

MicroRNAs as B-cell lymphoma biomarkers

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Abstract: B-cell lymphomas represent a group of more than 35 recognized mature B-cell neoplasms differentiated largely on the basis of immunohistochemical staining patterns that are often challenging to accurately diagnose. Despite having been only formally recognized just over 10 years ago, microRNAs (miRNAs) have become one of the trendiest topics in biology. Dysregulation of miRNAs is a ubiquitous feature of cancer in general, including B-cell lymphomas. Many of the miRNAs aberrantly expressed in B-cell lymphomas also play a crucial regulatory role in normal hematopoietic function. MiRNAs show great potential as novel biomarkers of cancer, as they can differentiate cancers according to diagnosis and developmental stage, even discriminating between cancers that are poorly separated histologically. Furthermore, they can be robustly measured from routinely prepared formalin-fixed paraffin-embedded biopsy material and biological fluids such as blood. Here, we consider the identity, function, and biomarker potential of miRNAs in B-cell lymphomas and, most importantly, the hurdles that remain to be overcome if they are really to become part of future clinical practice.

Keywords: microRNA, lymphoma, biomarker, B-cell

Introduction

The central dogma of molecular biology states that biological information flows in one direction from DNA to RNA to protein.¹ However, this view implies that noncoding RNA has little or no intrinsic value, despite accounting for more than 90% of eukaryotic transcriptional output.² Therefore, it is perhaps not surprising that microRNAs (miRNAs) were unknown to the scientific community until just over 20 years ago and were not formally recognized until 2001.¹⁻⁵ There are now over 2,500 human miRNAs that have been identified through cloning and/or sequence analysis (miRBase),⁶ and it is believed that more than two-thirds of all human genes are a target for direct miRNA regulation.⁷ MiRNAs play key regulatory roles in virtually every aspect of biology including developmental timing, cell differentiation, apoptosis, cell proliferation, metabolism, organ development, and hematopoiesis.⁸ The potential importance of miRNAs in cancer is implied by the fact that the majority of human miRNAs are located at cancer-associated genomic regions.⁹ There is now overwhelming evidence that dysfunctional expression of miRNAs is a common, if not ubiquitous, feature of cancer in general including lymphomas.^{10,11} The reasons for the dysfunctional expression of miRNAs in cancers are numerous, and can include chromosomal aberrations, epigenetic deregulation, aberrant expression of transcription factors that regulate promoter regions of miRNAs, as well as factors that change miRNA biosynthesis and/or function.¹²

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According to the National Cancer Institute, a biomarker is “a biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process or of a condition or disease.” Cancer biomarkers are most commonly defined in three broad categories as being used for differential diagnosis (diagnostic), distinguishing between “good outcome tumors” and “bad outcome tumors” in the absence of treatment (prognostic), or assessing the probability that a patient will benefit from a particular treatment (predictive). As we will see, miRNAs have potential in all three of these categories.

The potential of miRNAs as cancer (diagnostic) biomarkers is clear, as differences in the expression profile of miRNAs can distinguish cancers according to the diagnosis and developmental stage of the tumor to a greater degree of accuracy than traditional gene expression analysis, even discriminating between cancers that are poorly separated histologically.¹³ This characteristic is especially attractive to the field of B-cell lymphomas (BCLs), a group of more than 35 recognized neoplasms¹⁴ differentiated largely on the basis of immunohistochemical staining patterns that are often challenging to accurately diagnose.¹⁵ The usefulness of molecular methods to supplement traditional morphological classifications in BCL is exemplified by diffuse large BCL (DLBCL), where gene expression profiling has led to the identification of least two distinct subtypes that are prognostically and mechanistically very different, and which

respond differently to treatment.^{16,17} An additional attraction of miRNAs as cancer biomarkers is that they are much more stable than other RNA species and, as a result, can be purified and measured easily in routinely prepared formalin-fixed paraffin-embedded (FFPE) biopsy material¹⁸ and biological fluids such as blood¹⁹ (as will be discussed).

