

## ANNALS OF THE NEW YORK ACADEMY OF SCIENCES

**MicroRNAs of the immune system****Roles in inflammation and cancer**Jan Davidson-Moncada,<sup>1</sup> F. Nina Papavasiliou,<sup>1</sup> and Wayne Tam<sup>2</sup><sup>1</sup>Laboratory of Lymphocyte Biology, The Rockefeller University, New York, New York, USA. <sup>2</sup>Department of Pathology and Laboratory Medicine, Weill Cornell Medical College, New York, New York, USA

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MicroRNAs (miRNAs) are small noncoding RNAs that regulate gene expression by binding to complementary target mRNAs and either promoting their decay or inhibiting their translation. Most eukaryotic genomes studied encode miRNAs, which are processed from longer noncoding transcripts through pathways conserved from fungi to plants to animals. miRNAs are now understood to be key mediators of developmental transitions in a number of model organisms. With respect to the immune system, miRNAs affect all facets of immune system development, from hematopoiesis to activation in response to infection during both the innate and the adaptive immune response. At the same time, miRNA dysregulation is a central event in the development and pathophysiology of a number of cancers of the immune system. Here we will discuss our current understanding of this general regulatory mechanism, focusing on its involvement in inflammation and in oncogenesis.

**Keywords:** miRNA; immune system; inflammation; cancer

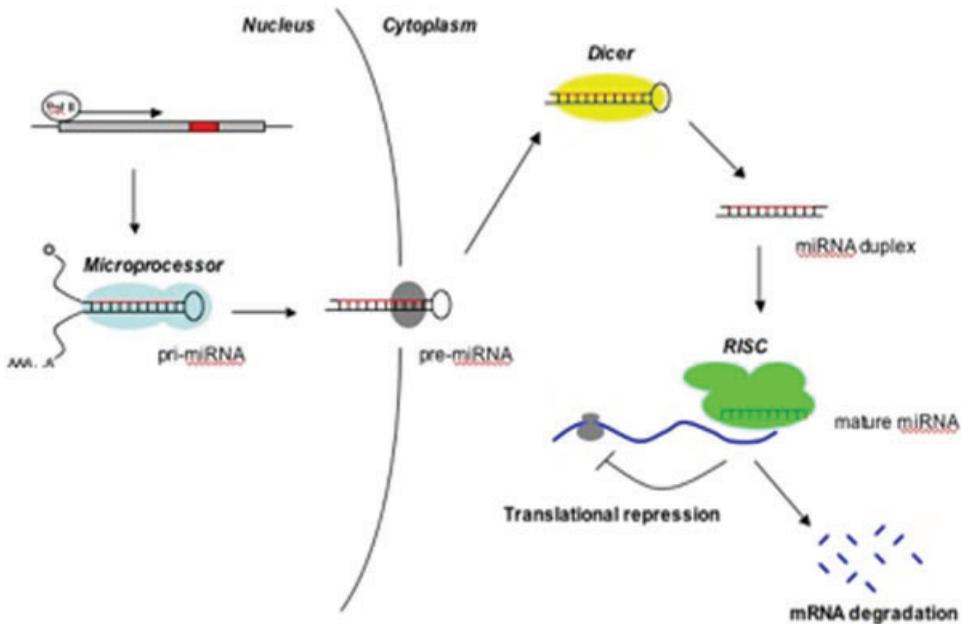
**Introduction**

Historically, the small RNA revolution began with the observation of an unexplained silencing phenomenon in floral pigmentation. Specifically, overexpression of a pigment biosynthesis gene in petunia plants, which was expected to produce more vividly colored flowers, resulted instead in the production of flowers with variegated pigmentation or even complete lack of color.<sup>1</sup> At the time, this phenomenon was termed *co-suppression* and it is the first example of what is now known as RNA interference. Far from being confined to plants, this gene-silencing phenomenon was also observed in fungi<sup>2</sup> and nematodes,<sup>3</sup> but the molecular mechanism behind it remained unclear until the landmark studies of Fire and Mello who demonstrated that it was specifically triggered by a double-stranded RNA.<sup>4</sup> Within the same decade, the field of microRNA (miRNA) began when *lin 4*, a small RNA hitherto unknown as an miRNA, was found in *Caenorhabditis elegans* and noted to downregulate expression of *lin-14* by antisense complementarity to the 3' untranslated region (UTR) of *lin-14*.<sup>5,6</sup> This kind of

RNA was then shown not to be an isolated peculiarity limited to nematodes but rather a member of a large family of small regulatory RNA particles that is widely expressed in many species.<sup>7</sup> Since the initial discovery, miRNAs have been continuously uncovered and implicated in many cellular processes, including but not limited to development,<sup>8,9</sup> cellular proliferation,<sup>10</sup> apoptosis, and cancer.<sup>11</sup> Many hundreds of miRNAs have been identified in humans<sup>12</sup> and they are predicted to regulate approximately 30% of all mRNAs coding for proteins.<sup>13,14</sup>

