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REVIEW

Sexual dimorphism of miRNA expression: a new perspective in understanding the sex bias of autoimmune diseases

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Abstract: Autoimmune diseases encompass a diverse group of diseases which emanate from a dysregulated immune system that launches a damaging attack on its own tissues. Autoimmune attacks on self tissues can occur in any organ or body system. A notable feature of autoimmune disease is that a majority of these disorders occur predominantly in females. The precise basis of sex bias in autoimmune diseases is complex and potentially involves sex chromosomes, sex hormones, and sex-specific gene regulation in response to internal and external stimuli. Epigenetic regulation of genes, especially by microRNAs (miRNAs), is now attracting significant attention. miRNAs are small, non-protein-coding RNAs that are predicted to regulate a majority of human genes, including those involved in immune regulation. Therefore, it is not surprising that dysregulated miRNAs are evident in many diseases, including autoimmune diseases. Because there are marked sex differences in the incidence of autoimmune diseases, this review focuses on the role of sex factors on miRNA expression in the context of autoimmune diseases, an aspect not addressed thus far. Here, we initially review miRNA biogenesis and miRNA regulation of immunity and autoimmunity. We then summarize the recent findings of sexual dimorphism of miRNA expression in diverse tissues, which imply a critical role of miRNA in sex differentiation and in sex-specific regulation of tissue development and/or function. We also discuss the important contribution of the X chromosome and sex hormones to the sexual dimorphism of miRNA expression. Understanding sexually dimorphic miRNA expression in sex-biased autoimmune diseases not only offers us new insight into the mechanism of sex bias of the disease but will also aid us in developing new sex-based therapeutic strategies for the efficient treatment of these diseases with a sex bias.

Keywords: sex differences, microRNA, immune regulation, autoimmune diseases, X chromosome, sex hormones

Introduction

It is now well-established that males and females have different immune capabilities, consequently displaying differing degrees of susceptibility to and severity of various diseases, including autoimmune diseases.[1–4] Autoimmune diseases arise from a devastating attack on self antigens and self tissues by a malfunctioning immune system. Thus far, more than 80 different human diseases with an autoimmune component have been recognized. These autoimmune-related disorders collectively affect approximately 5%–10% of the population of the Western countries.[5] Autoimmune disorders are often lifelong and seriously compromise quality of life, resulting in an enormous health care cost burden globally.[6]

Strikingly, about 80% of autoimmune patients are women. Autoimmune diseases are among the top ten causes of all deaths and are the fourth largest cause of disability

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among women.[6,7] The female-to-male patient ratio varies from 2:1 to 25:1 for those autoimmune diseases with female prevalence.[1,3] It must be noted that not all autoimmune diseases occur exclusively or preferentially in females. In some autoimmune diseases, both males and females are equally susceptible, or the diseases have a tendency to occur more often in males. Nevertheless, overall, a majority of autoimmune diseases occur in women. Despite extensive studies in past decades, the etiology of autoimmune diseases and the mechanism of female prevalence of autoimmune diseases remain elusive. Multiple factors including genetic predisposition, epigenetic dysregulation, and hormonal and environmental influence are thought to contribute to the pathogenesis of autoimmune disease. These factors likely interact with each other to confer susceptibility to autoimmune disease. Sex differences in immune responses and prevalence of autoimmune diseases are attributed to direct differences in male and female genome and sex hormone production, as well as sex-specific epigenetic regulation and gene expression.[8–11] One of the important epigenetic mechanisms is microRNA (miRNA) regulation of gene expression at the posttranscriptional level, which is a focus of this review in relation to sex differences in autoimmune diseases.

miRNAs are a group of endogenously expressed, small, non-protein-coding RNAs (about 22 nucleotides [nts] in size) that regulate gene expression posttranscriptionally. Since the discovery of the first miRNA, lin-4, in nematode *Caenorhabditis elegans* in 1993, the importance of miRNAs in the regulation of various biological functions including stem cell development and differentiation, organogenesis, early embryo development, and immune responses has been highly appreciated.[12–15] Dysregulated miRNA expression profiles have been observed in various human diseases, including female-predominant autoimmune diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjögren's syndrome, and multiple sclerosis, implying their contribution to the etiology of these diseases.[13,16–21] It is plausible that miRNAs are differentially expressed between males and females in the context of autoimmune diseases, leading to sex differences in disease susceptibility and severity. In support of this view, our laboratory recently demonstrated the sex differences in the expression of lupus-associated miRNAs in immune cells from NZB/W_{F1} mice, which manifest lupus predominantly in females.[22] In this review, we summarize the sexual dimorphism of miRNA expression that is observed in diverse gonadal and somatic tissues and further discuss the potential involvement of sexually dimorphic miRNA regulation in the development of sex-biased autoimmune disease, an aspect not addressed thus far.