The first demonstration that miRNAs could be useful as diagnostic biomarkers came from the 2002 seminal publication of Calin et al,²⁰ who made the connection between 13q14, a frequently deleted locus, and downregulation of the *miR-15a/16* cluster that is encoded within this region in chronic lymphocytic leukemia patients. Two years later, miRNAs were first demonstrated as prognostic biomarkers in lung cancer patients.²¹ Since then, the speed of miRNA cancer biomarker discovery has been quite astonishing, with over 8,500 publications (source: PubMed search [November 17, 2014] string = “[microRNA AND cancer] AND [prognosis OR diagnosis OR biomarker]”).

Here, we consider the identity, function, and biomarker potential of miRNAs in BCLs and, most importantly, the hurdles that remain to be overcome if they are really to become part of future clinical practice.

miRNAs and lymphopoiesis

Potential biomarker miRNAs of lymphomas are by definition aberrantly expressed, but many are also key regulators of lymphopoiesis (and hematopoiesis) (Figure 1 and Table 1).

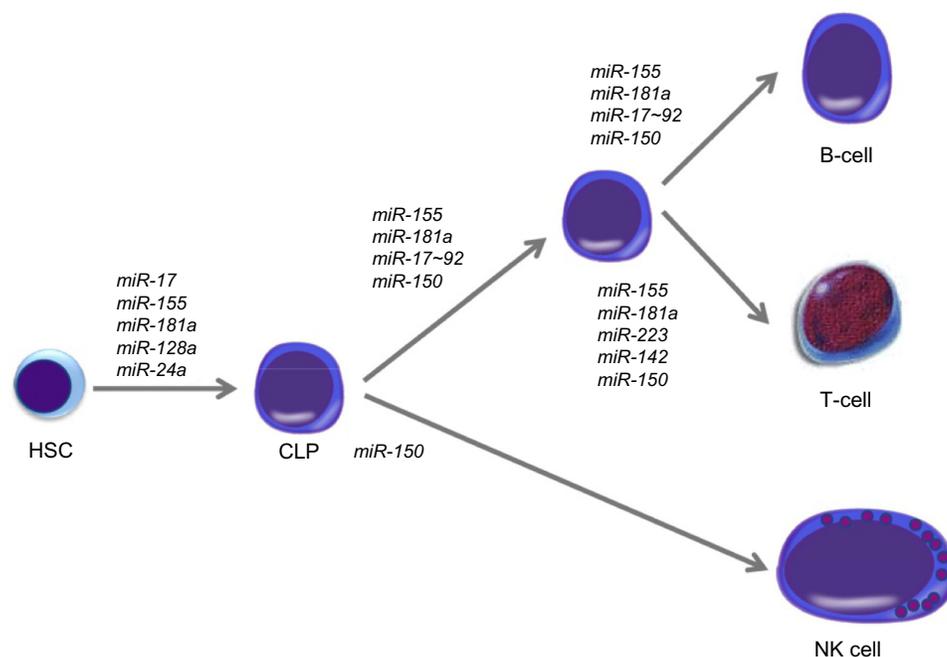


Figure 1 Role of miRNAs in lymphopoiesis.

Abbreviations: HSC, hematopoietic stem cell; CLP, common lymphoid progenitor; NK, natural killer; miRNA, microRNA.

Table 1 Major biomarker miRNAs aberrantly expressed in B-cell lymphomas showing a known role in hematopoiesis and their validated target genes