**MicroRNA biogenesis**

It is estimated that miRNAs comprise approximately 1% of the human genome.<sup>15</sup> Forty to seventy percent are encoded in introns of protein-coding genes or introns and exons of noncoding RNAs, implying two or more possible mechanisms for their transcription.<sup>16</sup> Intronic miRNA genes are generally thought to be transcribed by RNA polymerase II,<sup>17</sup> while exonic miRNA genes are thought to use RNA polymerase III for transcription.<sup>18</sup> Regardless of the transcribing polymerase, miRNAs are



**Figure 1.** Biosynthesis of microRNA. Primary-microRNA (pri-miRNA) transcription takes place in the nucleus by RNA polymerase II. Pri-miRNA is then processed in the nucleus by the microprocessor complex composed of Drosha and DGCR8, producing a precursor-miRNA (pre-miRNA), which is then exported out of the nucleus by exportin 5 and Ran-GTP. In the cytoplasm, further processing by Dicer and TRBP releases an miRNA duplex. One strand is degraded, while the mature miRNA is incorporated into the RNA-induced silencing complex (RISC), which leads to targeting to an mRNA, leading to its destabilization or repression of translation.

initially synthesized as primary-miRNA (pri-miRNA) transcripts, which can extend over 1 kb in length. These transcripts are processed by Drosha (a ribonuclease [RNase] III) and DGCR8 (an RNA-binding protein), which cleave the pri-miRNA to a ~70 nt precursor miRNA (pre-miRNA) within the nucleus.<sup>17,19,20</sup> The pre-miRNA is then exported out of the nucleus by exportin 5, in the presence of its cofactor Ran guanosine triphosphate (GTP), to be further processed in the cytoplasm; concordantly, depletion of exportin 5 by RNA interference leads to a decrease in mature miRNAs and a decrease in pri- or pre-miRNA in the cytoplasm.<sup>21,22</sup>

Once in the cytoplasm pre-miRNAs are cleaved by Dicer (an RNA endonuclease III) along with its partner protein trans-activator RNA-binding protein (TRBP) to a ~21–22 nt double-stranded RNA molecule;<sup>23</sup> inhibition of Dicer leads to the accumulation of pre-miRNA in the cytoplasm.<sup>24</sup> This mature miRNA molecule is loaded onto the RNA-induced silencing complex (RISC), which contains

Dicer, TRBP, and an Argonaute protein which is present in multiple isoforms in mammalian cells.<sup>25,26</sup> Once incorporated into the RISC, the single-stranded mature miRNA will guide the RISC to the target mRNA.<sup>27</sup> At least in vertebrate systems, it is thought that targeting of miRNAs results in either target RNA destabilization<sup>28</sup> or in repression of translation<sup>5,29,30</sup> (Fig. 1). However, recent data would also suggest that under certain conditions miRNA binding leads to a boost in translation.<sup>31,32</sup>

### miRNA function: mRNA destabilization or translational inhibition?

Mammalian miRNAs pair to the 3' UTR of their target mRNA. It is thought that perfect or near-perfect matching of the 5' end of an miRNA (the "seed" region) to the target site within the 3' UTR was the sole requirement for miRNA's function and led to translational inhibition. Although the idea that seed matching leads to interference with

translation is still current, the features within the 3' UTR of a particular mRNA that would accurately predict miRNA targeting have been expanded to include proximity of miRNA-binding sites, AU (adenosine, uracil) rich environments flanking the seed region, and preferential sites within the UTR at both ends. On the basis of these parameters Grimson and colleagues developed a database ([www.targetscan.org](http://www.targetscan.org)) to better predict experimentally focused miRNA : mRNA pairing.<sup>33</sup>

It should be noted, however, that an alternate pathway of miRNA : target binding exists, which appears to rely on matching of a different stretch of sequence within the miRNA to a 3' UTR. This second pathway is thought to lead not to translational inhibition but rather to mRNA destabilization through adenosine/uridine rich elements (ARE)-mediated decay.<sup>34</sup> These noncanonical types of miRNA : target interactions have not been extensively studied and thus cannot be bioinformatically predicted; however, they could be equally consequential to the better studied seed-matching mechanism leading to translational inhibition.

### miRNA in the immune system

The immune system provides a complex but well-orchestrated defense program poised against the myriad of pathogenic infections to which an organism is exposed. For example, an infection may trigger an inflammatory response, the initiation, propagation, and resolution of which must be carefully coordinated and balanced. Lack of proper initiation or propagation hampers the innate immune response; conversely, lack of proper resolution can lead to chronic disease states.