miRNA biogenesis and function

So far, 24,521 hairpin precursor miRNAs entries, which produce 30,424 mature miRNAs in 206 species, have been registered in the miRNA database (miRBase [http://www.mirbase.org], version 20, released June 2013). The majority of miRNA genes (about 80%) are located in the intergenic and intronic regions of protein-coding and non-protein-coding genes. The remaining 20% of the miRNA genes are located in the exonic regions of noncoding transcripts, of which some are considered a "mix" (either exonic or intronic miRNA, depending on the alternative splicing pattern).[23,24] As illustrated in Figure 1, miRNA genes are transcribed by RNA polymerase II or III to produce a long primary transcript (pri-miRNA), which may contain single (monocistronic) or clusters (polycistronic) of hairpin loop structures. The long pri-miRNA transcript is initially processed in the nucleus by a microprocessor complex comprising nuclear RNase III enzyme Drosha, generating a 60–70-nts-long stem-loop intermediate structure called precursor miRNA (pre-miRNA). The pre-miRNA is transported by the nucleocytoplasmic shuttle protein exportin 5 to the cytoplasm, where it is further cleaved by the RNase III enzyme, Dicer, to yield a short, imperfectly matched miRNA/miRNA* duplex. To regulate target gene expression, the miRNA/miRNA* duplex is loaded onto the Argonaute (Ago) protein to form the final effector complex, RNA-induced silencing complex (RISC). In RISC, only a single strand of miRNA duplex will remain as functional miRNA to interact with the target mRNA to regulate target gene expression; the other strand (miRNA*) will be degraded.[25–27]

Although not common, there are some special miRNA genes called mirtrons that are encoded within the intronic region of genes, but their biogenesis and maturation bypass Drosha processing in the nucleus.[28–30] After being transcribed, the mirtron is spliced out from primary transcript by spliceosome and then further goes through lariat debranching to generate pre-miRNA (Figure 1). Similar to classical miRNA, mirtron-derived pre-miRNA is also further cleaved by Dicer in the cytoplasm to generate functionally mature miRNAs.

The mature, single-stranded miRNA usually binds to the 3′ untranslated region (UTR) sequence of the target mRNA in the RISC, repressing target gene expression through either translational inhibition or mRNA destabilization. Although the perfect complementarity between miRNA and target mRNA is not necessary for miRNA-mediated