miRNA	Hematopoiesis	Malignancy	Target
<i>miR-155</i>	B-/T-cell development	DLBCL, BL, AML	<i>HGAL, RTKN2</i> ; ³⁷ <i>SMAD5</i> ; ⁵⁹ <i>PIK3R1</i> ; ⁶⁰ <i>SHIP</i> ; ⁵⁶ <i>PU.1</i> , <i>CD10</i> ; ⁴¹ <i>AID</i> ; ^{33,34}
<i>miR-17~92</i>	B-cell development	B-cell lymphoma, CML, ALL, MCL	<i>BIM, PTEN</i> ; ⁴⁴ <i>E2F1</i> ; ⁷³ <i>PP2A, PRKAA1</i> ; ⁴³⁻⁴⁶ <i>PHLPP2</i> ; ¹¹⁸ <i>CCND1</i> ^{119,120}
<i>miR-34a</i>	B-cell development	B-cell lymphoma	<i>SIRT1</i> ; ⁶⁷ <i>MYC</i> ; ¹²¹ <i>FOXPI</i> ⁶⁹
<i>miR-125</i>	Myelopoiesis	DLBCL, ALL, MDS, AML	<i>IRF4, PRDM1</i> ; ⁴⁰ <i>TNFAIP3</i> ; ⁴² <i>LIN28A</i> ⁸⁸
<i>miR-223</i>	Myelopoiesis, erythropoiesis, granulopoiesis, B-cell development	MALT, SzS	<i>MEF2C</i> ; ¹²² <i>LMO2</i> ; ⁴⁰ <i>NFIA</i> , <i>CEBPA</i> ; ¹²³ <i>E2A</i> ⁹⁷
<i>miR-150</i>	Myelopoiesis, Megakaryopoiesis, B-/T-cell development, NK cell development	MDS, MALT, NK/T-cell lymphoma	<i>MYB</i> ; ^{48,124} <i>NOTCH3</i> ; ¹²⁵ <i>DKCI, AKT2</i> ¹²⁶
<i>miR-181</i>	B-/T-cell development	AML, CLL	<i>AID</i> ; ^{33,34} <i>BCL2, CD69, TCRα</i> , <i>DUSP5, SHP2, PTPN22</i> <i>KIT</i> ¹²⁷
<i>miR-221/222</i> <i>miR-15a/16-1</i>	Erythropoiesis	CLL, B-ALL, ALCL	<i>BCL2, MCL1, CDK6</i> ; ¹²⁸ <i>TP53</i> ; ¹²⁹ <i>HIF1A</i> ; ¹³⁰ <i>CCND1</i> ^{119,120}
<i>miR-21</i>		B-cell lymphoma, NK/T lymphoma, SzS	<i>PDCD4, PTEN</i> ; ⁵⁷ <i>STAT3</i> ; ¹³¹ <i>ANP32A, SMARCA4</i> ¹³²
<i>miR-29a</i>	Early HSC development	AML, CLL, MCL	<i>HBPI</i> ; ¹³³ <i>TCLI</i> ; ¹³⁴
<i>miR-124a</i>	Myelopoiesis	AML, ALL	<i>EVII, CEBPA</i> ; ¹³⁵ <i>CDK6</i> ¹³⁶

Abbreviations: miRNA, microRNA; DLBCL, diffuse large B-cell lymphoma; BL, Burkitt lymphoma; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; CLL, chronic lymphocytic leukemia; ALL, acute lymphoblastic leukemia; MCL, mantle cell lymphoma; MDS, myelodysplastic syndrome; MALT, mucosa-associated lymphoid tissue; SzS, Sézary syndrome; NK, natural killer; B-ALL, B-cell acute lymphoblastic leukemia; ALCL, anaplastic large-cell lymphoma; NK/T, natural killer/T-cell; HSC, hematopoietic stem cell.

In order to fully understand the function of these miRNAs in lymphomagenesis, we must first understand their role under physiological conditions.

Deletion of components of the miRNA biosynthetic pathway in vivo demonstrates the critical role that miRNAs play in hematopoiesis. For example, deletion of *Dicer1* resulted in a severe block in peripheral CD8⁺ development, as well as in reduced numbers of CD4⁺ cells which, when stimulated, underwent increased apoptosis and proliferated poorly.^{22,23} However, when *Dicer1* was deleted in CD34⁺ hematopoietic stem cells (HSCs), this led to an increase in apoptosis coupled with a reduction in hematopoietic ability.²⁴ When *Dicer1* was ablated in early B-cell progenitors, the pro- to pre-B-cell transition was blocked as a result of *miR-17~92* targeting of BIM, which could be partially rescued by BCL2 expression.²⁵ HSCs remain pluripotent while at the same time, they respond to lineage-determining signals in order to differentiate into the various hematopoietic lineages including lymphocytes. This is a finely balanced process tightly controlled by a complex network of extrinsic and intrinsic stimuli, signaling pathways, growth factors, cytokines, transcription factors, and other molecular components. MiRNAs can target many of these factors and, in general, can determine HSC fate, differentiation state and function, the ability to self-renew, apoptosis levels, and the balance of myeloid and lymphoid progenitor cells.²⁶ Georgantas et al²⁶ identified 33 different

miRNAs commonly expressed in CD34⁺ HSCs derived either from bone marrow or peripheral blood. Using a combination of predictive algorithms as well as functional validation, the authors hypothesized that *miR-17*, *miR-24*, *miR-146*, *miR-155*, *miR-128*, and *miR-181* act to prevent the differentiation of early-stage progenitors; *miR-16*, *miR-103*, and *miR-107* act later on; and *miR-221*, *miR-222*, and *miR-223* can control the end stages of hematopoietic development (Figure 1).