Propagation of the innate response activates the adaptive immune system, which relies upon a pre-determined program of DNA rearrangements in lymphocytes, to generate specific antibodies toward the pathogen that initiated the immune response as well as cellular memory. This process of DNA rearrangements in lymphocytes is stringently regulated; a failure in coordination of cutting and repairing the DNA leads to chromosomal translocations and the seeds of oncogenesis.

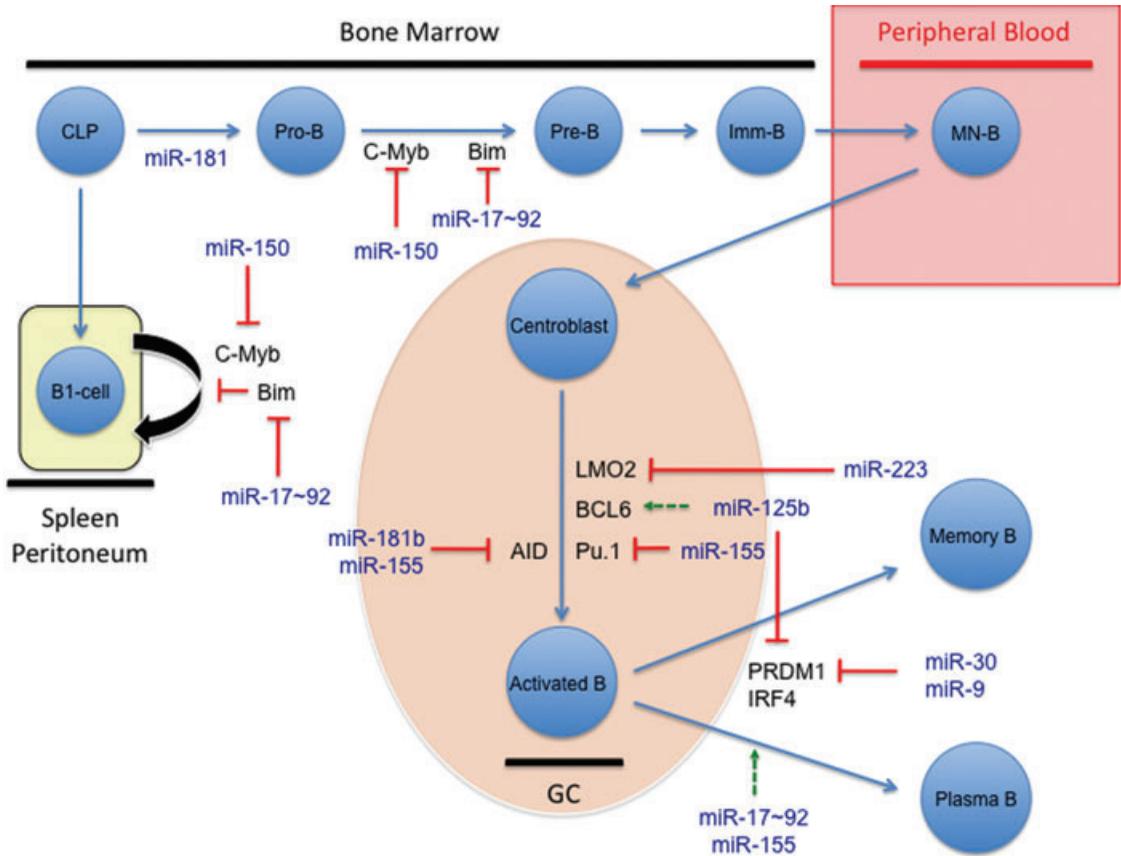
Clearly the immune response (both innate and adaptive) is extremely highly regulated. Recent work from a number of laboratories has revealed that miRNAs play an important role in this in-

tricate system (Fig. 2). The first step toward this understanding was the identification of multiple miRNAs in hematopoietic cells, many of which are expressed specifically in cells and tissues of immune relevance. In fact, cells of the hematopoietic system can be selectively identified from other tissues by their miRNA expression profile; they all express five highly specific miRNAs: miR-142, miR-144, miR-150, miR-155, and miR-223.<sup>35</sup> Different lineages of immune cells can also be distinguished by their unique miRNA expression profiles. For example, erythrocytes show higher expression of miR-451, whereas B and T lymphocytes express miR-223.<sup>36,37</sup> Additionally, although it may seem that similar expression patterns of miRNAs exist in two different cell types, expression levels and spatio-temporal control can be different. For example, in comparing B and T lymphocytes, Merikova and colleagues reported similar expression patterns of miRNAs in peripheral blood samples of both these cell types (particularly the expression of miR-16, miR-142-3, and miR-150); however, miR-342 expression was 10-fold higher in T cells than in B cells.<sup>36</sup> Others have shown differential temporal and spatial expression of miRNA in these cells, which have a distinct role on B and T cell development and function. Therefore, miRNA profiles can help in delineating the different cells of the immune system;<sup>38</sup> yet within each cell type, varying expression of selected miRNA can help hematopoietic cells navigate through their developmental stages.

