MicroRNAs in the key events of systemic lupus erythematosus pathogenesis

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Background. Small non-coding RNA molecules (miRs) are involved in immune cell maturation and function and might influence immunopathological processes of systemic lupus erythematosus (SLE) pathogenesis.

Methods and Results. This paper presents the results of a literature search for publications dealing with the relationship between miRs and pathological factors related to SLE such as genetic background, immune dysregulation and gender-associated differences participating in SLE development. In SLE, distinct miRs are differentially expressed in SLE cells of innate and adaptive immunity. The miR-146a and miR-155 genes, among others, interfere with intracellular signalling pathways downstream of toll-like receptors 7 and 9 (TLR-7, TLR-9) and influences interferon (IFN)-type I synthesis in plasmacytoid dendritic cells. In T and B cells, miR-126, miR-21, miR-146a, miR-155, miR-1246 and others might influence gene expression by epigenetic modifications, support abnormal cytokine release, differentiation of cell subsets, B cell hyperactivity and autoantibody production. Besides, estrogen might up- and downregulate immunologically active miRs, which are potential mediators of hormonal influences in SLE development. Moreover, SLE genetic basis included some polymorphisms of the miR-146a gene, which varies across populations.

Conclusion. Distinct miRs are differentially expressed in both SLE mice models and human patients and promote autoimmune features of immune processes. MiRs are important molecules modulating susceptibility to SLE as well as its onset, clinical diversity and progression.

Key words: microRNA, SLE, innate immunity, adaptive immunity, genetic predisposition, estrogen

INTRODUCTION

Systemic lupus erythematosus (SLE) is a severe autoimmune disease with various clinical manifestations including multiorgan involvement, an unpredictable course with alternation of flares and remissions requiring long-term treatment using glucocorticoids, immunosuppressive and biologic agents or both1-3. The worldwide overall incidence rates of SLE range from 1-10 per 100,000 person-years, and SLE affects predominantly females (the female to male ratio is 9:1) in reproductive age4, although there appears to be a delay of the peak of disease onset after 40 years of age in the Caucasian population5. Interestingly, the severity of some organ manifestations, like lupus nephritis (LN) with consequences for morbidity and mortality6, varies among populations. The clinical diversity of SLE is reflected in its complex pathogenesis. The risk conditions for SLE development, such as genetic predisposition, environmental and hormonal stimuli, dysregulation of innate as well as adaptive immunity, cumulate to cause the onset of the disease or influence its course7.

MicroRNAs and SLE

The evolutionarily conserved small non-coding ribonucleic acid (microRNA) was discovered by Lee et al. in 1993 in the nematode Caenorhabditis elegans as a lin-4 transcript containing a complementary sequence to another protein-coding messenger RNA (mRNA), lin-14, whose function is regulated upon binding of these small molecules and causes disruption of larval development8. In subsequent years, small non-coding RNA became of key interest in many studies focused on the regulation of plant, animal and human gene expression, and the number of known microRNAs (miRNAs or miRs) increased gradually. Thus, a database of 218 miRs, miRBase (www.mirbase.org), comprising miRs sequences and annotations9-11 was established in 2002 and has since been expanding exponentially; it now contains more than 35,800 mature miRs products from 223 species including over 2500 human miRs (miRBase release 21, June 2014).

Due to their binding to complementary mRNA in the 3’-UTR region, 18-25 nucleotides long miRs alter protein expression, and are thus involved in almost all biological processes of the cell. In human medicine, the regulatory functions of miRs in cell metabolism, survival and apoptosis as well as cell proliferation and differentiation12 have been supposed to participate in the pathogenesis of illnesses such as several types of cancer or degenerative, autoimmune and metabolic diseases13-15. MiRs are potent regulators of immune cell development and possibly interfere with several immunological processes16. In the pathogenesis of SLE, the central role belongs to dysregulation of adaptive immunity mechanisms, while pathological communication between T and B lymphocyte subsets leads to abnormal cytokine synthesis and production of autoantibodies, which are in some cases directly pathognomonic. Besides this, abnormalities in innate immunity, particularly in the early phases of the
inflammatory response during autoantigen presentation and processing by dendritic cells and phagocytes, are involved in SLE immunopathology. MiRs are involved in the majority of immunopathological mechanisms in SLE. Furthermore, hormonal factors affect miRs expression particularly in immune cells, and the miR-146a gene is located within SLE-risk areas (see Fig. 1). Moreover, miRs are not located only intracellularly, but their presence in tissues and body fluids, such as the plasma and the serum, makes them potential blood-based biomarkers of disease development and activity as well as for predicting therapeutic responses. In SLE, different patterns of miRs expression have been detected in the plasma, serum, and urine as well as in peripheral mononuclear cells (PBMCs) (ref.24-28). Interestingly, underexpression of several miRs is more common in SLE (Table 1). A certain degree of variability in the SLE miRs pattern can be observed also in clinical manifestations such as renal involvement (e.g. miR-146a) (ref.22,23) or even SLE onset during childhood (miR-516a-3p, miR-629, miR-525-5p) (ref.29), and some miRs positively (e.g. miR-21) or inversely (e.g. miR-146a) correlate with the disease’s activity.

Here we overview the genesis of miRs and potential approaches to treating SLE. Furthermore, we focus on potential links between miRs and SLE pathogenesis, in particular the distinct genetic association between miRs expression in SLE and hormonal influences on SLE and the connection to miRs dysregulation. We also review the role of miRs in immunopathological reactions linked to SLE, especially changes in innate immunity and the function of T and B cells.

**MiRs biology and regulation**

**Biogenesis of miRs**

The multistep process of miRs synthesis begins in the nucleus, and maturation is completed in the cytoplasm.
Table 1. List of miRs differentially regulated in body fluids and peripheral mononuclear cells of Systemic lupus erythematosus.