The first study to suggest the importance of miRNAs in normal lymphocyte development was performed by Chen et al²⁷ in 2004, who cloned ~150 miRNAs from murine bone marrow and found that *miR-181*, *miR-223*, and *miR-142* were preferentially expressed in B-cells. Furthermore, HSCs expressing *miR-181* transferred to lethally irradiated mice significantly increased B-cell and cytotoxic CD8⁺ T-cell levels. *miR-181* can also regulate levels of CD69, BCL2, and T-cell receptor (TCR) α during T-cell development,²⁸ and is responsible for TCR sensitivity.²⁹ *miR-155* deletion in mice made them immunodeficient, with B-cells producing reduced levels of immunoglobulins (Igs) in response to antigen treatment, and T-cells producing lower amounts of interleukin (IL)-2 and interferon gamma. Both of these effects were caused by PU.1 targeting.^{30,31} In vitro activation of B-cells or CD4⁺ T-cells upregulate *miR-155*, while the deletion of *miR-155* in activated B-cells causes a reduction of tumor necrosis factor (TNF) and lymphotoxin by two-thirds of their

normal levels, and biases T-cell differentiation towards the T_H2 phenotype.³²

Both *miR-155* and *miR-181* are key regulators of germinal center (GC) B-cell differentiation mediated through the targeting of activation-induced cytidine deaminase (AID), an important regulator of B-cell differentiation.^{33,34} The specific deletion of *Dicer1* in activated B-cells dramatically decreased GC B-cell formation and that *Dicer*-deficient GC B-cells expressed higher levels of BIM.³⁵ GC B-cells are defined by the expression of surface proteins including BCL6, CD10, HGAL, and LMO2, and the absence of activated B-cell markers such as PRDM1/BLIMP1 and XBP1.³⁶ MiRNAs can regulate the expression of all these. For instance *miR-155* targets both HGAL and Rhotekin 2,³⁷ the *miR-30* family, *miR-9* and *let-7a*, target BCL6 and PRDM1/BLIMP1,³⁸ while *miR-223* regulates the expression of LMO2,³⁹ and *miR-125b* controls the expression of IRF4 and PRDM1/BLIMP1.⁴⁰ It has also been demonstrated that *miR-155* plays a role in activated B-cells via nuclear factor-kappa B (NF- κ B) regulation by the inhibition of PU1 and CD10,⁴¹ and *miR-125a/b* regulates TNFAIP3 enhancing NF- κ B activation.⁴²

The *miR-17-92* cluster is another important regulator of lymphopoiesis that when deleted blocks pro- to pre-B-cell development via BIM targeting.⁴³ In addition members of this cluster can target PP2A, PTEN and AMP-activated kinase (PRKAA1), all important immune regulators.^{43–46} Similar to the *miR-17-92* cluster, forced expression of *miR-34a* in HSCs can inhibit the transition of pro- to pre-B-cells, this time by FOXP1 inhibition,⁴⁷ as does *miR-150* expression via MYB downregulation.⁴⁸

Aberrant expression of miRNAs in B-cell lymphomas and their use as biomarkers

As in normal lymphopoiesis, there is a general requirement for miRNA biosynthetic components in the development and maintenance of BCLs. Deletion of *Dicer1* in a MYC-lymphoma model resulted in the decreased development of lymphoma, and those that did arise were of a very early B-cell precursor stage.⁴⁹ Similarly, in a p53-null model, *Dicer1* deletion reduced lymphomagenesis and conferred prolonged survival to the mice.⁵⁰

Diffuse large B-cell lymphoma (DLBCL)