We will focus on the B-cell lineage to further illustrate the contribution of miRNAs to normal development and maturation of cells in the immune system.

### B cells and miRNA

miRNA profiling has revealed differences in miRNA expression between the various maturation states of B cells (Fig. 2). Malumbres and colleagues showed that the differentiation of naive B cells to centroblast and of centroblast to memory B cell is marked by radical changes in the miRNA profile, while naive and memory B cells exhibit marked similarities.<sup>39</sup> For example, miR-125b is highly expressed in centroblasts, possibly preventing these from further differentiation (miR-125b has been shown to repress positive regulatory domain 1 (PRDM1) and interferon regulatory factor (IRF) 4 two key



**Figure 2.** Involvement of miRNAs in B-cell differentiation. MiRNAs are involved in B-cell differentiation and maturation. MiRNAs near the arrows have been shown to be involved in that developmental stage by downregulating (red arrows) or indirectly stimulating (green arrows) the labeled factors (black) involved in that developmental transition. IMM, immature; CLP, common lymphoid progenitor; MN, mature naïve; AID, activation induced cytidine deaminase; BCL, B cell lymphoma; GC, germinal center; LMO2, LIM domain only 2.

factors in post-germinal center (GC) reaction,<sup>40</sup> while allowing for expression of BCL6, which is important in GC reaction).<sup>39</sup> Similarly, a correlation between miR-223 and B-cell development has been argued. *LMO2*, a target of miR-223, is required for hematopoiesis during mouse embryogenesis<sup>41</sup> and is also expressed in GC B cells.<sup>42</sup> In addition, Zhang and colleagues reported high expression of miR-223 in both naïve and memory cells compared to GC cells, which they postulated control the expression of *LMO2*, allowing for repression of this transcription factor once the cell has exited the GC.<sup>43</sup> Similar correlations are made between miR-9 and members of the miR-30 family on *PRDM1*, which is essential for post-GC B-cell development and function.<sup>44</sup> Thus, aside from marking distinct developmental

transitions, miRNAs are also vital for maintaining a cell at its proper developmental stage.

Although a correlation can be made between miRNAs expressed in certain cells and the potential target genes they may regulate, direct demonstration that these genes are in fact targets of miRNA regulation has relied upon genetic approaches. These have come in three flavors: (a) ablation of miRNAs (gene knockouts); (b) enforced expression of miRNAs (usually by targeted knockin approaches); and (c) ablation of miRNA target sites from 3' UTRs of putative target genes. Although bioinformatic algorithms suggest that miRNAs target many hundreds of genes within a single cell, and therefore genetic gain- and loss-of-function studies (a and b above) may be predicted to have pleiotropic effects, this is

not necessarily the case. Studies with some miRNAs have shown that only a few of the target mRNAs may be critical for a particular biologic process. Alternatively, there are hints that a particular miRNA may target multiple components that belong to a common regulatory pathway;<sup>45</sup> therefore, the impact of miRNA control of a process may be additive and much more important than the moderate effects on individual target protein concentrations suggest. These two considerations have eased fears that such studies will be hard to interpret.

Below we will concentrate on a handful of miRNAs of specific relevance to B cells.

### miR-150

miR-150, which is expressed selectively in mature B and T cells,<sup>46</sup> also prevents transition through early stages of B cell development, specifically transition from pro-B- to pre-B-cell stage through the down-regulation of *c-Myb*;<sup>47</sup> targeted deletion of *c-Myb* was shown to block B-cell development at the pro-B- to pre-B-cell transition. Furthermore, miR-150 plays a vital role in B-cell survival in the spleen, and targeted deletion of *c-Myb* mirrors that phenotype.<sup>48</sup>

Overexpression of miR-150 in B cells results in decreased levels of *c-Myb*, both at the mRNA and protein levels. Transgenic mice overexpressing miR-150 are phenotypically similar to mice with a haploinsufficiency for *c-Myb*. Xiao and colleagues also showed that ectopic miR-150 expression in B-cell progenitors blocks B-cell development at the pro-B-cell stage, and these mice also had decreased levels of B1 cells, a subset of mature B cells present in the spleen and peritoneal cavity as a result of increased apoptosis. Upon miR-150 deletion there was an expansion of splenic and peritoneal B1 cells plus an increase in IgA levels, the preferential isotype related to this B-cell subset.<sup>49</sup>