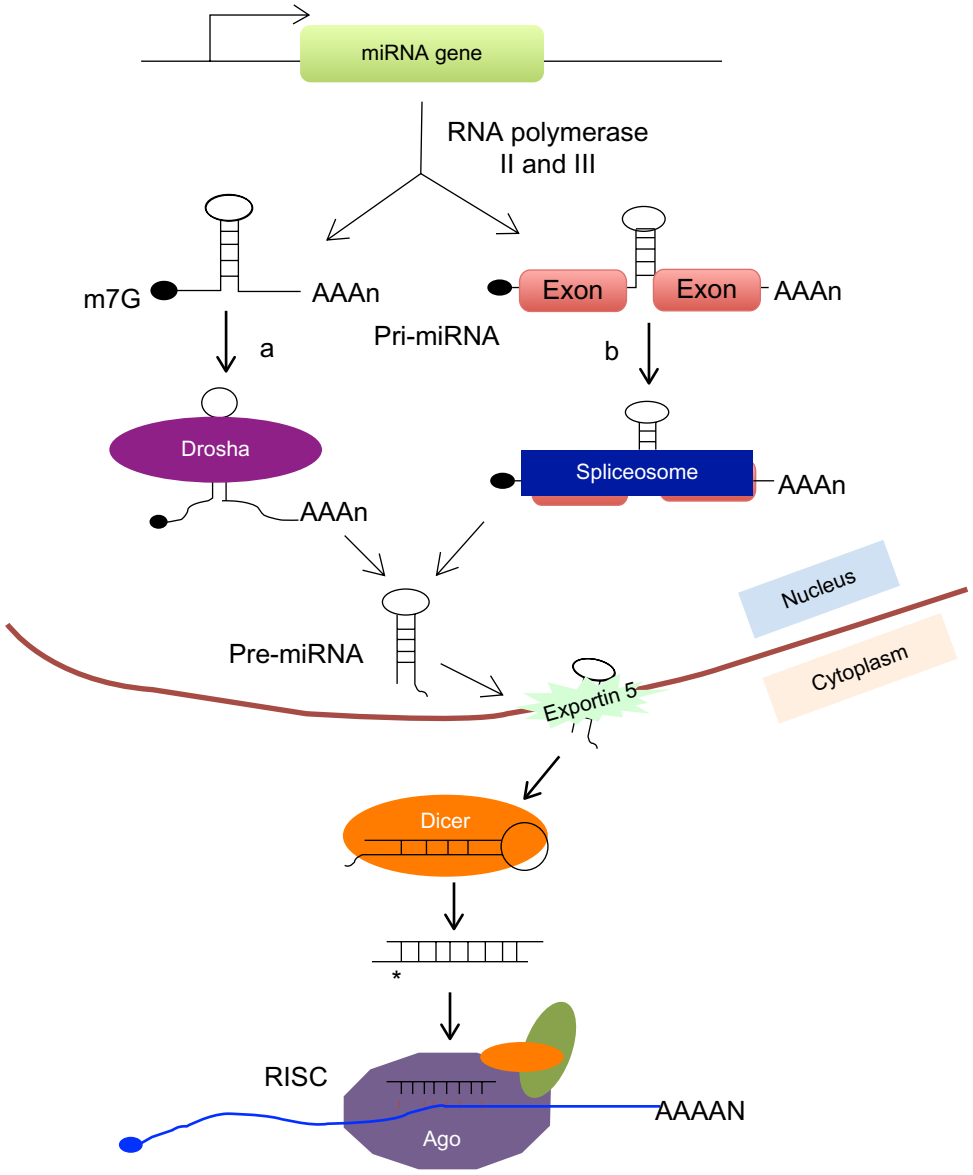

Figure 1 microRNA (miRNA) biogenesis.
Notes: The miRNA gene is transcribed by RNA polymerase II and III into primary miRNA (pri-miRNA). Classically, the pri-miRNA is cleaved by Drosha (a) in the nucleus to generate precursor miRNA (pre-miRNA). Biogenesis of nonclassical miRNA, mirtron (b), bypasses Drosha processing to form pre-miRNA through splicing out from the pri-miRNA. The pre-miRNA is exported to the cytoplasm by Exportin-5. In the cytoplasm, the pre-miRNA is further processed by Dicer to yield ~22 nts long, imperfectly matched miRNA/miRNA* duplex. This duplex is loaded into Argonaute (Ago) protein to generate effector complex, RNA-induced silencing complex (RISC), assisted by other cofactors. In RISC, the mature miRNA interacts with its target messenger RNA to regulate gene expression via repression of translation or destabilization of messenger RNA. The complementary strand of miRNA, miRNA* (also called passenger strand), is degraded.
Abbreviations: nts, nucleotides; Ago, Argonaute; pri-miRNA, primary miRNA; precursor miRNA, pre-miRNA; RISC, RNA-induced silencing complex.

gene regulation, the seed region of 2–8 nts located at the 5′ end of miRNA is critical for target gene recognition.[31] One miRNA can target hundreds of genes in mammalian cells, but the inhibitory effect of a miRNA on each target gene is typically mild or moderate.[32,33] Although miRNA is largely identified as negative regulator of gene expression, recent studies revealed that in certain circumstances, miRNA could also stimulate gene expression via either directly binding to the target gene or indirectly, suppressing the suppressor of gene expression.[34,35] In addition to interacting with the 3′ UTR, miRNA may also bind to the 5′ UTR of target mRNA to promote or repress target gene expression.[35,36]

miRNAs are ubiquitously expressed and evolutionally conserved in different species. Computational genome analysis revealed that miRNAs compose about 3% of human genome, and they are computationally predicted to regulate 30%–90% of human genes.[31,37–39] The crucial role of miRNAs in the regulation of almost all biological pathways in both physiological and pathological conditions has been extensively documented in numerous reports.[13,14,40,41]

Importantly, miRNAs have now emerged as new diagnostic markers and therapeutic targets for various diseases.[42–44]

miRNA regulation of immunity and autoimmunity

The expression and function of miRNAs is tightly regulated during immune cell development (lineages commitment and differentiation) and function (innate and adaptive immune responses). The proper expression and function of miRNAs are prerequisite for immune system homeostasis. Interruption or malfunctioning of miRNA regulatory machinery could cause the breakdown of immune homeostasis and self-tolerance and, consequently, lead to the development of autoimmunity. Disruption of miRNA biogenesis by depleting Dicer or Drosha in early lymphocyte progenitors impaired T- and B-cell development and function.[45–48] In mice with Dicer or Drosha deficiency, specifically in the lymphocyte progenitor cells, there were reduced T-cell numbers in the central and peripheral lymphoid organs, a complete developmental block of B cell at the pro- to pre-B transition stage, impaired antibody diversity, aberrant T helper cell differentiation, and cytokine production.[45–48] Several research groups reported that depletion of Dicer or Drosha specifically in T regulatory cells (Tregs) in mice led to a complete loss of immunoregulatory functions of Tregs, resulting in the development of inflammatory diseases and fatal autoimmunity.[47,49–51] These studies with Dicer- or Drosha-deficient mice revealed the important contribution of general miRNA biogenesis to immune system development and function. Moreover, functional contribution of individual miRNA to immunity and autoimmunity has also been well-documented in numerous research reports and has been extensively reviewed elsewhere.[13,14,17,40,52–63] Therefore, this review will not discuss the functional significance of individual miRNA in immunity and autoimmunity. Rather, we focus on the sexual dimorphism of miRNA expression and its potential contribution to sex differences in immune responses and autoimmune diseases.

Sexually dimorphic gene expression and sex differences in immune responses

There are marked differences in immune capabilities between males and females.[4,64] Compared with their male counterparts, females typically mount stronger innate and adaptive immune responses to various pathogens and toxins. The better immune capability presumably contributes to the greater survival rate in females compared with males. However, the enhanced immune capability in females is a double-edged sword. Females also tend to respond vigorously to self antigens and, consequently, become more susceptible to a majority of autoimmune inflammatory diseases. The sex differences in immune responses are attributed to multiple factors including a direct genetic difference such as the X chromosome and X chromosome-linked genes, sex hormones, and sex-specific regulation of immune-related genes.[2,4,10,65]