DLBCL, the most common form of BCL accounting for nearly 40% of all lymphoid tumors,⁵¹ was one of the first cancers to be linked with dysfunctional miRNA expression – namely, the observation of *miR-155* overexpression (Figure 2).^{18,52,53}

This miRNA is a key “oncomir” in lymphomagenesis, and ectopic expression of *miR-155* in mice resulted in the development of a high-grade BCL similar to DLBCL,⁵⁴ with oncogenicity mediated by SHIP1 and C/EBP β targeting.^{55–57} Remarkably, when *miR-155* overexpression was removed in this model, using either an inducible promoter or antagomir against *miR-155*, the tumor quickly disappeared, and after 1 week, no detectable disease was found,⁵⁸ demonstrating the potential efficacy of miRNA-based therapeutics against lymphoma. In addition, *miR-155* suppresses the in vitro growth-inhibitory effects of TGF- β 1 and BMP2/4 in DLBCL cells by SMAD5 downregulation,⁵⁹ and it controls PI3K–AKT pathway activity by PIK3R1 targeting.⁶⁰ Furthermore, overexpression of this miRNA promotes TNF α -dependent proliferation of DLBCL cells in vivo in xenotransplanted mice.⁵⁶

Recently, it has been demonstrated that SHIP1 is differentially expressed between the two molecular subtypes of DLBCL, GC B-cell-like, and activated B-cell-like (ABC) DLBCL.¹⁷ This is consistent with previous reports of differences in *miR-155* expression levels between ABC- and GC-type DLBCL.^{18,53} Moreover, CD10, indicative of GC-type DLBCL,⁶¹ and the constitutive expression of NF- κ B, a hallmark of ABC-type DLBCL,⁶² are linked via the *miR-155/PU.1* regulatory loop.⁴¹ Interestingly, the lymphoma-inducing herpes viruses, Kaposi’s sarcoma-associated herpes virus and Marek’s disease virus, encode *miR-155* orthologs,^{63,64} whereas Epstein–Barr virus – also intimately involved in lymphomagenesis – can deregulate *miR-155* via LMP1 transactivation.⁶⁵

miR-34a is a well-described tumor suppressor miRNA that forms part of the p53 network,⁶⁶ with a positive feedback loop between the p53 induction of *miR-34a* expression and activation of p53 by *miR-34a* through SIRT1 inhibition.⁶⁷ Mice xenotransplanted with U2932 (an ABC-type DLBCL cell line), along with exogenous *miR-34a*, had reduced tumor growth – an effect mediated by Foxp1 targeting,⁶⁸ it is also a molecule that is linked to the high-grade transformation of lymphoma via myc-mediated *miR-34a* repression.⁶⁹

Overexpression of the *miR-17-92* cluster in conjunction with MYC accelerates lymphoma development and increases the aggressiveness of tumors^{70,71} due to the presence of a MYC/*miR-17-92*/E2F circuit in this malignancy.⁷² MYC upregulates the *miR-17-92* cluster which, in turn, inhibits E2F1, and E2F3 (pro-proliferative) regulates the *miR-17-92* cluster.⁷³ Using a E μ -myc murine model, *miR-19* was proposed to be the key oncogenic component of the *miR-17-92* cluster and was demonstrated to activate the AKT–mTOR