As opposed to most other miRNAs, it has been reported that expression of miR-150 is higher in normal B cells compared to lymphoma cells.<sup>50</sup>

### miR-181

miR-181 comprises a family of four noncoding RNAs (miR-181 a through d), each of which is processed from a distinct transcript, but all of which contain an identical seed sequence, therefore potentially targeting the same set of mRNAs for

translational repression. miR-181 is preferentially expressed in the thymus, lung, and brain with detectable levels in bone marrow and spleen. Within the bone marrow miR-181 is detectable in undifferentiated progenitor cells but upregulated in differentiated B lymphocytes in mice. Ectopic expression of miR-181 in these progenitor cells leads to an increase in the production of B-lymphoid cells over T cells, as measured by increased expression of CD19. This was also seen *in vivo* upon infection of mouse bone marrow B cells with an miR-181-expressing retrovirus and transfer into irradiated recipients,<sup>51</sup> therefore implicating this miRNA as an early determinant in B-cell, hematopoietic, lineage differentiation.

One of the members of the miR-181 family, miR-181b, has also been implicated in regulation of B-cell function by affecting class-switch recombination (CSR). CSR is an intrachromosomal, deletion, recombination event that leads to a switch in the effector portion of the Ig gene and thus to the expression of a new Ig isotype. CSR is catalyzed by activation-induced cytidine deaminase (AID)-dependent mutation,<sup>52</sup> and AID may contain what has been described as an alternative target site for miR-181b on its 3' UTR (i.e., a target site that does not depend on seed match and therefore is not predicted by robust algorithms, such as TargetScan). Specifically, De Yebenes and colleagues have shown that infection of mouse primary B cells with retrovirus expressing miR-181b impaired class switching to IgG1 upon lipopolysaccharide (LPS) and IL-4 stimulation. AID mRNA and protein levels were both diminished in transfected B cells, and the 3' UTR of AID was fingered as a specific target site for miR-181b by *in vitro* luciferase reporter assays. Although these *in vitro* data do not exclude a more pleiotropic contribution of miR-181b to the CSR reaction *in vivo*, the interpretation preferred by the authors of that study is that miR-181b helps in fine regulating CSR by directly regulating AID.<sup>53</sup>

### miR-17~92

The miR-17~92 cluster consists of six miRNAs that are processed from a common precursor transcript and which are grouped together on the basis of their seed sequence as well as function.<sup>54</sup> miR-17~92 cluster has been implicated in the pathogenesis of B-cell lymphomas, in particular diffuse large B-cell

lymphoma; this will be discussed in further detail below.

What is miR-17~92 cluster's contribution to normal B-cell physiology? Targeted deletion of the miR-17~92 cluster in embryonic stem cells shows normal levels of progenitor B cells in the fetal liver but a decrease in pre-B cells due to increased apoptosis, specifically in the B-cell compartment. Adult B-cell development is also compromised. Ventura and colleagues treated lethally irradiated mice with fetal liver cells of wild-type (WT) or miR-17~92-deleted mice in order to reconstitute their hematopoietic system.<sup>55</sup> They found that, compared to WT, reconstitution with cells from miR-17~92-deficient mice leads to a contraction of the lymphoid compartment, and this was a result of a specific reduction in circulating B cells, splenic B cells, and peritoneal B1 cells. They attributed this to increased expression of the pro-apoptotic gene *Bim*, which has been shown to keep cell proliferation in check in Myc-induced lymphoma models.<sup>56</sup> Interestingly, the 3' UTR of *Bim* contains four predicted sites for the members of the miR-17~92 cluster, and increased levels of *Bim* are found in pro-B and pre-B cells of transgenic mice fetal liver and in acute deletion of miR-17~92 in adult hematopoietic cells.<sup>55</sup>

## miR-155

miR-155 is probably the best characterized miRNA involved in B-cell maturation and function. Original studies<sup>57</sup> showed that co-expression of B-cell integration cluster (*BIC*), a common site of insertion for avian leukosis virus (which induces lymphomas), and *c-Myc* cooperate to induce cellular proliferation involving mostly cells of the hematopoietic system. Lymphomas were found to be from an early B-cell progenitor and lacked rearrangement of the  $\lambda$ -light chain locus.<sup>57</sup> Subsequent studies identified that *BIC* encodes miR-155,<sup>58,59</sup> which is then processed by the Drosha/Dicer pathway.

miR-155 was also shown to be essential for normal immune function. Mice deficient in *BIC* showed reduced humoral and cellular response to infection, succumbed more readily to *Salmonella* infection, had a reduced number of GC B cells, an overall reduction of Ig levels, deficient antigen presentation by dendritic cells, failure to activate T cells, and an overall failure to mount a memory immune response and build protection against re-infection.<sup>60,61</sup>