The salient difference between the male and female genome is that the male possesses one X chromosome and one Y chromosome, whereas the female has two copies of the X chromosome. There are about 1,100 genes in the human X chromosome, but only about 100 genes in the human Y chromosome. Among them, only 54 genes are homologous between the Y and X chromosomes.[10,66] While the initial sex determination is dependent on the Y chromosome-specific *Sry* gene, subsequently, X-chromosome and X-chromosome linked genes, together with sex hormones are critical contributors to the differences in physiology, behavior, gene expression/regulation, and immune functionality between males and females.[67–69] Because the female possesses two copies of the X chromosome, during the early stage of female embryonic development, one X chromosome is randomly inactivated (XCI) to balance the dosage of X chromosome-linked genes between the male and female. However, about 15% of X chromosome-linked genes might escape XCI and therefore contribute to the sexual dimorphism of X chromosome-linked genes.[70] In addition to XCI escape, other mechanisms such as skewed XCI (nonrandom silencing of the X chromosome) and imprinting expression of X chromosome genes may also contribute to the sex differences in the expression of X chromosome-linked genes.[10]

Many immune-related genes are located on the X chromosome, including toll-like receptor (TLR) family members *TLR7* and *TLR8*, *Foxp3* gene (key transcription factor regulating Tregs development and function), CD40 (cluster of differentiation 40) ligand (*CD40L*, a B-cell costimulatory molecule), key components of TLR and nuclear factor–kappa B signaling pathways such as Bruton's tyrosine kinase, interleukin 1 (IL-1) receptor–associated kinase 1, IκB kinase γ, cytokine receptors such as IL-9 receptor (*IL-9R*), IL-2 receptor γ chain (*IL-2RG*), and IL-13 receptor α1 chain (*IL13RA1*), among others.[10,69] The differential expression or mutation of these immune-related, X chromosome-linked genes could contribute to the sex differences in immune response and disease incidence. For example, varied X chromosome-linked *TLR7* gene copy number, increased TLR7 expression, and *TLR7* functional polymorphisms are associated with SLE susceptibility in both

human and murine lupus.[71,72] Demethylation and overexpression of X chromosome-linked *CD40L* in $CD4^+$ T cells from female lupus patients is believed to contribute significantly to the striking female predominance of lupus.[73] In contrast, the pathogenesis of immune dysregulation, polyendocrinopathy, enteropathy, and X-linked syndrome, a rare inflammatory disease that occurs exclusively in males, is attributed to the mutation of the X chromosome-linked *Foxp3* gene.[69]

Although the sex chromosome-linked genes contribute significantly to sex dimorphism of gene expression, they can only count for a small number of sexually dimorphic genes. In fact, many sexually dimorphic genes are located on autosomes, which possess 95% of the annotated human genome. The differential expression of autosomal genes between males and females is subjected to X chromosome-mediated epigenetic controls and sex hormone regulation.[68,74] The contribution of sexually dimorphic expression of immune-related protein-coding genes to sex differences in immune responses and in susceptibility to autoimmune disease is now appreciated. With recognition of the immune regulatory role of miRNAs, we propose that miRNAs are differentially expressed in immune cells between males and females, which disparately regulate immune-linked genes in males and females and subsequently contribute to the sex differences in immune capabilities and in susceptibility to autoimmune diseases.

Sexually dimorphic miRNA expression in gonadal and nongonadal (somatic) tissues

The miRNA expression has been extensively profiled in testis and ovary in various species.[75–81] In a recent report, 770 known and 5 novel miRNAs were identified in normal human testis.[82] Further computational bioinformatic analysis indicated that select miRNAs expressed in testis regulate many important biological pathways that are implicated in spermatogenesis, including meiosis and p53-related pathways.[82] A study using ovine fetuses identified a number of differentially expressed miRNAs in testis and ovary during gestation. These were further predicted to target genes that have been previously implicated in mammalian gonadal development such as *ESR1*, *CYP19A1*, and *SOX9*.[80] Differential expression of miRNAs in porcine testis and ovary was also reported.[83] After a further survey of the expression of X chromosome-linked miRNAs in different normal porcine tissues, the authors predicted that X chromosome-linked miRNAs were expressed in a testis-preferential or testis-specific pattern.[83] By using a small RNA cloning method, 141 miRNAs were identified in testis and 122 miRNAs were identified in mouse ovary.[78,79] In a separate study, 49 miRNAs were exclusively detected in adult testis and 48 miRNAs were exclusively detected in ovary.[77] There were also 24 miRNAs that were differentially expressed in testis and ovary with at least fivefold changes. The authors further suggested that most male-biased expressions of miRNAs were located on the X chromosome.[77] Overall, these studies documented that miRNAs are differentially expressed in testis and ovary in diverse species, suggesting the important role of miRNA in driving gonadal tissue development and function.

Sex differences of miRNA expression have been observed not only in gonadal tissues but also in diverse somatic tissues such as liver,[84] lung,[85] and brain.[74,86,87] Among the somatic tissues, the brain is the most extensively studied organ to address the contribution of sexually dimorphic miRNA expression to sex differences in brain development and function. In a recent report, by using the RNA-Seq method, the spatial–temporal expression of miRNA in a human developing brain spanning from infancy to adolescence was studied.[87] The authors demonstrated that 40 miRNAs displayed significant sex-biased expression pattern in the prefrontal cortex, particularly during adolescence. Moreover, these sex-biased miRNAs in the human prefrontal cortex targeted genes that are related to *Wnt* signaling and transforming growth factor-beta pathways, which are implicated in neurological disorders with marked sex bias in prevalence.[87] A different study has shown that 149 miRNAs were differentially expressed between male and female mouse neonatal brain.[74] Of these 149 sex-biased miRNAs, 47 miRNAs were regulated by a sex chromosome-mediated mechanism, and 72 miRNAs were regulated by sex hormones.[74,88] Overall, the mentioned studies documented that miRNAs are differentially expressed between males and females in both gonadal and nongonadal tissues, suggesting the important role of miRNA in the sex differences of diverse physiological system development and function.