<table>
<thead>
<tr>
<th>Overexpression</th>
<th>Underexpression</th>
<th>Origin</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-223, miR-142-3p</td>
<td>miR-150</td>
<td>plasma</td>
<td>18</td>
</tr>
<tr>
<td>miR-21</td>
<td></td>
<td>plasma</td>
<td>20</td>
</tr>
<tr>
<td>miR-142-3p, miR-181a</td>
<td>miR-106a, miR-17, miR-20a, miR-203, miR-92a</td>
<td>plasma</td>
<td>19</td>
</tr>
<tr>
<td>miR-126, miR-21, miR-451, miR-16, miR-223</td>
<td>miR-125a-3p, miR-155, miR-146</td>
<td>plasma</td>
<td>21</td>
</tr>
<tr>
<td>miR-200a, miR-200b, miR-429, miR-205, miR-192</td>
<td>miR-200a, miR-200b, miR-429, miR-205, miR-192</td>
<td>serum</td>
<td>22</td>
</tr>
<tr>
<td>miR-146, miR-155</td>
<td></td>
<td>serum</td>
<td>23</td>
</tr>
<tr>
<td>miR-189, miR-61, miR-78, miR-342, miR-142-3p, miR-299-3p</td>
<td>miR-196a, miR-17-5p, miR-409-3p, miR-141, miR-383, miR-112</td>
<td>PBMC</td>
<td>24</td>
</tr>
<tr>
<td>miR-127-3p, miR-1271-5p, miR-1301, miR-136-5p, miR-379-5p, miR-381-3p, miR-382-5p, miR-758-3p, miR-1466-5p, miR-154-3p, miR-154-5p, miR-31-5p, miR-409-5p, miR-410, miR-421, miR-543, miR-431-5p, miR-432-5p, miR-654-3p, miR-181a-2-3p, miR-337-5p, miR-376-3p, miR-376b-3p, miR-376c-3p, miR-485-3p, miR-487b, miR-493-5p, miR-495-3p, miR-539-5p</td>
<td>miR-31, miR-95, miR-99a, miR-130b, miR-10a, miR-134, miR-146</td>
<td>PBMC</td>
<td>25</td>
</tr>
<tr>
<td>miR-21, miR-25, miR-148a, miR-214, miR-148b, miR-494, miR-198, miR-155, miR-324-3p, miR-342, miR-373, miR-106b, miR-544</td>
<td>miR-296, miR-196c, let-17-5p, let-16, let-15a, miR-383, miR-184, miR-379, miR-150, miR-7a, miR-7d, miR-7g, miR-98, miR-832</td>
<td>PBMC</td>
<td>26</td>
</tr>
<tr>
<td>miR-157, miR-64, miR-147, miR-160, miR-65, miR-120, miR-100, miR-194, miR-217, miR-173</td>
<td>miR-41, miR-42, miR-27, miR-8, miR-21, miR-3, miR-40, miR-28, miR-9, miR-49</td>
<td>PBMC (only top ten novel dysregulated miRs)</td>
<td>28</td>
</tr>
</tbody>
</table>

The over- or underexpression to healthy controls. miR – microRNA. PBMC – peripheral mononuclear cells
sanger strand (ref.40,41), see Fig. 2, 6th point. The miR star strand usually undergoes degradation39, however, in some cases might keep regulatory function. Thus, according to sequence derived from 5' or 3' arms of pre-miR precursor can be miR/miR* also termed as -5 or -3p (ref.10). The single stranded mature miR - Ago2 becomes part of the RNA induced silencing complex (RISC) (ref.50,41), see Fig. 2, point 7. RISC complex bind to target mRNA (or other non coding RNAs) and causes its inhibition of translation (see Fig. 2, point 8A) or decay and degradation (see Fig. 2, point 8B). The target is located in the 3'UTR of mRNA and is recognized by the "seed region" of miR, complete complementarity is not essential 40-46.

Each step of miRs genesis can be controlled. Like other genes, promoters of miRs are under the regulation of multiple transcriptional factors, and alterations to their binding sites, be it by genetic variations or epigenetic modifications, may lead to aberrant expression. In SLE, the risk G allele of single nucleotide polymorphism (SNP) rs57095329 in the miR-146a promoter causes decreased binding of Ets-1 and subsequently decreased miR-146a expression47. Similarly, epigenetic modifications like histone acetylation48 or CpG island hypomethylation may influence some miRs expression and increase the risk of diseases. Genetic variation in the miRs machinery (e.g. Dicer, Drosha, Ago proteins, Exp5) may cause global changes in miRs synthesis. On the other hand, miRs are able, through a feedback loop, to regulate the microprocessor complex or other parts of the miRs machinery. The SNP rs3742330A>G in 3'UTR of the Dicer gene is located in the binding site for mirRNA-5582-5p and miRNA-3622a-5p and is associated with survival of patients suffering from T cell lymphomas49. However, not only genetic variation, but also other regulatory molecules can affect expression or directly bind to the Ago or Dicer proteins and alter their functions. Estrogens and progesterone increase the expression of Exp5 and Dicer50, and, interestingly, anti-Su antibodies occurring in SLE patients might have a role in miRNA biogenesis and RISC silencing because they recognize Ago proteins and Dicer51. The mechanisms and factors influencing expression of the miRNA machinery may depend on individual cells and organs and has been of immense research interest.

**MiRs function in general**

MiRs bind to the target miRNA-responsive element (MRE) of protein-coding mRNA, usually located in the 3'UTR region (ref.32,40), but occasionally in other regions such as 5'UTR (ref.45). Additional, miRs can bind to other regulatory RNAs or non-coding RNAs (ncRNAs) (ref.41,44). The miRs target is recognized by the "seed region", a 2-8 nucleotides long locus within the 5'terminal region of the miR (ref.35,46). For this reason, each miR can connect to hundreds of miRNAs, and mRNA might have multiple binding sites for miRs46. The potential interaction between mRNA and miR can be modelled and predicted by a range of different computational approaches (miRBase, DIANA-microT-CDs, miranda-misVR and TargetScan) (ref.52). Matched miR-mRNA either causes mRNA destabilization, deadenylation and degradation or leads to inhibition of translation41. Although complete complementarity miR-mRNA is not essential, the functions of miRs are influenced by genetic variation either in miRs "seed regions" or in target regions of mRNA (ref.13). In addition, RNA editing can alter binding sites and affect the stability of miR (ref.54).

MiRs manipulate approximately of 60% of gene expression46-48 and are important regulators in post-transcriptional modifications. Moreover, miRs can regulate not only the original cell, but are involved in intercellular communication. Between cells over short distances, miRs may be exchanged via the gap junction52, but circulating miRs can influence intercellular communication of tumour, immune and healthy cells over long distances.

**Extracellular miRs**

Since mature miRs are found in the extracellular fluid, secretion outside the cell takes place by some as yet insufficiently known mechanisms, which is currently being studied. In the Fig 2 are described some known ways how miRs can leave viable cells - miRs become components of multivesicular body and by exocytosis are secreted outside the cell16 (point 9A), or are components of encapsulated cell membrane and leave the cell as microvesicles57 (point 9B). Moreover, miR leaves the cell by transporter protein and is re-updated by receptor (point 9C, Fig 2). To date, there is only one example of this mechanism: HDL – miRs complexes are released from the cell by ABCA1 transporter and recipient cells use scavenger receptor class B type I (SR-B1) receptor for uptake54. Additionally, miR might leave the cell via gap junction during intercellular communication on short distance55, see point 9D Fig 2.