Although humoral immunity was clearly impaired in miR-155-deficient animals, there was no deficiency in the antibody diversification processes of somatic hypermutation or CSR, both of which are catalyzed by AID. However, the levels of Ig in the blood were clearly reduced, and the number of memory B cells 42 days post immunization were lower as well.<sup>62</sup>

The mechanism behind this impairment is complex. It partly involves a defect in cell differentiation and maturation by failure of B cells to differentiate into plasmablasts, as the miR-155<sup>-/-</sup> phenotype was partly recapitulated by overexpression of Pu.1 (an important protein in B-cell development<sup>63</sup> that is highly expressed in GC B cells and downregulated in post-GC cells<sup>40</sup> and that has been identified as a possible target for miR-155 by transcriptome profiling<sup>62</sup>). The plasmablast can also be partly attributed to a lack of downregulation of AID, itself a direct target of miR-155; directed mutation of the miR-155 target site within the 3' UTR of *AICDA* (the gene which encodes AID) leads to a failure of translational repression of AID by miR-155. Consequently, mice that contain these mutations have higher levels of CSR and aberrant affinity maturation, recapitulating the miR155<sup>-/-</sup> phenotype.<sup>64,65</sup>

As is clear from these recent studies, we have only begun to scratch the surface of what promises to be a fertile field of investigation of miRNA-mediated gene-expression control mechanisms during the immune response (Fig. 2).

## miRNAs and lymphoid malignancies

Alterations of miRNAs have been observed in hematologic malignancies, including lymphomas, by global miRNA expression profiling as well as expression analysis of specific miRNAs. Apart from identifying abnormal expressions of specific miRNAs, the former method also allows a global view of the miRNomes. Global miRNA expression profiling revealed, in the miRNA profiles of B-cell lymphomas, genetic fingerprints of normal B-cell counterparts and aberrations of a small number of miRNAs, presumably acquired during transformation.<sup>39,43,66</sup> In addition, miRNA profiling identified miRNA signatures that can distinguish distinct types of lymphomas<sup>66,67</sup> or subgroups of a lymphoma type, for example, the GC B cell versus the activated B-cell type of diffuse large B-cell lymphomas

(DLBCL).<sup>39,50</sup> These miRNA signatures may have ancillary diagnostic roles to the current methodology based on morphology and immunophenotype. Moreover, these signatures may provide mechanistic insights to the biologic differences between lymphoma subgroups and subtypes.

Among the miRNAs that are shown to be abnormally expressed in lymphoid malignancies, two, miR-155 and miR-17~92, deserve more detailed discussions. As briefly highlighted above, miR-155 and miR-17~92 have important functions in the normal immune system and are also implicated in the pathogenesis of lymphomas.

### miR-155 and lymphomas

Given the role of miR-155 in normal B-cell differentiation, the involvement of miR-155 in B lymphomagenesis is not surprising. Elevated levels of miR-155 compared to normal B cells have been observed in many lymphomas, including DLBCL, primary mediastinal B-cell lymphoma, Hodgkin lymphoma, and chronic lymphocytic leukemia/small lymphocytic lymphoma.<sup>67–70</sup> miR-155 expression in these lymphomas can be heterogeneous and associated with specific subgroups that have prognostic significance. For example, in DLBCL, miR-155 expression is highest in the activated B-cell-like subgroup, which has a worse clinical outcome.<sup>50,69,71</sup> In chronic lymphocytic leukemia, miR-155 expression tends to be higher in cases with unmutated immunoglobulin heavy chain variable region (IgVH), a poor prognostic marker, although statistical significance has not been reached.<sup>70</sup> In addition, miR-155 expression may have prognostic significance that is independent of its association with other prognostic markers. An miRNA signature that includes high miR-155 expression correlates with a shorter interval needed for treatment intervention in chronic lymphocytic leukemia (CLL).<sup>72</sup> Although miR-155 expression does not appear to correlate with overall survival in DLBCL as a group, high miR-155 in the activated B-cell subgroup is associated with better survival.<sup>73</sup>

The exact mechanisms that mediate miR-155 overexpression in these lymphomas are not known. miR-155/*BIC* induction has been linked to the B-cell receptor and the transforming growth factor- $\beta$  pathways.<sup>74,75</sup> The former is believed to play an important role in lymphomagenesis<sup>76</sup> and is thought

to induce miR-155 through the activator protein-1 and nuclear factor (NF)- $\kappa$ B-binding sites.<sup>75,77</sup> A causal role of abnormal NF- $\kappa$ B activity in driving miR-155 overexpression is supported by the association of higher miR-155 expression with higher NF- $\kappa$ B activity in DLBCL cell lines and higher miR-155/*BIC* levels in activated B-cell-type DLBCLs<sup>71</sup> in which NF- $\kappa$ B is often constitutively activated.<sup>78</sup> miR-155 is also induced by the latent membrane protein of Epstein–Barr virus (EBV).<sup>79,80</sup> Increased miR-155 may contribute to the pathogenesis of EBV-associated lymphoid malignancies by modulating endogenous transcription regulatory factors and by maintaining viral latency via attenuation of NF- $\kappa$ B activities.<sup>80,81</sup>