Sex-specific regulation of miRNA expression in response to stimuli

In addition to the inherent sex differences in miRNA expression, males and females also display sex-specific miRNA expression in response to environmental stimuli and/or pathological changes. For example, a study has shown that ionizing radiation induced sex-specific miRNA expression change in the mouse brain.[89] Specifically, miR-29 family members (miR-29a and miR-29c) were exclusively downregulated in female, but not male, mouse frontal cortex brain region in response to ionizing radiation. Of relevance,

miR-29 was confirmed to target DNA methyltransferases (DNMT)3a and DNMT3, which provided a mechanistic understanding of ionizing radiation induction of epigenetic changes in brain.[89] Further, sex-specific regulation of the X-linked inhibitor of apoptosis (XIAP) gene in response to stroke was known to contribute significantly to sex differences in cerebral ischemia.[90] A recent study revealed that miR-23a, which was differentially regulated in male and female ischemic brains, targeted XIAP expression directly in the brain of mice.[90] Specifically, miR-23a was decreased in the male ischemic brain but significantly increased in female ischemic brains when compared with their sham controls. The increased expression of miR-23a was associated with reduced expression of XIAP protein in female ischemic brains. This study provided direct evidence for the contribution of sex-specific regulation of miRNA to the sex differences in response to stroke.[90] In addition, males and females have different pharmacologic and toxicologic responses to drugs, which may be partly caused by the sex differences in the expression of cytochrome P450 superfamily members, including Cyp2b9, a 16α-hydroxylase enzyme specifically expressed in the female mouse liver.[91] The cytochrome P450 superfamily is a large group of enzymes that catalyze the metabolism of drugs and carcinogens. A current study revealed that multiple miRNAs were associated with the sexual dimorphism of Cyp2b9 expression. There was a negative association between the expression levels of these miRNAs and Cyp2b9 in mouse liver. The female-specific expression of the Cy2b9 gene may be contributed towards by the reduced expression of these miRNAs in female mouse liver when compared with the male.[91] Interestingly, the sex-specific miRNA expression in response to stress is also observed in insects. The red flour beetle *Tribolium castaneum* exhibited both sex- and stressor-specific expression of immune genes and miRNAs. Strikingly, more miRNAs were induced in female *T. castaneum* in response to stress, thereby supporting the theory of female immune advantage in response to bacterial antigen challenge.[92] Because miRNA plays an important role in regulation of gene expression, the sex-specific regulation of miRNA in response to stimuli may subsequently lead to sex-specific control of gene expression and cellular functions.

Sexually dimorphic miRNA expression in immune cells in the context of autoimmune disease

It is now well acknowledged that miRNAs have a critical role in immunity and autoimmunity.[13,14,40,93,94] Nevertheless, there is paucity of data with regard to sex differences in miRNA expression in cells of the immune system. The potential contribution of sexually dimorphic miRNA expression to sex differences in immune response and autoimmune disease is under-investigated thus far. SLE is a prototypic autoimmune disease with marked female bias. Numerous dysregulated miRNAs have been identified in both human and murine lupus, thereby implicating their role in lupus pathogenesis.[13,17,19] To determine the contribution of sexually dimorphic miRNA expression to the sex bias of autoimmune disease, we recently investigated the sex differences in the expression of lupus-associated miRNAs, including the miR-182-96-183 cluster, miR-155, miR-31, miR-148a, miR-127, and miR-379, in splenocytes of male and female NZB/W_{F1} mice.[16,22] We found that the sex differences in the expression of aforementioned lupus-associated miRNAs were markedly evident after the onset of lupus, especially at 30 weeks of age when female NZB/W_{F1} mice manifested moderate to severe lupus. Before the onset of lupus, only miR-127 and miR-379 displayed slight but significant increase in female NZB/W_{F1} mice when compared with age-matched male counterparts.[22] To our knowledge, this is the first report to show sexual differential expression of miRNA in splenocytes in the context of autoimmune disease. The significance of these lupus-associated miRNAs in lupus pathogenesis has been suggested in other recent reports. Increased miR-148a level in lupus T cells was linked to lupus pathogenesis because it targeted DNMT1, resulting in DNA hypomethylation and induction of autoimmune-associated genes in lupus T cells.[95] miR-31 was shown to target Foxp3, suggesting its role in the regulation of Tregs development or function.[96] Unlike in murine lupus, where miR-31 was upregulated, there was reduced miR-31 expression level in human lupus peripheral blood T cells. Further, miR-31 was shown to regulate IL-2 production in human lupus T cells by targeting ras homolog family member A (RhoA).[97] miR-182 is a dominant miRNA in the regulation of T helper cell activation and proliferation. Inhibition of miR-182 in T cells suppressed ovalbumin (OVA)-induced arthritis in mice, which suggests a potential link between miR-182 dysregulation and autoimmune inflammation.[61] miR-155 is one of the most extensively studied miRNAs that plays an essential role in the regulation of both innate and adaptive immune response.[57,63,98,99] Dysregulated miR-155 expression or function has also been implicated in the pathogenesis of various autoimmune-related disorders.[58,100–102] There is limited knowledge with regard to the immune regulatory function of miR-127 and miR-379. A recent study revealed that miR-127

inhibited lung inflammation by targeting immunoglobulin G (IgG) FcγR1 (CD64).[103] Together, our recent finding of a differential expression of lupus-associated miRNA between male and female lupus mice suggests a potential contribution of sexually dimorphic miRNA expression to female predominance of lupus. However, there is a pressing need to mechanistically determine the contribution of these sexually dimorphic miRNAs to the sex differences in autoimmune disease development and progression.