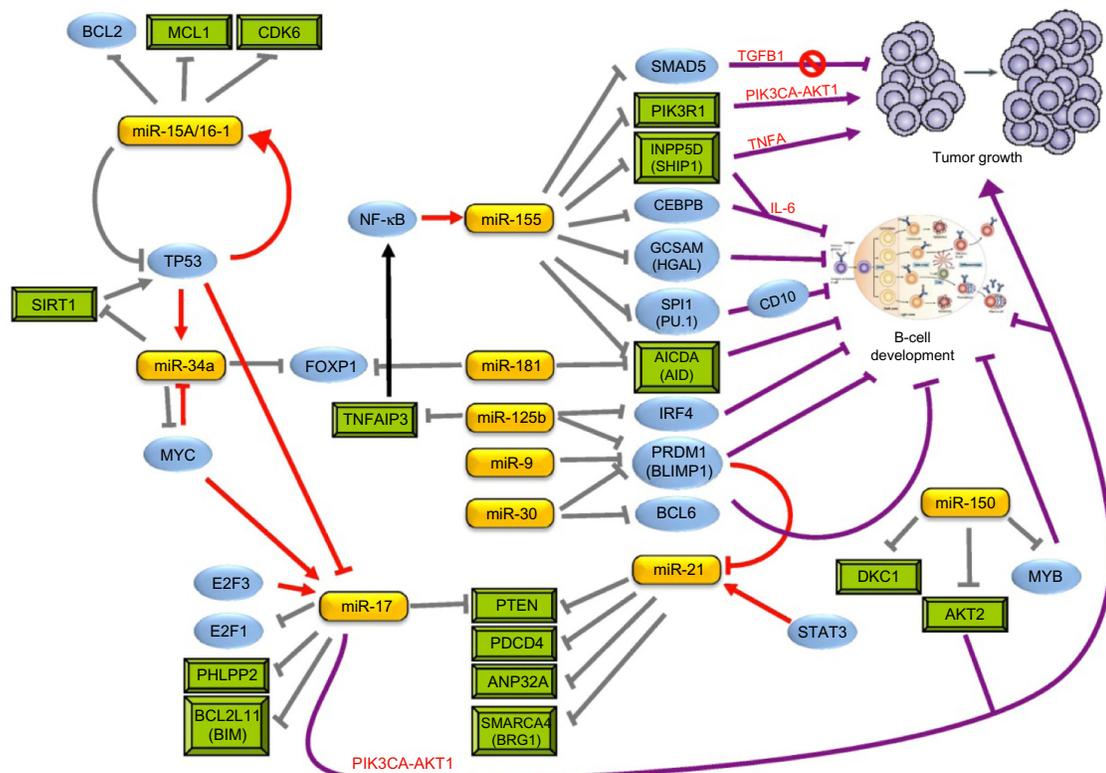


Figure 2 MiRNA involvement in lymphomagenesis.

Notes: Functionally validated target genes of miRNAs are depicted by gray lines, and red lines indicate the transcription factors that are known to regulate the miRNAs. Purple lines show the proposed phenotypic effect of miRNA dysregulation in lymphomagenesis, and the implicated pathways are indicated in red text.

Abbreviations: NF-κB, nuclear factor-kappa B; TNF, tumor necrosis factor; AICDA, activation-induced cytidine deaminase; IL-6, interleukin-6; STAT3, signal transducer and activator of transcription 3; miRNA, microRNA.

pathway via antagonizing PTEN, leading to the promotion of cell survival.⁷⁴

In terms of biomarkers for DLBCL, several early studies linked the expression of miRNAs with the molecular subtypes of DLBCL and prognostic outcomes.^{18,40,75} A retrospective study of 258 DLBCL cases defined a 9-miRNA signature that was independently indicative of both overall survival and progression-free survival in this cohort.⁷⁶ In one study of 176 DLBCL patients uniformly treated with R-CHOP (rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone),⁷⁷ levels of *miR-18a* were identified as independent variables associated with overall survival and levels of *miR-181a* and *miR-222* with progression-free survival. More recently, a signature of 52 miRNAs was identified in patients with hepatitis C virus-associated DLBCL, and the downregulation of *miR-138-5p* and upregulation of *miR-147a*, *miR-147b*, and *miR-511-5p* was correlated with poor prognostic outcomes.⁷⁸

Follicular lymphoma (FL)

Even though it is the most common type of indolent lymphoma, there are only a few miRNA biomarker studies in FL.

The expression levels of 153 miRNAs were measured in 46 FL samples compared to normal lymph nodes or DLBCL cases.⁷⁹ We identified a 26-miRNA signature that could differentiate between FL cases (number [n]=18) and de novo cases of DLBCL (n=64).⁷⁵ As part of this study, we also found six miRNAs (*miR-223*, *miR-217*, *miR-222*, *miR-221*, *let-7i*, and *let-7b*) that could distinguish FL cases that underwent high-grade histological transformation from those that did not, something not possible by histoimmunohistochemical means. Other biomarker studies have identified miRNA signatures able to differentiate between FL and nodal marginal zone lymphoma,⁸⁰ and between FL and follicular hyperplasia patients.⁸¹ The *miR-17~92* cluster has been proposed as useful to differentiate between the potentially confounding diagnoses of GC-DLBCL and clinical stage III FL.⁸² Seventeen miRNAs were identified that could distinguish between FL cases harboring the t(14;18) translocation from t(14;18)-negative cases (about 10% of FL cases). Recently, however, a study of 44 FL cases failed to derive a significant miRNA signature that could distinguish between differing subtypes of FL (FL with BCL6 locus rearrangement [FL{BCL2+/BCL6+}, FL{BCL2-/BCL6+}] or FL with a diffuse growth pattern).⁸³