Besides the aforementioned lists of circumstantial evidence that support a role of miR-155 in lymphoma development, miR-155 has been demonstrated to cause lymphomas in animal models. As previously mentioned, *BIC* overexpressed by retroviral vectors cooperates with *c-Myc* to promote lymphomagenesis in chickens.<sup>57</sup> In addition, mice overexpressing miR-155 in B cells developed polyclonal pre-B-cell proliferation followed by full-blown, high-grade, B-cell lymphoma at 6 months of age.<sup>82</sup>

Obviously, further investigations are necessary to better understand how miR-155 contributes to lymphoma development. On the basis of the animal studies, miR-155 could promote B-cell proliferation. In normal B cells, miR-155 deficiency seems to disrupt the production of high-affinity B-cell clones that give rise to plasma cells and memory cells.<sup>83</sup> Thus, an overexpressed miR-155 may “arrest” the lymphoma cells in an activated proliferative state characteristic of these B cells prior to entry into terminally differentiated B cells. In addition, mouse models that specifically disrupt the interaction of miR-155 with *AICDA* 3' UTR demonstrated that miR-155 can target *AICDA* in normal B cells *in vivo*.<sup>64,65</sup> Considering the role of *AICDA* in lymphomagenesis,<sup>84</sup> it will also be important to determine the relevance of these interactions in lymphoid malignancies.

Overall, identification of the full complement of miR-155 target genes will be instrumental to mechanistically delineate the role of miR-155 in B-cell lymphomas. Several potential targets for miR-155 with relevance to lymphomagenesis have been identified through the use of gene expression arrays

generated by manipulating miR-155 levels in cell lines,<sup>81</sup> by subgrouping primary lymphoma samples based on miR-155 levels (i.e., high versus low),<sup>71</sup> or by comparing cell lines with differential miR-155 expression.<sup>67</sup> Further studies should aim to dissect the relative contribution of each of these targets in miR-155-mediated lymphomagenesis for specific lymphoma types.

### miR-17-92 and lymphomas

miR-17-92 (also called oncomir-1) is a polycistronic miRNA cluster located in 13q31-32 that encodes six miRNAs (miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, and miR-92a-1). miR-17-92 and its paralogues are known to act as oncogenes in a variety of tumors, including lymphomas.<sup>85</sup> miR-17-92 was first implicated in lymphomagenesis when a minimal amplicon containing the primary transcript of this polycistron (at that time called *C13orf25*) was identified in B-cell-lymphoma cell lines and primary-lymphoma samples, which are associated with increased expression levels of this transcript.<sup>54,86</sup> In a subsequent study, upregulation of miR-17-92 was observed in 65% of the B-cell-lymphoma patients, including those without detectable amplifications.<sup>87</sup>

A direct causal relationship between increased miR-17-92 and lymphomagenesis was demonstrated in several mouse models. In the first one, enforced miR-17-92 expression in Em-*Myc* transgenic mice resulted in acceleration of lymphoma formation.<sup>87</sup> The tumors in these double-transgenic mice did not exhibit the high degree of apoptosis normally associated with lymphoid tumors in the Em-*Myc* mice, suggesting that miR-17-92 helps to suppress *c-MYC*-induced apoptosis. In the second model, transgenic mice that had elevated miR-17-92 levels developed a lymphoproliferative disease and autoimmunity and died prematurely. Both the peripheral B- and T-cell compartments were expanded.<sup>88</sup> These studies indicate that miR-17-92 can lead to abnormal expansion of the lymphoid compartment but may require cooperating genetic lesions for full-blown malignancy.