Mechanism of sexual dimorphism of miRNA expression

X chromosome-linked miRNAs (X-linked miRNAs)

X chromosome and X chromosome-linked genes contribute significantly to the sex differences in immunity and autoimmunity.[10,68,69] The potential contribution of X chromosome-linked miRNAs to sex differences in immune response and autoimmunity is beginning to be appreciated, although direct experimental data are still lacking.[10,104,105] It is noteworthy that the X chromosome is highly enriched in miRNAs. One hundred and thirteen miRNAs, representing about 7% of total human miRNAs, are located on the X chromosome, whereas only 2 miRNAs have been identified so far on the Y chromosome.[104,105] Although the function of most X chromosome-linked miRNAs remains unclear, some X chromosome-linked miRNAs have been shown to be involved in immune regulation. The most well-studied X chromosome-linked miRNA is miR-223. miR-223 is highly expressed in bone marrow myeloid cell lineages and plays a key role in the regulation of granulocytes differentiation and function.[106–108] Reduced expression of miR-223 was commonly observed in various types of cancer and was correlated with the progression of these cancers.[109,110] Altered expression of miR-223 is also associated with different autoimmune pathological conditions. miR-223 was increased in human RA or type 2 diabetes patients[111,112] but decreased in human lupus patients.[113]

In addition to miR-223, several other X chromosome-linked miRNAs such as miR-221/222 cluster, miR-98, miR-106a, miR-424, and miR-18b, have also been implicated in immune regulation. miR-221/222 was increased in synovial fibroblasts from tumor necrosis factor transgenic mice and from human RA patients.[114] miR-221 was shown to regulate proliferation and survival of immune cells.[115,116] miR-221 expression level was inversely correlated with the expression of cell cycle inhibitor protein, cyclin-dependent kinase inhibitor 1B ($p27^{kip1}$), during dendritic cell development and maturation. Silencing miR-221 resulted in $p27^{kip1}$ protein accumulation and dendritic cell apoptosis.[115] In a different study, miR-221 was shown to be highly upregulated in activated $CD4^+$ T lymphocytes.[116] Inhibition of miR-221 in $CD4^+$ T lymphocytes promoted cell proliferation by suppressing proliferation suppressors such as insulin receptor substrate 2, phosphatidylinositol 3-kinase regulatory subunit alpha, I-kappa-B kinase epsilon, and FBJ murine osteosarcoma viral oncogene homolog (FOS).[116] miR-98 was reduced in lipopolysaccharide (LPS)-activated macrophages, and negatively regulated LPS-induced IL-10 production in macrophages, by directly targeting IL-10 3′ UTR.[117] Enhancing miR-98 function with miR-98 mimic inhibited TLR4-triggered IL-10 production and promoted cyclooxygenase 2 production, but did not affect tumor necrosis factor α and IL-6. Further, the authors showed that LPS-mediated downregulation of miR-98 contributed to endotoxin tolerance in macrophages.[117] In addition, four other X chromosome-linked miRNAs, miR-106a, miR-424, miR-503, and miR-542, have been shown to be involved in monocyte differentiation.[104]

Importantly, a recent report revealed that 18 X chromosome-linked miRNAs including miR-188, miR-421, miR-503, Let-7f2, and miR-98 were overexpressed in $CD4^+$ T cells from female lupus patients when compared with male lupus patients.[105,118] These data add support to the view that X chromosome-linked miRNAs contribute to the sex differences in the susceptibility and severity of lupus. Interestingly, miRNA microarray analysis of murine lupus splenocytes has shown that two X chromosome-linked miRNAs, miR-222 and miR-98, were dysregulated in lpr lupus mice when compared with control mice.[16] miR-222 was upregulated in lpr lupus mice, whereas miR-98 was downregulated.[16] In addition, we also found that miR-223 was significantly increased in lpr lupus mice but not in NZB/W_{F1} mice when compared with control mice (Rujuan Dai, unpublished data 2010). However, whether miR-222, miR-98, and miR-223 are differentially expressed between male and female lupus mice is not known so far.

Sex hormone regulation of miRNAs in the immune system

In addition to the direct genetic contribution by the X and Y chromosomes, sex hormones markedly influence the sex differences in gene expression and immune function. The sexual dimorphic expression of immune-related genes after puberty in mice strongly suggested a role of sex hormones in sex-specific gene regulation.[106] The profound effect of

sex hormones on immune system development and function has been extensively documented.[2,64,65,119–126] There are an increasing number of reports indicating that sex hormones regulate miRNA expression in various normal human tissues, including the lymphoid organ, and also in hormone responsive cancer cells, via the activation of the hormone nuclear receptors,[74,127–130] which suggests the involvement of miRNA in sex hormone-mediated regulation of biological functions. It has been shown that sex hormones regulate miRNA expression at either the transcriptional or the posttranscriptional level. We next discuss recent data with regard to the regulatory effect of estrogen and androgen on miRNA expression.