Circulating miRs are highly stable in conditions, such as high and low pH, boiling or extracellular RNase activity, and are probably capable, by some unknown mechanism, to maintain stability outside the cell. Wang et al. demonstrated in experimental cell lines that the RNA-binding protein nucleophosmin 1 protects synthetic miR-122 from degradation58, and in the human plasma, ribonucleoprotein Ago 2 maintains miRs stability48. MiRs in the whole blood, serum or plasma exist either as protein-bonded complexes or as microvesicles or exosomes56,59,60. Both these packages protect against extracellular RNase activity and allow the miRs to be accepted by other cells. The majority of circulating miRs are bound to proteins – the Argonaute family, particularly Ago 2 (ref.29), nucleoplasmin58 or high density lipoproteins (HDL) (ref.34). The mechanism of cellular release and uptake requires a transporter and receptor system and is not yet definitely known. A minority of circulating miRs may be kept either by microvesicles or exosomes56,57 from viable cells or are part of apoptotic bodies from cells undergoing apoptosis. Microvesicles are formed by encapsulation of the cell membrane, but exosomes are vesicles that are actively secreted by fusion of exocytic multivesicular bodies with the plasma membrane. Apoptotic bodies are large vesicles containing cytoplasmatic components, miRs and fragmented DNA. In general, host vesicles are
taken up by recipient cells via endocytosis, phagocytosis or fusion of plasma membranes. Microvesicles and apoptotic bodies are supposed to be involved in cell-to-cell communication over long distances and between organs. Recently, however, Chevillet et al. asked whether to revise this model in the case of exosomes. They demonstrated by quantitative and stoichiometric analysis that exosomes prepared from the plasma, seminal fluid, dendritic cells, mast cells and ovarian cancer cells do not carry biologically significant numbers of miRs (ref.35). On the other hand, exosomes containing miRs are involved in the formation of immunological synapsis of T cells and antigen-presenting cells (APC). The unidirectional transfer of exosomes containing miR-335 from T cells to APC is induced after antigen recognition37. The miRs in exosomes probably have a distinct role in the microenvironment of inflamed tissues, such as in the case of LN. Interestingly, in SLE, the majority of urine miRs are packaged in exosomes, especially in active LN. Exosomes in SLE contain miR335-5p, miR-302, miR-200c and in active LN the highest levels of miR-146a (ref.34). Mir-146a is highly expressed in the glomerulus and is involved in local inflammation. Similarly, miR-26a in urine exosomes reflects renal injury in LN (ref.35).

Genetic predisposition to SLE and miRs

Predisposing genetic factors are a prerequisite for the disease to manifest. Family studies indicate 66% heritability, and the concordance rate in monozygotic and dizygotic twins is in the range of 24-69% and only 2-5% (ref.44), respectively. Although genome-wide association studies (GWAS) reliably determine SLE risk locations, some genetic predisposing factors may differ between populations. The complex genetic background of SLE pathogenesis resides in variation of gene expression or in functional variants of members of multiple signalling pathways, immunologically active molecules or both44. Some association studies focused on particular SNPs of miRs genes and autoimmune diseases have already been performed, but failed to find any association with SLE. For example, miR-499 rs3746444 is associated with decreased risk of rheumatoid arthritis development44, but there is no association with SLE (ref.45,46). The miR-146a gene, however, has become the centre of interest, as the novel genetic locus 5q33.3 has been ascertained in recent GWAS studies of SLE patients of European and Asian ancestry45,70. The miR-146a gene exhibits at least three functional SNPs important for autoimmunity.

Within the miR-146a promoter region, there is the A/G SNP rs57095329, and the G allele causes reduced promoter activity; furthermore, the GG genotype shows the lowest miR-146a mRNA levels compared to the AA genotype, $P = 0.019$ (ref.48). A meta-analysis of SLE in patients of Asian ethnicity (Hong Kong, Bangkok and the Chinese mainland) has demonstrated a significant association with the G allele ($P < 0.0001$, OR 1.29 [CI 95% 1.18-1.40]) (ref.47). In patients of European ancestry, however, the frequency of this allele of rs57095329/rs2277920 was found to be very low, so it cannot be presented as an SLE-associated genetic marker71. Future studies should examine whether differences in allele frequencies of miR-146a SNPs across ethnicities may be part of the explanation of population differences in SLE development and severity. The functionality of the rs57095329 SNP could be explained by an interaction with the Ets-1 transcription factor. Although rs57095329 is not located in the core sequence of Ets-1, the G allele, in particular, influences its binding affinity and reduces miR-146a transcription in vitro47. Interestingly, GWAS have established variations of Ets-1 (rs1128334 SNP) as a genetic factor in SLE (ref.39), and Luo et al. performed an interaction analysis of both risk SNPs (ref.40). No cumulative effect was found using a conditional logistic regression test with the interaction between the two variants treated as a covariate using PLINK. However, individuals carrying two or more risk alleles are at greater risk of developing SLE, and the OR increases in the process; for example, the combined risk for miR-146a homozygotes with no risk allele of Ets-1 (GG-GG) has an OR value of 1.75 (CI 95% 1.12-2.73), the risk for miR-146a homozygotes with one risk Ets-1 allele (GG-AG) has an OR value of 2.03 (CI 95% 1.35-3.05), and the combination of both risk homozygotes has an OR value of 4.79 (CI 95% 1.9-12.09) (ref.40). On the other hand, Leng et al. performed a gene-gene interaction analysis of miR-146a (rs57095329) and IL-21 (rs907715 and rs2221903), IRF-5, IKZF-1 and Ets-1 (rs6590330), using direct counting and a chi-square test with a 2×2 factorial design, and found no interaction of miR-146a, in particular with Ets-145. The Ets-1 rs6590330 SNP is in high linkage disequilibrium with the rs1128334 SNP ($r^2 = 0.97$) (ref.47) and has been associated with earlier onset of the disease and certain SLE manifestations, including malar rash, photosensitivity, arthritis, serositis and renal involvement72. The clinical diversity of SLE should probably be considered in further studies to elucidate genetic interactions, at least between Ets-1 and miR-146a.

The other miR-146a SNP, rs 24311697, is located between the pituitary tumour–transforming 1 (PTTG1) gene and the miR-146a gene, 15.3kb upstream of the miR-146a transcription starting site46. Löfgren SE et al. demonstrated a significant association of SLE with the T allele ($P < 0.001$, OR 1.23 [CI 95% 1.10-1.38]) and the TT genotype ($P < 0.001$, OR 1.49 [CI 95% 1.17-1.90]) in patients of European ancestry71. Similarly, the T allele of rs2431697 has been established as a risk factor in the Chinese Han population, $P < 0.01$, OR 1.30 (CI 95% 1.08-1.56) (ref.47). This SNP seems to be functional whereas TT homozygotes, compared to the CC genotype, express in PBMC 1.6- and 2.2-fold lower mature and primary miR-146a levels (both $P < 0.01$) (ref.47), respectively. Interestingly, the association between the T allele and SLE in patients with anti-dsDNA antibodies ($P < 0.001$, OR 2.510 [CI 95% 1.545-4.077]), and in particular in SLE females ($P = 0.006$, OR 1.538 [CI 95% 1.113-2.093]), has recently been proven (ref.73). The recent study of Tang et al., moreover, performed a meta-analysis of six studies involving 8642 SLE patients and 10947 healthy controls supporting the association of the T allele in patients
MiRs and hormonal influences in SLE

The predominance of females over males suffering from SLE and the increased incidence of the disease during childbearing age apparently shows the role of sex hormones and other sex-biased factors in the disease’s pathogenesis.