Mantle cell lymphoma (MCL)

A number of miRNA signatures have now been described for MCL.^{84–87} Overexpression of members of the *miR-17~92* cluster, for example, has been associated with high MYC levels in aggressive MCL,⁸⁶ and inhibition of chemotherapy-induced apoptosis in MCL cell lines,⁸⁸ while downregulation of *miR-29* has been proposed as a potential prognostic marker for this malignancy.⁸⁴

Burkitt lymphoma (BL)

Mice carrying mutations in the 3'-UTR *miR-155* binding site of AID contain increased levels of MYC-IgH (t[8;14]) translocations – the characteristic genetic hallmark of Burkitt lymphoma (BL).⁸⁹ Although the three major subtypes of BL (sporadic, endemic, and HIV-associated) share a very similar miRNA profile, all three were shown to be distinct from DLBCL.⁹⁰ Although *miR-155* is overexpressed in pediatric cases,⁹¹ in adult BL cases, this does not appear to be the case.^{52,92,93}

Other B-cell lymphomas

In Hodgkin lymphoma (HL), low *miR-135a* levels have been associated with poorer prognostic outcomes.^{94,95} Mucosa-associated lymphoid tissue (MALT) lymphoma has been distinguished from gastric DLBCL, a potentially difficult differential diagnosis by a 27-miRNA signature.⁶⁹ In another study, five miRNAs (*miR-150*, *miR-550*, *miR-124a*, *miR-518b*, and *miR-539*) were proposed to be able to distinguish between gastritis and MALT lymphoma,⁹⁶ and in another study, *miR-223* levels correlated with increased E2A expression in MALT lymphoma.⁹⁷ Bouteloup et al⁹⁸ analyzed 15 splenic marginal zone lymphoma (SMZL) cases by microarray and reported eight miRNAs including *miR-21* that distinguished between aggressive (n=2) and indolent (n=4) cases of SMZL. More recently, purified B-cell populations from 31 cases of SMZL were differentiated from the spleens of reactive lymphoid hyperplasia, and spleens from patients with other BCLs by a 51-miRNA signature.⁹⁹

miRNAs as noninvasive biomarkers of B-cell lymphoma

The standard protocol for the diagnosis of lymphoma (and other cancers) remains the histopathological review of tumor material obtained by invasive biopsy – a procedure that is typically expensive, uncomfortable, and sometimes risky for patients. Therefore, there has been great interest in the field of circulating nucleic acids in plasma and serum as noninvasive cancer biomarkers.^{100,101} An additional benefit of blood-based

testing is the ability to carry out screening and repeat sampling on patients undergoing therapy, or monitoring disease progression allowing for the development of a personalized approach to cancer patient management.

Unlike other RNA classes, the vast majority of which are degraded by high levels of RNases found in the blood,¹⁰² miRNAs appear stable in the blood and are surprisingly resistant to fragmentation by either chemical or enzymatic agents.¹⁰³ In 2007, we first reported the presence of miRNAs in the blood of cancer (lymphoma) patients,¹⁰⁴ and in 2008 observed the upregulation of *miR-155* and *miR-210* in the blood (sera) of DLBCL patients compared to healthy controls and the prognostic potential of *miR-21*¹⁰⁵ – an observation validated independently some years later.¹⁰⁶ As well as confirming the upregulation of *miR-155* in DLBCL sera, a recent study additionally reported the upregulation of *miR-15a*, *miR-16*, and *miR-29c* and downregulation of *miR-34a*.¹⁰⁷ A further study, this time in plasma rather than sera, observed reduced levels of *miR-92* that varied in response to chemotherapy in DLBCL, FL, and T-cell non-HL patients.¹⁰⁸ In HL patient plasma, *miR-494* and *miR-1973* were identified as indicators of both relapse and interim therapy response.¹⁰⁹ Plasma *miR-221* has been found to be a good diagnostic and prognostic marker for extranodal natural killer T-cell lymphoma.¹¹⁰ Clearly, although the results are very preliminary, the ability of miRNAs to act as noninvasive biomarkers of BCLs is a promising prospect.