Estrogen regulation of miRNA expression

After estrogenic activation, estrogen receptor (ER)α and ERβ regulate the expression of estrogen-responsive genes through genomic and nongenomic mechanisms.[121,131] Estrogen regulation of miRNA expression via activation of ER is important for fine-tuning of estrogen-mediated cellular responses.[127,132–134] It has been shown that estrogen treatment induced 21 miRNAs and repressed expression of 7 miRNAs in MCF-7 breast cancer cells. These estrogen-regulated miRNAs could potentially affect the expression of hundreds of estrogen-regulated and non-estrogen-regulated protein-coding genes at the posttranscriptional level.[132] Importantly, estrogen-induced miR-21, miR-98, and let-7 miRNA family members target C-Myc and E2F, two estrogen-regulated transcription factors that play a critical role in the regulation of ERα activity and estrogen responses.[132] Estrogen was also reported to upregulate pri-miR-17-92 in MCF-7 cells.[133] The induction of pri-miR-17-92 upon estrogenic stimulation was dependent on the direct transcriptional regulation by estrogen-induced c-Myc.[133] Further, estrogen-induced miR-17-92 family members such as miR-18a, miR-19b, and miR-20b targeted and reduced ERα protein expression.[133] Together, these reports demonstrated that estrogen-activated ERs, estrogen-regulated miRNAs, and estrogen-regulated mRNAs (protein-coding genes) form a negative feedback circuit to fine-tune estrogen action and estrogen-mediated cellular responses (Figure 2).

Akin to estrogen regulation of the protein-coding gene, estrogen also regulates miRNA expression through genomic and nongenomic mechanisms. Estrogen-bound ERα binds directly to the estrogen receptor binding site (ERE) on the promoter region of estrogen-responsive miRNAs, such as miR-21, miR-23a, miR-221/222, to stimulate the expression of these miRNAs.[132,135] Nevertheless, not all estrogen-regulated miRNAs contain ERE sites on their promoter. As we

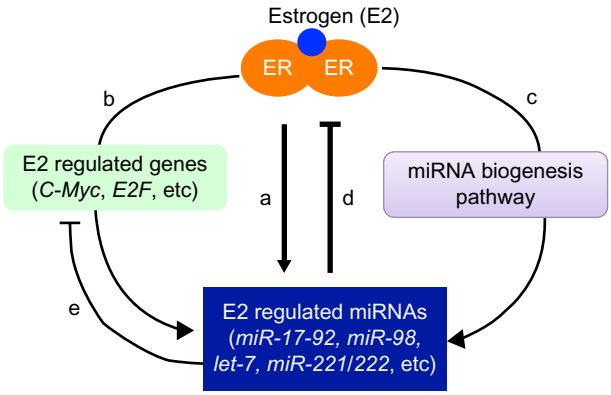

Figure 2 A regulatory circuitry of estrogen-activated estrogen receptor (ER), estrogen-regulated microRNA (miRNA), and messenger RNA in estrogen action.
Notes: Estrogen regulates miRNA expression at the transcriptional level through the direct binding of activated estrogen receptor to estrogen receptor binding site of the promoter of miRNA genes (a) or through the induction of estrogen-regulated transcription factors such as *c-Myc* (b). In addition, estrogen could regulate miRNA at the post-transcriptional level via affecting miRNA biogenesis (c). On the other hand the estrogen-regulated miRNAs can target estrogen receptor (d) and estrogen-responsive genes (e), thereby form a negative feedback loop to fine-tune estrogen-mediated cellular responses.
Abbreviations: E2, estrogen; ER, estrogen receptor.

mentioned earlier, the induction of miR-17-92 by estrogen was dependent on the estrogen-induced coactivator c-Myc, as miR-17-92 does not have ERE on its promoter.[133] In addition, estrogen may also regulate miRNA expression/function at the posttranscriptional level by affecting pri-miRNA processing (Figure 2). Estrogen-bound ERα can interact with Drosha-containing microprocessor complexes and attenuate the processing of pri-miRNAs into pre-miRNAs, leading to the inhibition of a set of miRNAs in the mouse uterus.[136] In addition, estrogen was shown to regulate the expression of Exportin-5, Dicer, and Ago 2, which are key components of miRNA biogenesis and functional pathways.[132,137,138] Moreover, it has been shown that estrogen reduced expression levels of a set of mature miRNAs without affecting the expression of these miRNAs at precursor level, providing direct experimental evidence of estrogen regulation of miRNA at the posttranscriptional level.[136]

Our laboratory was the first to demonstrate that estrogen regulated miRNA expression in splenocytes from normal C57BL/6 mice, which contributed to estrogen-mediated promotion of inflammation.[130] It is noteworthy that several estrogen-regulated miRNAs, such as miR-146a, miR-125b, and miR-148a, have been implicated in autoimmune disease pathogenesis. This suggests that estrogen might contribute to the sex bias of autoimmune disease and affect disease course by regulating miRNA expression and function. In a recent follow-up study, we addressed this question directly with a female-biased NZB/W_{F1} lupus model. We found that estrogen treatment of orchidectomized male NZB/W_{F1} mice promoted

the expression of lupus-associated miRNAs, including miR-182-96-183 cluster, miR-379, and miR-148a, but did not affect miR-155 in splenocytes.[22] To further substantiate the role of estrogen and other female hormones in the expression of lupus-associated miRNAs, it would be interesting to determine the expression of these miRNAs during pregnancy when estrogen and other female hormones are markedly increased. Together, estrogen regulation of miRNA provides a new paradigm for understanding estrogen-induced immune modulation and potential contribution to the sex differences in autoimmune disease.