Estrogens, in contrast to testosterone, might support the development of autoimmune diseases and influence the immunopathological mechanisms of SLE-like autoimmune B cells selection, autoantibody production and aggravation of Th2 reactions. Additionally, sex steroid hormones might influence miRs expression. Recently, Dong G et al. found increased expression of interferon (IFN) type I inducible genes, including toll-like receptor (TLR)-7 and the signal transducer and activator of transcription (STAT)-1, but reduced levels of let-7e-5p, mir-145-5p and mir-98-5p, in PBMCs of premenopausal females compared to age-matched men. The effect of estrogens, in particular 17β-estradiol, on the development and function of cells of innate and adaptive immunity has already been well documented. Estrogen enhances the innate immunity response of splenic lymphocytes to lipopolysaccharide (LPS) while at the same time it downregulates expression of miR-146a, which inhibits LPS-induced IFNγ and LPS-induced nitric oxide in vivo. Interestingly, the effect of 17β-estradiol on miRs expression and enhanced activity of IFN type I in B cells has recently been elucidated. 17β-Estradiol, via its estrogen receptor (ER) α, down-regulates expression of let-7e-5p, mir-98-5p and mir-145-5p in vitro, and all three miRs reduce the mRNA of inhibitor of kappa B kinase ε (IKKε, gene IKBKE), targeting it directly on 3'UTR (ref.80). IKKε is an essential factor for 17β-estradiol amplification of the IFN type I response in B cells, and gender differences of its expression have been found in vivo. This finding supports the role of IKKε in SLE pathogenesis. In addition, the functional single nucleotide variant of the IKBKE gene (rs12142086) has been found to be associated with SLE (ref.82).

The influence of gender on the contribution of miRs to autoimmunity, SLE in particular, has been clarified in mice models. Female Dicer-deficient mice (CD19-Creki/+; Dicerki/fl/fl) develop during ageing autoimmune features such as production of autoantibodies against cardiolipin, anti-dsDNA antibody production, and autoimmune kidney involvement, because lymphocyte infiltration and overall damage to the glomerulus architecture appears in 50% of them. On the other hand, as demonstrated in orchietomized lupus-prone NZB/W F1 mice, long-term estrogen treatment causes development of SLE and elevation of miR182-96-183 cluster, mir-379 and mir-148a levels, but no correlation between anti-dsDNA and these miRs was found after 26 weeks of estrogen treatment. Female NZB/W F1 lupus mice typically express the disease earlier than males. Similarly, before the onset of SLE, the two genders do not differ in their expression of miRs (miR-182-96-183, miR-31 and miR-148a, miR-155) except mir-127 and mir-379. After SLE onset, however, the levels of miR-182-96-183, miR-31, miR-127, mir-379 and mir-148a are elevated in 30 weeks old females compared to age-matched males without SLE features. Furthermore, estrogen-treated orchietomized 32 weeks old mice express similar levels of these miRs as their female counterparts. All these data explain the modulation of miRs by estrogen and support selected miRs as mediators of the alteration of immune reactions to autoimmunity by sex hormones.

To understand the differences of miRs expression between genders in relationship to SLE, it is necessary to keep in mind the potential role of other sex-biased factors. The sex-associated differences in miRs regulation have recently been robustly reviewed by Khan et al. (ref.85). The X sex chromosome comprises 113 miRs genes, in particular some that are involved in immune cells maturation and differentiation. Some of them are linked to the SLE, for example, miR-223, miR-222, miR-221, miR-98, miR-106a, the miR17-92 cluster, miR-503 and miR-542 (ref.82). Recently, Chen et al. found 46 dysregulated miRs located on chromosome X/Y in SLE compared to healthy controls, but when they considered fold changes of >1 or <1 and P ≤ 0.01, the number dropped to 11 up- and downregulated miRs (ref.28), respectively. In SLE, the dosage effect of chromosome X on disease development and severity has been found in an experiment on mice. Similarly in humans, men with Klinefelter’s syndrome (XXY) show an increased risk of disease development similar to women.

MiRs, the immune system and SLE

MiRs participate in immune cell life processes like survival, metabolism and apoptosis and are potent regulators of immune cell development, maintenance and functions. Both innate and adaptive immunity is influenced by these molecules, and moreover, due to intercellular communication during immune reactions, miRs might modulate the immune response. In the pathogenesis of SLE, modulation of immune processes leading to the perpetuation of a pathological inflammatory response, particularly the production of autoantibodies, might have a critical role.

Innate immunity and miRs and SLE

Humoral and cellular innate immunity jointly responds to foreign organisms, including viruses, either...
Cells of the innate immune system are equipped with several families of pattern recognition receptors (PRRs) to recognized molecules derived from pathogens or damaged cells. Dendritic cells (DC), in particular plasmacytoid cells (pDCs), express endosomal toll-like receptors (TLR)-7 and TLR-9 recognizing host single stranded RNA and bacterial DNA, respectively, as well as endogenous complexes of nucleic acids derived from apoptotic or necrotic cells. After activation, pDCs become a source of interferon (IFN) type I and IL-6. Prolonged production of these cytokines promotes processes essential for SLE development like B cell survival, antibodies production and T cell abnormalities. Recently, the involvement of pDCs was observed in the inhibition of apoptotic cell clearance in marginal zone macrophages. The pDCs, after TLR-7 and TLR-9 stimulation, express mir-146a, which through negative feedback accomplishes the survival of pDCs while down-regulating BCL2-a1 levels and causing apoptosis. The next mir-146a target in pDCs was observed in the inhibition of apoptotic cell clearance in marginal zone macrophages. The pDCs, after TLR-7 and TLR-9 stimulation, express mir-146a, which through negative feedback accomplishes the survival of pDCs while down-regulating BCL2-a1 levels and causing apoptosis. The next mir-146a target in pDCs was observed in the inhibition of apoptotic cell clearance in marginal zone macrophages.