miR-17-92 is likely to contribute to lymphoma development through promotion of cell cycle entry and suppression of apoptosis. B and T lymphocytes isolated from miR-17-92 transgenic mice have enhanced proliferation and reduced apoptosis, which

correlates with downregulation of two miR-17-92 target genes, the tumor suppressor *Pten* and the proapoptotic *Bim*.<sup>88</sup> miR-17-92 may also regulate cell cycle and apoptosis of lymphocytes through regulation of other target genes. miR-17-92 targets E2F transcription factor (E2F) and is an integral component of the E2F/*c-MYC* regulatory circuit controlling cell cycle and apoptosis. Complex positive and negative feedback loops exist among E2F, *c-MYC*, and miR-17-92, which ultimately determine the levels of E2F and whether the cells would progress from G1 into S phase, arrest at G1, or undergo apoptosis.<sup>89-92</sup> The critical role of miR-17-92 in this circuitry may be particularly relevant in the pathogenesis of *c-MYC*-mediated lymphoid malignancies. In the presence of a deregulated *c-Myc*, which can upregulate *E2F* (particularly *E2F1*), overexpression of miR-17-92 may modulate its level to facilitate cell cycle progression rather than apoptosis. miR-17-92 may also promote cell proliferation by targeting *CDKN1A/p21*, a negative regulator of the G1-S checkpoint<sup>93,94</sup> or by downregulating transforming growth factor- $\beta$  signaling.<sup>95</sup> Interestingly, miR-17-92 may contribute to lymphomagenesis by downregulating distinct targets in different lymphoma subtypes depending on the genetic constitution of the tumor cells.<sup>94</sup> In lymphomas with co-existing genetic events that downregulate *Bim* (e.g., a deletion) or upregulate *BCL2 BIM* (e.g., a translocation), miR-17-92 may promote lymphoma development by downregulating p21 and promoting cell cycle progression. On the other hand, in lymphomas where there is already high proliferative potential (e.g., *c-MYC* driven), miR-17-92 may facilitate lymphoma formation by downregulating *BIM*.

### miRNAs and inflammation

miRNAs provide a new layer in the multitiered regulation of cellular physiology. Thus far we have tried to illuminate how miRNAs partake in cellular development and differentiation by highlighting their involvement in normal B-cell maturation. We have also touched upon the role of two miRNAs important for B-cell oncogenesis. Although we did not discuss the roles of miRNAs in development and maturation of other immune cell types (T cells, dendritic cells, macrophages), some of the miRNAs we did discuss are important in the inflammatory response. For example, Baltimore and colleagues have shown

that miR-155 is induced early in macrophages as a consequence of exposure to a broad range of inflammatory mediators.<sup>96</sup> However, all the miRNAs we highlighted are thought to interact with their cognate target mRNAs in a canonical fashion—that is to say through their seed region through base complementarity with the target 3' UTR. Therefore, in all these cases miRNA regulation is through translational repression.

But what of miRNAs with base complementarity to a 3' UTR that does not require interaction with the canonical seed region? One such example exists in the literature and centers on the interaction of miR-16 with mRNAs whose 3' UTRs contain AREs.<sup>97</sup> miR-16 contains an 8-nt sequence (UAAAUAUU) that is complementary to the ARE sequence; within the 3' UTR, this sequence targets mRNAs for ARE-mediated degradation (requiring a number of mRNA-binding proteins, such as tristetraprolin [TTP] and BRF1).<sup>98</sup> Indeed, Jin and colleagues reported that miR-16 is required for targeting specific ARE-containing mRNAs (the very labile mRNAs of TNF-alpha and cox-2) for degradation by delivering it to the RISC in a process that requires both Dicer and TTP. Complementing this pioneering work is a recent study by Calin and colleagues<sup>99</sup> whereby exogenous expression of miR-16 followed by array expression profiling revealed that mRNAs with ARE elements are more frequently downregulated upon miR-16 overexpression. On the basis of these two studies, we hypothesize that an miR-16-like, noncanonical, mRNA targeting mechanism is not uncommon, although at present bioinformatics tools do not exist to directly predict such occurrences.

Should such a noncanonical mechanism be widely operative, the best system to study it would appear to be the immune inflammatory response. There is clear evidence in the literature that ARE-mediated degradation directly influences the initiation, propagation, and resolution of the inflammatory response. Most recently, an elegant study by Hao and Baltimore clearly showed that differences in mRNA stability exert a strong influence on the temporal order of gene expression after stimulation of fibroblasts or macrophages with proinflammatory stimuli (TNF and LPS). Of direct relevance was the observation that mRNA expressed early in the response had abundant AREs in their 3' UTRs, whereas those expressed later had fewer or

none.<sup>100</sup> Although this study focused on the notion that mRNA instability is an intrinsic property of gene structure, in light of the miR-16 study detailed above, the implication is that ARE-mediated mRNA instability during the inflammatory response could be either miR-16 mediated or mediated by an uncharacterized miR with ARE-base complementation properties. We anticipate future studies aimed at finding such miRNAs; perhaps the inflammatory response will offer a paradigm in this regard.

## Acknowledgments

We apologize to the many investigators whose work we were unable to cite because of space limitations. We thank Dr. G. Teng for developing the diagram presented in Figure 1 and Rebecca Delker for help with editing. This work was supported by the U.S. National Institutes of Health.

## Conflict of interest

The authors declare no conflicts of interest.

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