Androgen regulation of miRNA expression

Androgen usually functions through the activation of androgen receptors (ARs) to regulate the expression of androgen-responsive genes. Androgen and the AR signaling pathway is critical for male reproductive organ function, such as spermatogenesis. The AR signaling pathway is also involved in the development of prostate cancer (CaP). A recent study demonstrated that testosterone regulated the expression of a group of miRNAs in mouse Sertoli cells, of which some are preferentially expressed in testis.[139] One of the androgen-responsive miRNAs, miR-471, targeted Forkhead/winged-helix transcription factor (*FoxD1*) and the desmosomal cadherin (*Dsc1*) gene, which are highly expressed in Sertoli cells and germ cells and are important for androgen-mediated regulation of spermatogenesis.[139] Numerous miRNAs have been implicated in the development and progression of CaP.[127,140] Deep sequencing revealed that 83 miRNAs were differentially expressed in an androgen-dependent (LNCaP) and androgen-independent (LNCaP-AI) prostate cancer cell line, suggesting the involvement of miRNAs in the transition of prostate cancer cells from androgen-dependent state to androgen-independent state.[141] Further bioinformatic analysis suggested that most of these differentially expressed miRNAs target genes involved in mitogen-activated protein kinase (MAPK) pathways, which are implicated in the promotion of tumor growth and in the development of hormone-refractory disease.[141] In a different report, miR-125b was shown to differentially express in LNCaP and LNCap-AI cell line, as well as in benign and malignant prostate tissues.[142] The authors further reported that androgen upregulated miR-125b expression via direct binding of AR to its promoter. Administration of synthetic miR-125b mimics stimulated androgen-independent growth of LNCaP cells by reducing the expression of the proapoptotic protein, Bcl-2 antagonist killer 1.[142] Moreover, androgen was shown to promote the expression of miR-21, miR-19a, miR-27a, and miR-133 in CaP cancer cells through the direct binding of activated AR to the promoter of these miRNAs.[143–145]

In addition to regulating male reproductive organ function, androgen and AR signaling also play an important role in regulation of somatic tissues development and function. It was shown that testosterone induced the expression of 6 miRNAs in mouse liver, of which most were induced via nongenomic mechanism.[146] Androgens are usually considered as immune suppressors, thereby exerting protective effect on different autoimmune diseases such as SLE, multiple sclerosis, and RA.[147–149] Nevertheless, further investigation is warranted to investigate AR regulation of miRNA expression in immune cells, which may provide a plausible explanation for AR-mediated suppressive effect on SLE.

Conclusion and perspectives

The disparities between males and females in immune capabilities, in susceptibility to diverse diseases, and in responses to drugs are apparent. Nevertheless, the sex factor is usually neglected in biomedical research and in drug clinic trials, which has led to the withdrawal of 8 of 10 prescription drugs in the US market.[150] Thankfully, the importance of defining gene regulation and functional mechanism in the context of different sexes and pathological conditions is increasingly appreciated by biomedical researchers and pharmaceutical drug development scientists.[4,69,151] Dissecting the sex differences in autoimmune disease shall provide optimal disease management for male and female patients and will aid us in developing efficient, sex-based therapy.

Despite their short history due to recent discovery, miRNAs have provided us with a new paradigm for understanding genome regulation and function in diverse biological systems. miRNAs have emerged as key regulators of immune system development and immune responses and have been linked to the development of various immune disorders, including autoimmune diseases. Given that most autoimmune diseases display marked sex differences, it is the time to incorporate sex factors in studies pertaining to miRNA regulation and functions. Further investigations are warranted to determine the functional contribution of sexual dimorphism of miRNA expression to the sex differences in autoimmune disease. It is also equally important to delineate the underlying basis of sex differential miRNA expression.

Epigenetic mechanisms such as DNA methylation also play an important role in the regulation of both non protein-coding miRNA and protein-coding mRNA expression. Sex-specific epigenetic control of miRNAs could also contribute to the sexual dimorphism of miRNA

expression. It should be noted that sex chromosomes, sex hormones, sex-specific environmental exposures, and other sex-biased factors do not work as individual components. Rather, they interact with each other to cause sex-biased action in gene networks and, consequently, lead to sex differences in physiological traits and health outcomes.[151] We believe that incorporating sex factors in miRNA and autoimmune research is critical for better understanding of the sex bias of autoimmune diseases. This is a prerequisite for developing a sex-based, efficient, miRNA interfering therapeutic approach to treat autoimmune diseases with marked sex bias.

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Disclosure

The authors report no conflicts of interest in this work.

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