Firstly, mirs act as a modulator of epigenetic mechanisms directly, or by producing inflammatory cytokines and cooperating with the adaptive immune system, particularly B cells. The prolonged inflammatory answer, however, may crucially support the development of autoimmunity such as SLE, and SLE-associated miRs, particularly miR-146a and miR-155, are supposed to be involved in these processes. In experiments, mice deficient in mir-146a (miR-146a−/−) respond hypersensitively to lipopolysaccharide (LPS) stimulation and produce large numbers of interleukins (IL), such as IL-6, IL-1β, IL-10 and tumour necrosis factor (TNF)-α, and moreover, during aging develop an autoimmune disorder characterized as tissue damage by multiorgan lymphocytic and monocytic infiltrates and formation of anti-dsDNA autoantibodies. Interestingly, Xing et al. demonstrated in a recent study of miR-155 knockdown lupus-prone Fas−/− mouse models a milder SLE phenotype than in wild-type strains. miRs are differentially expressed in SLE patients. While the M2c subset together with the M2a subset are reduced in LN, the M2b subset matures after stimulation by immune complexes, and its amount correlates with LN relapse. The experimental mouse model of induced lupus, following the administration of activated lymphocyte-derived DNA (ALD-DNA) to BALB/c mice, exhibits infiltration of macrophages, particularly of the M2b type, in the kidneys. Xiao et al. recently demonstrated a critical role of miRs in M2b polarization in this model. They performed mirs microarrays of bone marrow-derived macrophages (BMDMs) after ALD-DNA induction over time and found dynamic mirs expression corresponding with BMDMs polarization. The M2b subset expand after six hours of stimulation, but decreased after thirty-six hours, and 43 and 42 miRs, respectively, were found to be differentially expressed. However, expression of only six miRs was similar at both times, but the rest were up- or downregulated. Interestingly, miR-155, miR-29b and miR-30e were continuously up-regulated, while miR-129-5p, miR-466j, miR-222* or the miR-29 family showed transient up-regulation. Early miRs are involved in the immune response, immune cell trafficking and cellular development whereas later miRs are associated with cell development, death and survival. In light of these findings, it is necessary to keep in mind that the miRs network in the innate immune response is complex and time-dependent. Breakdown of this tight regulation may result in the perpetuation of pDC or macrophage activity and promote autoimmunity.

**Pathologies in adaptive immunity in SLE and miRs**

**T cells, miRs and SLE**

Abnormalities in T cell populations contribute importantly to SLE pathogenesis either by abnormal distribution of pro-inflammatory Th17 and anti-inflammatory T regulatory (Treg) cells or by abnormal production of cytokines such as IL-2 and IL-10 or even by abnormal communication with B cells leading to the production of autoantibodies. MiRs are differentially expressed in SLE T cells and contribute to their abnormalities by influencing several mechanisms (Table 2). Firstly, miRs act as a modulator of epigenetic mecha-
nisms, yet changes in their expression correspond to epigenetic modifications. Whereas epigenetic modifications are among the key pathogenetic events in SLE, miRs are in the centre of interest. In SLE, decreased activity of the DNA methyltransferase 1 (DNMT1) is associated with abnormal gene expression and disease development. MiRs alter DNMT1 activity in a direct or indirect manner. Two miRs, miR-126 and miR-148a, directly inhibit the translation of DNMT1 by binding to the 3’UTR of the transcript. Both subsequently regulate the expression of CD11a and CD70 (ref.30,104), and moreover, experimentally over-expressed miR-126 instigates CD4+ cells to stimulate IgG production in cocultured B cells104. On the other hand, DNMT1 levels are indirectly alleviated in SLE by two other miRs: MiR-29b reduces Sp1, the DNMT1 transactivator105 and miR-21 reduce the activity of the Ras-MAPK-DNMT1 signalling pathway106. Although miRNA-21 is expressed equally in SLE with and without nephritis, it correlates with disease activity106, and abnormalities in the “micrRNA expression pattern” have been supposed to depend on the SLE phenotype. Zhao M. et al. recently demonstrated differences in methylation in CD4+ T cells within miRs as well as other genes in three SLE patients groups suffering from skin involvement only, both skin and renal involvement and involvement of all skin, kidneys and joints106. Although the study is limited by the number of SLE patients (n = 4 in each group), relevant data show differences not only in miRs target genes, but also in up-regulation and down-regulation of miRs, such as miR-126, miR-451 and miR-181b, and miR-142-3p, miR-505 and miR-324-5p (ref.106), respectively. Similarly, expression of some miRs such as miR-142 might be alleviated through another epigenetic mechanism, which is histone modification107.

Table 2. The selected T cells abnormalities influenced by miRs in systemic lupus erythematosus.

<table>
<thead>
<tr>
<th>Epigenetic Modifications</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA hypomethylation</td>
<td>miRNA-126, miRNA-29b, miRNA-21, miRNA-148a</td>
</tr>
<tr>
<td>Histone acetylation</td>
<td>miRNA-142</td>
</tr>
<tr>
<td>Intracellular signaling and transcription factor expression</td>
<td></td>
</tr>
<tr>
<td>STAT-1</td>
<td>miRNA-145, miRNA-146a</td>
</tr>
<tr>
<td>SOCS-1</td>
<td>miRNA-155</td>
</tr>
<tr>
<td>KLF13</td>
<td>miRNA-125a</td>
</tr>
<tr>
<td>Jagged-1</td>
<td>miRNA-524-5p</td>
</tr>
<tr>
<td>NFkB</td>
<td>miRNA-146a</td>
</tr>
<tr>
<td>NFAT</td>
<td>miRNA-31</td>
</tr>
<tr>
<td>STAT-3</td>
<td>miRNA-125b</td>
</tr>
<tr>
<td>TRAF6, IRAK1</td>
<td>miRNA-146a</td>
</tr>
<tr>
<td>Ets-1</td>
<td>miRNA-125b</td>
</tr>
<tr>
<td>IRF-4</td>
<td>miRNA-224</td>
</tr>
</tbody>
</table>

| Abberation in cytokine and surface activation markers production                         |          |
| IL-10                                                                                 | miRNA-21, miRNA-142-3p, miRNA-142-5p | 27,107   |
| IL-2                                                                                  | miRNA-155, miRNA-31                | 111,112  |
| IL-17A                                                                                | miRNA-146a                          | 108      |
| IFN-γ                                                                                 | miRNA-524-5p                        | 114      |
| CD40L                                                                                 | miRNA-21                            | 27       |
| RANTES                                                                                | miRNA-125b                          | 119      |
| CD-84                                                                                 | miRNA-142-3p, miRNA-142-5p          | 107      |
| CD-69                                                                                 | miRNA-146a                          | 108      |

| Apoptosis, survival and proliferation                                                  |          |
| miRNA-224                                                                              | 115      |
| miRNA-146a                                                                             | 108      |
| miRNA-21                                                                               | 27       |

| CD4+ T cells differentiation                                                           |          |
| Treg                                                                                  | miRNA-31, miRNA-155                  | 111,112  |
| potential: miRNA-126 via PI3K/Akt                                                      | 116      |
| potential: miRNA-125                                                                   | 109      |
| TFH                                                                                   | miRNA-10a, miRNA-17-92 cluster        | 121,103  |
| potential:                                                                             | 120,122  |

STAT- Signal transducer and activator of transcription, SOCS- suppressor of cytokine signalling, KLF- Kruppel factor, NFkB- nuclear factor kappa B, NFAT- nuclear factor of activated T cells, TRAF- TNF receptor-associated factor, IRAK- IL-1 receptor-associated kinase, IL- interleukin, IFN- interferon, CD- cluster of differentiation, RANTES-regulated on activation, normal T cell expressed and secreted (Chemokine ligand 5), Treg-T regulatory cells, TFH- T follicular helper

Recently, Tang Q et al. have shown escalated H4 acetylation contributing to increasing transcription of miR-142 in CD4+ T cells after treatment by mycophenolic acid (MPA), a drug commonly used for treating SLE (ref. 48).