Summary and future directions

The identification of miRNAs that are aberrantly expressed in tumor cells compared to their respective normal counterpart cells was the initial goal of many early miRNA studies.^{13,111} However, although of great scientific interest, this approach has limited diagnostic utility, as studies are almost exclusively retrospective and based upon the ability to confirm previously made clinical diagnoses. Much more useful is the ability of miRNAs to differentiate between diagnoses of benign and malignant conditions, between confounding pathologies, and to confirm or add to the molecular classification of tumors, which is often limited by the prerequisite for fresh or frozen material. For example, the *miR-17~92* cluster has been suggested as a useful diagnostic differentiator between the potentially conflicting diagnostic classifications of GC-DLBCL and grade 3 FL cases.⁸² In addition to facilitating diagnosis, one of the most clinically useful features of miRNAs is their potential as prognostic biomarkers. This can be a result of acting as a surrogate marker of a previously recognized prognostic marker (eg, clinical, cytogenetic, or molecular)

or as an independent prognosticator. The majority of studies to date fall within the former category. For example, Calin et al¹¹² identified a 13-miRNA signature on the basis of comparisons between chronic lymphocyte leukemia groups with typically good outcomes (low ZAP70, mutated IgH) with those with a typically poor outcome (high ZAP70, unmutated IgH). In the latter category, a miRNA signature was derived that could predict prognostic outcome independently of other chromosomal or clinical parameters in DLBCL patients treated with R-CHOP.⁷⁷

There has been a great deal of interest in the use of miRNAs as indicators of response to treatment in solid tumors.¹¹³ We identified *miR-27a* as being important in bortezomib action in myeloma,¹¹⁴ whereas *miR-125b* is the main determinant of sensitivity in cutaneous T-cell lymphoma.¹¹⁵ In HL, *miR-21* and *miR-30d* were demonstrated to be responsible for doxorubicin-induced apoptosis, although normally this drug is only used as part of combination therapy.¹¹⁶

The miRNA BCL biomarker studies described here are by no means an exhaustive list, but are instead intended to illustrate the rapid growth of the field. An especially useful characteristic of miRNAs as biomarkers is their remarkable stability, which means that they can be robustly measured from routine FFPE biopsy material.¹⁸ Consequently, retrospective miRNA studies can be carried out on the huge collections of pathology laboratories worldwide, something that is not feasible when investigating traditional genes. Indeed, most miRNA studies have been carried out on FFPE material, and this characteristic has greatly facilitated the rapid pace of discovery of miRNA involvement in all but the rarest of malignant pathologies. Another attractive feature of miRNAs as biomarkers is the ability to detect these molecules in biological fluids including blood, tears, urine, saliva, amniotic fluid, breast milk, cerebrospinal fluid, and seminal fluid,¹¹⁷ offering the possibility of noninvasive cancer biomarkers that could be used for monitoring disease progression and treatment response.

However, it should be noted that the vast majority of miRNA biomarker studies to date are single-center and retrospective. Consequently, the reliability of much of these data remains contentious and should be treated with some caution, as the degree of discordancy between seemingly identical studies is troubling and, in reality, very few of the biomarker studies published will ever make it into clinical practice. These differences are primarily due to biological and technical variation between the studies, such as the starting material used in experiments (eg, the purification of cells, cell types, the control populations used, RNA extraction,

etc), as well as the technological platforms (eg, microarray, quantitative real-time reverse transcription polymerase chain reaction, versus next-generation sequencing, etc) and differing statistical methodologies used. Consequently, there is a clear need for a standardized approach to be taken in future miRNA biomarker studies in order to rationalize these confounding factors. In particular, a systematic approach should be taken in a similar fashion to that achieved for other “omic” disciplines (ie, transcriptomics and genomics).

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Disclosure

The authors report no conflicts of interest in this work.

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