Molecular Subclassification

- microRNAs might identify the molecular mechanisms of various clinical phenotypes (inflammation, exocrine dysfunction) and might prove helpful as determinants and classifiers of pathogenetically distinct subclasses

Disease Activity Monitoring

- The presence of microRNAs in biological fluids might allow for a quick, non-invasive disease activity monitoring, through microRNAs that are altered during disease activity changes.

Therapeutic Targeting

- microRNAs that target genes that regulate the salivary or lachrymal flow might be manipulated to restore exocrine function
- microRNA targeting inflammatory pathways in the target tissues or circulating lymphocytes might be manipulated to control inflammation
- Utilizing the central role microRNAs play in development it is possible that microRNAs can orchestrate the regeneration of affected tissues

Figure 1.

Table 1

microRNAs associated with specific autoimmune diseases

Disease	microRNA
Type I Diabetes	miR-342[31], miR-191[31], miR-510[31]
Multiple Sclerosis	miR-326[13], miR-17-5p[32]
Rheumatoid Arthritis	miR-146a[16, 17]
Primary Biliary Cirrhosis	miR-122a[33], miR-26a[33], miR-328[33], miR-299-5p[33]
Sjögren's Syndrome	mir-17-92[23]
Systemic Lupus Erythematosus	miR-146a[20], miR-516-5p[19], miR-637[19]
Psoriasis	miR-203[34], miR-146a[34], miR125b[34], miR-21[34]