Secondly, abnormalities in the expression of cytokines or other signalling molecules are caused either by the regulatory effect of miRs on several intracellular signalling pathways or directly by binding to target molecules, see Table 1. The SLE-associated miR-146a is down-regulated in SLE CD4+ T cells20,48, but MPA treatment restores its expression48. As demonstrated in miR-146a−/− mice, miR-146a plays multiple roles in T cells such as proliferation, apoptosis and cytokine production, for example, IL-17A (ref. 108). The target pathway NFκB is modulated in a negative feedback manner via the T-cell receptor (TCR) pathway. TCR stimulation causes aggravation of miR-146a expression (ref. 108). MiR-146a is involved in the differentiation of Th1 and Th17 cells, while Th1 cells show alleviated levels of miR-146a (ref. 109).

In SLE, cytokines IL-2 and IFN-γ produced by T cells are down-regulated, but the levels of IL-10 are elevated and the ratio IL-10: IFN-γ determines the severity of SLE110. The regulation of IL-2 is complex, but miR-31 and miR-155 contributes to its expression. Fan et al. demonstrated that decreased miR-31 expression positively correlates with decreased IL-2 synthesis in SLE T cells111. Further experiments showed that miR-31 increases IL-2 promoter activity by altering the expression of the nuclear factor of activated T cells (NFAT) (ref. 111). Similarly, miR-155 can bind directly to the suppressor of cytokine signalling 1 (SOCS1) and down-regulate IL-2 release112. In SLE CD4+ T cells, both elevated miR-21 and down-regulated miR-142-3p/5p contribute to enhanced expression of IL-10, either indirectly via suppression of programmed cell death-4 (PDCD4) or directly by inhibiting translation7100, respectively. Furthermore, an in vivo study of B6.Sle123 lupus mice with silencing of miR-21 found approximately 20% de-repression of PDCD4 in naïve CD4+ T cells and amelioration of the disease113. On the contrary, in SLE, miR-524-5p over-expressed in T cells enhances IFN-γ production in the activated T cell line114 and positively correlates with SLEDAI in SLE patients115. Since miR-524-5p affects the expression of the surface molecule Jagged-1 (ref. 114), a ligand for the Notch-1 receptor and target for miR-21 with a function during maturation of monocyte-derived dendritic cells, the role of this miRNA in SLE pathogenesis should be clarified. Other signalling molecules, like surface activation markers (e.g. CD40L) or chemokines (e.g. RANTES), are regulated by miRs (Table 2).

Thirdly, miRs influence cell survival, proliferation and apoptosis. In SLE, the prolonged cell survival and persisting immune response promote immunopathological activity. Lu et al. demonstrated in CD4+ T cells increased miR-224 expression. In vitro miR-224 decreases its target apoptosis inhibitory protein S (API S) at the protein level and could affect activated T cell survival115. Martinez-Ramoz recently confirmed elevated levels of miR-224 in CD4+ T cells obtained from inactive SLE patients103.

Fourthly, differentiation of T cells, particularly into Treg cells or T follicular helpers (TFH), which play an important role in SLE, might be influenced by several miRNAs. In short a nutshell, mir-150, mir-21 and mir-155 are involved in CD8+ genesis, but mir-150 or mir-146a and mir-147 or mir-155 and mir-326 in Th2, Th1 and Th17 (ref. 109), respectively. We should, however, keep in mind that Th differentiation requires an extraordinarily managed orchestra of transcription factors and other regulatory molecules. For example, IRF-4 is involved in Th2 and Th17 differentiation and has been computationally identified as a target for miR-224, which is overexpressed in CD4+ T cells of inactive SLE (ref. 109). The differentiation of Treg cells requires the transcription factor forkhead box protein 3 (Foxp3), which is the target of “SLE-associated” miR-126 (ref. 104,116). As mentioned above, IL-2 – a cytokine that is essential for Treg survival – is down-regulated by miR-31 or miR-155 in SLE (ref. 111,112). Moreover, miR-31 can suppress Foxp3 expression directly by binding to 3'UTR mRNA (ref. 117). Elevated levels of miR-125 have been found in Treg cells and is involved in their development109 whereas in SLE T cells both miR-125a and miR-125b are alleviated (ref. 118,119). Another subset of CD4+ T cells, TFH, is critical for cooperation with B cells and autoantibody production. TFH cells are characterized by the transcription factor B cell lymphoma-6 (Bcl-6) and secretion of IL-21 and are supposed to interfere with SLE pathogenesis. SLE model mice depleted of miR-155, miRNA-1553-Fas98,99 develop a phenotype with a reduced proportion of spleen TFH and subsequent lower levels of IgG and IgM titres8. Similarly, reduced levels of Bcl-6 and IL-21 in mouse spleens and lower levels of IL-21 in the mouse serum are found in this model8. Recently, two miRNAs, cluster miR-17-92 and miR-10a, were described as modulators of TFH genesis, Bcl-6 expression in particular (ref. 120,121). Experimentally, the miR-17-92 transgenic mouse model develops spontaneous TFH cell differentiation120 and miR-10a directly suppresses Bcl-6 on 3'UTR (ref. 121). Upregulation of miR-10a has been found in PBMC SLE cells103 and miR-17 and miR-19a or miR-19b in PBMCs of SLE patients with anti-dsDNA or anti-ENA autoantibodies122, respectively. Both these links to SLE should be elucidated in future studies. Recently, the mir-146a has been found to be highly expressed in TFH cells and plays a role in TFH cells development123. MiR-146a directly represses multiple TFH cell miRNAs, including ICOS and STAT-1, and downregulates TFH accumulation, while miR-146a−/− mice exhibited a 5-fold increase of TFH cells compared to wild-type littermates123.

B cells, miRs and SLE

Disturbances of B cells are a hallmark of SLE pathogenesis. They are manifested either as hyperactivity in autoantibody production, in the functioning of antigen-presenting cells or even as a source of inflammatory cytokines. Autoantibodies are involved in SLE immunopathological events and might serve as markers for monitoring SLE activity and organ manifestations2. The multistep process of B cells maturation in bone marrow
and the peripheral compartment is supported by tight regulation of function of B cell receptor (Bcr) and antibodies synthesis. The survival and function of B cells is determined by several crucial points either of immunoglobulin gene rearrangement by VdJ recombination during pro-B to pre-B cells transition in bone marrow or differentiation of immature B cells into marginal zone (mZ) or follicular (Fo) B cells, or even somatic hypermutation (sHm) and class switch recombination (csr) during activation in the periphery. The mechanisms of central and peripheral tolerance guarantee that only functional Bcr B cells undergo the next step of development. Each step of B cells maturation, however, may tip over and produce autoreactive B cells.

different patterns of mirs are expressed in each stage of B cell development, particularly in bone marrow and peripheral lymphoid tissue or the circulatory system, and can critically regulate the maturation process in a positive or negative manner, see Fig. 3. experimentally, in vitro or in vivo ablation of Dicer leads to disruption of mirs synthesis and causes the blocking of the development process in the early stages as well as maturation of B cells into MZ or follicular FO B cells or terminal maturation of B cells into memory and plasma cells during T cell-dependent immune response. During the early development of B cells in bone marrow is highly expressed mirR-181a (ref. 126), but alleviated levels of mir-132 are required for early transition of pro-B cells. Similarly, the over-expressed mirR-17-92 cluster is essential for survival of B cell precursors, differentiation from pro-B to pre-B cells and latter into immature B cells; there are variations between adult and paediatric age. On contrary, studies based on over-expressed experiments established important role of mirR-34a, mirR-150, mirR-125 and mirR-210 for correct transition of pro-B to pre-B cells. The first two mirs target transcription factor Foxp1 and c-myc, respectively, and regulate apoptosis and survival pro-B or pre-B cells.

In periphery, immature B cells progress into mature MZ or FO B cells. Interestingly, Cd19-CreERT2/DicerloxPlox mice develop reduced total numbers of mature B cells, but MZ B cells are favouring over FO B cells. MZ B cells mostly reside in the spleen and can be generated from B cells with autoreactive BCR signalling rather than FO B cells that circulate, form B cells follicles and undergo further maturation in a T-dependent manner in germinal centres (GC). The mir-185 is responsible for MZ or FO B cells differentiation, while targeting bruton tyrosine kinase (Btk), the downstream of Bcr (ref. 83), see Fig. 3. In GC, mir-181b, mirR-155 mirR-93 are involved in the setting up of antibodies isotype and BCR affinity maturation, see Fig. 3. All in stimulated B cells directly regulate activation-induced cytidine deaminase (AID), enzyme critical for CSR (ref. 132-134). The mir-155, moreover, directly suppress PU.1 transcription factor, playing a role in the CSR and during initiation of plasma cell differentiation. The mir-217 positively regulates in Gc csr and sHm as well as differentiation of plasma and memory B cells. The other GC mir-125b and mir-223, target transcriptional factors irF-4 or blimp-1 and lmo2, respectively. The first two factors are essential for plasma cells differentiation. Moreover, blimp-1 is directly targeted by two others mirRNAs involved in the GC B cells to plasma cells transition, mirR-9 and mirR-30 family. The regulation of apoptosis during GC B cells maturation is tightly regulated, the anti-apoptotic bcl-2 protein is down-regulated in GC B cells and both GC elevated mirR-15a and mirR-16 directly inhibit it. Mature
B cells express miR-150 and miR-185, but expression of both is after activation reduced\textsuperscript{131,137}. The miR-185 indirectly supports B cell activation such as production of IgG and TNF-\(\alpha\) cytokine while negatively regulate the EphB2 expression\textsuperscript{137}. On contrary, miR-210 is induced after B cell activation\textsuperscript{130}. The two members of cluster miR-17-92 (miR-17-5p, miR-20b), miR-28 and miR-181b are involved in transition GC B cells to memory B cells\textsuperscript{131}. Tree miRs – miR-101c, miR-150 and miR-29c are highly expressed in memory B cell compared to GC centroblast\textsuperscript{136}.

In the MRL/lpr mouse model of SLE, it has been demonstrated that deficiency of AID ameliorates disease course, especially autoantibody production and kidney involvement\textsuperscript{138}. Moreover, AID overexpression has been found to be regulated by miR-155, miR-17 and miR-181b in SLE patients with high disease activity\textsuperscript{86}. The importance of MiRs in AID activity and subsequently B cells functions has been elucidated in mouse models. Dicer\textsuperscript{140/PtecdaCre}\textsuperscript{\textsuperscript{146a}} mice that have ablated Dicer in activated B cells exhibit defective GC formation and absence of memory and plasma cells\textsuperscript{125}. Moreover, Dicer deficiency in antigen-activated B cells reduces the generation of high-affinity class-switched antibodies\textsuperscript{122}. On the other hand, in the absence of Dicer in Cd19-Creki/+Dicer fl/fl mice, B cells with skewed BCR formation occur, and female mice are prone to producing anti-dsDNA and anti-cardiolipin autoantibodies during ageing\textsuperscript{131}. Similarly, the other miR-210 knockdown mouse model spontaneously develops autoantibodies such as ANA or anti-dsDNA during ageing\textsuperscript{131}, and the ectopic overexpression of miR-210 in vitro leads to B-cell functional abnormalities such as impaired isotype switching to IgG1 (ref.\textsuperscript{130}). On the other hand, in miR-146a transgenic mice during GC formation, miR-146a directly targets Fas on 3′-UTR, and these mice develop increased levels of IgG autoantibodies, but anti-dsDNA or ANA were not detected\textsuperscript{139}. Thus, miRs are critical for GC processing of B cells, and moreover, have a spatiotemporal dose-dependent effect on antibody production and GC function. Disharmony of the miRs orchestra within GC should prefer the autoreactive fate for B cells during their terminal differentiation.

In view on the fact that miRs can drive B cell functions towards autoimmunity, several studies focused on miRs analyses of B cells in SLE-prone mouse models and in particular on CD19\textsuperscript{+} B cells obtained from SLE patients.

Several miRs are differentially expressed in splenic B cells in mice models of SLE. Elevated levels of miR-21 and miR-146a in B6.Sle123 lupus mouse B cells correlate with disease severity, and treatment with anti-miR-21 ameliorates splenomegaly\textsuperscript{115}. Two other mouse models of SLE, MRL/MpJ-Fas\textsuperscript{\textsuperscript{th}}/J (MRL-lpr) and congenic lupus strain B6.MRL-Fas\textsuperscript{\textsuperscript{th}}/J (B6-lpr) have exhibited dysregulation of miRs expression in splenocytes compared to controls. B cell splenocytes express elevated levels of miR-155 in both MRL-lpr and B6-lpr strains, but downregulation of miR-150 has been found in MRL-lpr (ref.\textsuperscript{140}). On the other hand, the mouse model of SLE with deficiency in miR-155 (miRNA-155–Fas\textsuperscript{\textsuperscript{th}}/J) exhibit lower levels of IgG and IgM autoantibodies, but the proportions of spleen B cells (B220\textsuperscript{+}, B220\textsuperscript{+}IgM\textsuperscript{+}, B220\textsuperscript{+}IgD\textsuperscript{−}) did not differ in Fas\textsuperscript{\textsuperscript{th}}/J (ref.\textsuperscript{88}). The autoimmune condition is more likely caused by abnormal communication between T\textsubscript{H} cells and B cells than by direct changes in B cell splenocytes in this model. Further insight into the spleen B cell subpopulation is provided by a study that used (NZB X NZW) F1 mice (B/W) treated with IFN-\(\alpha\), which develop anti-dsDNA antibodies and changes in the splenic miRs profile, mainly overexpression of miR-15a (ref.\textsuperscript{137}). MiR-15a is directly involved in apoptotic processes by targeting bcl-2. In B/W mice, there is an imbalance between regulatory spleen B cells and hyperactive B-2 subsets. MiR-15a is overexpressed in B regulatory cells, and miR-15a-induced loss of these cells may lead to autoantibody production\textsuperscript{127}. The two members of cluster mir-17-92 (mir-17-5p, mir-20b), mir-28 and mir-181 (mir-15a, mir-15b, mir-15c) are highly expressed in GC B cells, and the expression of these miRs increases in GC B cells treated with iFN-\(\alpha\), which develop anti-dsDNA antibodies and lower levels of anti-dsDNA antibodies\textsuperscript{141}. The diminished B cell proliferation in this model is caused mainly by enhanced activation of SH2 domain-containing inositol 5′-phosphatase 1 (SHIP-1) and subsequent ERK phosphorylation after BCR – FcγRIIB signalling. SHIP-1 is downregulated and ERK is enhanced in activated Fas\textsuperscript{+} B cells, but not in miR-155\textsuperscript{−/−} B cells, and more importantly, miR-155 deficiency in Fas\textsuperscript{+} mice restores SHIP-1 expression, moderate ERK phosphorylation and IgG antibody production\textsuperscript{142}. The other miR supposed to directly support B cell hyperactivity, miR-30a, has been found to be elevated in human SLE B cells. MiR-30a directly binds to the 3′UTR of Lyn mRNA and inhibits its expression in B cells. Subsequent in vitro studies demonstrate that miR-30a, via the Lyn pathway, caused B cells to overproduce IgG and enhanced their proliferation\textsuperscript{142}.

In human SLE, different patterns of miRs expression in B cells could serve as a marker of disease activity or clinical manifestations and might provide new insights into the pathogenesis of SLE. Fig. 3 illustrates differentially expressed miRs in CD19\textsuperscript{+} B cells in human SLE. Martinez-Ramos et al. performed a microRs assay including 377 miRs in CD19\textsuperscript{+} B cells obtained from 46 SLE inactive patients. Further validations have shown that only miR-10a and miR-345 are overexpressed in SLE compared to healthy controls, and have been identified by computational means as targets for IL-8 and IRF-8 (ref.\textsuperscript{115}). These findings, in accordance with previous studies, demonstrate the positive correlation between serum levels of IL-8 with SLE disease activity, autoantibodies production and renal involvement. Recently, a high-throughput miR microarray measuring the activities of 371 miRs identified six differentially expressed miRs (3 up- and 3 downregulated), but only miR-1246 was found to be alleviated in CD19\textsuperscript{+} B cells of active SLE patients in contrast to inactive SLE or healthy controls. Further in vitro study confirmed no influence of treatment, most notably by prednisone, on this observation, and moreover, identified a pathogenetic mechanism of miR-1246 in B cells. MiR-1246 inhibits, by interacting with its 3′-UTR, early B cell factor 1 (EBF1), which is necessary for B...
cell maturation (pro-B cells, MZ B cells and peripheral B cells) and signalling, including the expression of co-
stimulatory molecules such as CD40, CD80 and CD86 (ref.145). In active SLE, B cells showed activation of the PI3K/AKT-p53 signalling pathway leading to alleviation of miR-1246 and enhanced EBF-1 expression144. The other miRs aberrantly expressed in CD19+ B cells in SLE patients compared to controls in the study by Stagakis et al., are, unfortunately, not related to disease activity. MiR-21, miR-25 and miR-106b, which are elevated in B cells, are upregulated in T cells as well, and the positive correlation with SLEDAI in total PBMCs should not be ascribed to B cells145. However, elevated miR-21 in B cells has been confirmed in further studies to be associated with disease severity146 and lupus nephritis145. Recently, Wu et al. demonstrated elevated levels of miR-7, miR-21 and miR-22 in B cells of newly diagnosed treatment-naive SLE patients144. All three miRs are upregulated by IL-21 and could downregulate phosphatase and the tensin homologue (PTEN), whose levels are alleviated in immature, naïve and plasma SLE B cells144. PTEN regulates BCR signalling and suppressing the activity of the PI3K pathway. Furthermore, conditional deletion of PTEN leads to aberrant CSR and augmentation of autoreactive fate of B cells, in particular maturation of MZ B cells146. Additionally, an miR-7 antagonist is able, via IL-21-induced PTEN expression, to elicit a positive effect of IL-21 on plasma cells maturation144. Very recently, a study done on two ethnicities, Chilean and French, determined the miRs expression in B cell subsets of untreated active SLE patients145. Although the number of patients is limited, both ethnicities show the same dissimilarities in miRs expression in naïve CD27- and memory CD27+ B cells. Using microarray analyses comparing the expression levels of 782 miRs, out of which 11 (2 up- and 9 down-regulated) and 6 (1 up- and 5 down-regulated) miRs differentially expressed in naïve CD27- and memory CD27+ B cells, respectively (Fig. 3). Moreover, in the lupus nephritis group compared to the SLE group, miR-145 was upregulated, yet levels of miR-18b, miR-21, miR-29c, miR-345 and miR-365 were alleviated145.

Conclusion and future perspectives

Although the amount of available information about miRs in the pathogenesis of autoimmune diseases is growing exponentially, in this review we examine the participation of miRs in SLE development in light of recent findings. Although new therapies for SLE are coming5, the treatment of SLE flares with regard to miscellaneous clinical features remains challenging23. It is necessary to discover new ways how to treat and monitor the disease and predict flares. MiRs are molecules with the potential to serve as markers of the disease as well as a future therapeutic option. SLE mouse models with knockdown miR-155 exhibit a milder disease with smaller amounts of IgG antibodies88. Whereas miR-155 is one of the fully occupied miRs in innate as well as adaptive immune responses, further studies to exclude infection, malignancies and other adverse effects following inhibition of miR-155 in vivo are intensively required. Interestingly, in mouse models of amyotrophic lateral sclerosis, treatment by anti-
miR-155 prolonged survival147, but the adverse effects are not known. In human medicine, anti-miR therapies, e.g. anti-miR-122 in the treatment of hepatitis C, anti-miR-208 in heart failure or anti-miR-33 in atherosclerosis represent potential future therapeutic options, which are being pursued in ongoing clinical trials148. In SLE, however, no clinical trials have so far focused on anti-miR treatment.

The clinical diversity is reflected in different patterns of miRs expression. Interestingly, in the serum or in PBMCs, miR-146a, miR-126, miR-451, miR-142 and miR-21 correlate with disease activity or clinical manifestations22,25,30,94,98,106. Further studies will show whether miRs could be used as markers for monitoring the disease, predicting flares or determining the best treatment.

ABBREVIATIONS

DNA, Deoxyribonucleic acid; CI, confidence interval; DC, Dendritic cells; miR, (miRNA), Micro ribonucleic acid; OR, Odds ratio; PBMC, Peripheral mononuclear cells; RNA, Ribonucleic acid; SLE, Systemic lupus erythematosus; SNP, Single nucleotide polymorphism; UTR, Untranslated region.

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