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Role of microRNAs in Lymphoid Biology and Disease

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Structured Abstract

Purpose of the Review—To provide a state-of-the-art knowledge on the implications of microRNA (miRNA) dys-regulation in lymphoid malignancies.

Recent Findings—Several new studies have broadened our understanding of how aberrancies of the miRNome contribute to the development of a malignant lymphoid phenotype. Recently, a new pathogenetic model involving miRNAs and protein coding genes (such as TP53 and ZAP-70) has been identified and as the prognostic implications of the most recurrent chromosomal abnormalities in human B-chronic lymphocytic leukemia. Moreover, new recent studies have addressed the role of miRNAs in human lymphomas and acute lymphoblastic leukemias.

Summary—The recent advances in our understanding of the role of miRNAs in lymphoid malignancies demonstrate that miRNAs can effectively be used as tumoral biomarkers with diagnostic, prognostic and predictive-of-response-to-therapy implications.

Keywords

microRNAs; lymphoma; leukemia; biomarkers

Introduction

MicroRNAs (miRNAs) are non-coding RNAs (ncRNAs) that regulate gene expression and affect several biological processes, spanning from development, differentiation, cell cycle regulation to senescence and metabolism (1-5).

The biogenesis of miRNAs has been described in several excellent reviews (6, 7). The full spectrum of miRNAs expressed in a specific cell type (the miRNome) varies between normal and pathologic tissues (8), and specific signatures of deregulated miRNAs harbor diagnostic and prognostic implications (9, 10). The first link between miRNAs and cancer came from the discovery of loss of miR-15a and miR-16-1 in chronic lymphocytic leukemia (11) and from the discovery that these ncRNAs are frequently located in cancer associated genomic regions (CAGRs), which include minimal regions of amplification, loss of heterozygosity, common breakpoint regions in or near oncogenes (OGs) and tumor suppressor genes (TSGs), and fragile sites (preferential sites of chromatid exchange, deletion, translocation, amplification or integration of plasmid DNA and tumor-associated viruses) (12). Since then, a plethora of studies have investigated the aberrancies of the

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miRNome in almost all types of human cancer, both solid tumors and hematological malignancies (for reviews see (10, 13-18)). In particular, while some miRNAs act mainly as TSGs, others exert a predominantly oncogenic function, although labeling a miRNA as exclusively a TSG or an OG is inappropriate, given that the overall function of any given miRNA seems to depend on its cellular context. The present review will focus on the most recent discoveries in the field of miRNAs and lymphoid malignancies, which led to the identification of new pathogenetic molecular networks with prognostic significance and potential therapeutic implications.

microRNAs in Lymphoid Leukemias

Specific signatures of de-regulated miRNAs have been described in both acute and chronic lymphocytic leukemias (ALL and CLL, respectively). Table 1 summarizes the main de-regulated miRNAs in human lymphoid malignancies further discussed in this review. High levels of miR-128b have been described in ALL (19), and high expression of miR-128a and miR-128b can distinguish between ALL and AML (acute myeloid leukemia) (20), with an accuracy of 98% (20), making these two miRNAs diagnostic biomarkers for acute leukemia. More recently, Zhang et al., have identified a miRNA signature in children with ALL complicated by central nervous system (CNS) relapse. High levels of miR-7, -198 and -663, and low expression of miR-126, -345, -222, and -551a associate with a higher risk of CNS relapse (21). Intriguingly, some of the predicted targets of this “miRNA cascade” include neuron function- and neurotransmitter- related genes, although an experimental validation of this targeting is still needed. The miR-17-92 cluster has been correlated with the development of mixed lineage leukemia (MLL)-rearranged acute leukemia (22). Up-regulation of this cluster was observed not only in MLL-associated AML, but also in ALL, and is possibly due to both DNA copy number amplification at 13q31, and to direct up-regulation by MLL fusions (22). Kaddar et al., have investigated the prognostic significance of miR-16 in childhood ALL and its relationships to normal and malignant lymphocyte proliferation (23). Overall, they found that low levels of miR-16 are associated with a better outcome, an unexpected discovery, since it has been shown that miR-16 directly targets several anti-apoptotic genes (such as BCL2, MCL1 and CDK6 (24)), therefore harboring proapoptotic effects. Interestingly, we have recently demonstrated that in addition to anti-apoptotic genes, the miR-15a/16-1 cluster also directly targets the tumor suppressor gene TP53, in chronic lymphocytic leukemia (CLL) (25), suggesting that the cluster has a dual role, both as an OG and as a TSG. It will be interesting to investigate whether miR-16 also targets TP53 in ALL, which could provide a molecular explanation for the positive prognostic implications of this miRNA in pediatric ALL. MiRNAs are also involved in viral-induced leukemias. The human T-cell leukemia virus type-1 (HTLV-1) is responsible for adult T-cell leukemia, a very aggressive CD4+ malignancy. It has been demonstrated that several miRNAs are de-regulated in HTLV-1-infected T-lymphocytes when compared to uninfected cells. Among them, miR-146a is strongly up-regulated by the viral infection (26). In particular, the virus encoded trans-activator protein *tax* is able to induce miR-146a, which in turn affects the expression of several target genes (such as IRAK1, TRAF-6, IL-8, CXCR4, and MMP9) (26). How the *tax*-miR-146a trans-activation leads to a proliferating effect on infected lymphocytes still needs to be established but this interaction might cast a new light on the mechanism of HTLV-1-mediated leukemogenesis.

CLL is characterized by common and recurrent chromosomal aberrations (27). The most frequent of these abnormalities is the 13q14 deletion. Based on the analysis of a large number of CLL cases with monoallelic 13q14 deletion, a minimal deleted region (MDR) has been defined. This MDR includes a long non-coding RNA, called DLEU2 (deleted in leukemia 2), strongly conserved among vertebrates, and the first exon of the DLEU1 gene, another non-coding RNA (28, 29). The miR-15a/16-1 cluster is located within intron 4 of

DLEU2, and genetic alterations affecting DLEU2 mRNA expression would also affect miR-15a/16-1 cluster expression (11). Therefore, the expression of miR-15a/16-1 is reduced in the majority of CLL patients carrying the 13q deletion (11). One of the main molecular signatures of B-CLL is the increased expression of the anti-apoptotic BCL2 in the mostly non-dividing malignant B lymphocytes, and we first demonstrated that BCL2 is a direct target of miR-15a/16-1 (24), explaining the indolent course of the disease in patients who carry the 13q deletion. In fact, restoration of miR-15a/16-1 expression induces apoptosis in leukemic cell lines both in vitro and in vivo (24, 30). Interestingly, we also identified a signature of de-regulated miRNAs that distinguishes between the indolent and the aggressive form of CLL (9). CLL is characterized by an extremely variable clinical course and the available clinical staging systems are relatively poor predictors of disease behavior. MiRNAs can effectively stratify CLL patients based on the biological aggressiveness of their disease. Surprisingly, high levels of miR-15a/16-1 (and of the paralogous cluster miR-15b/16-2 on chromosome 3), are associated with the *de novo* aggressive CLL phenotype (9). This finding prompted us to investigate other possible molecular mechanisms, able to explain this apparent paradox. We identified a new pathogenetic network linking the three most common chromosomal abnormalities in CLL (the 13q, the 17p and the 11q deletions), to their prognostic implications (good prognosis for CLLs with the 13q deletion, bad prognosis for CLLs with the 17p and 11q deletion alone or in association with the 13q deletion). We demonstrated that the miR-15a/16-1 cluster (on chromosome 13q), the tumor suppressor TP53 gene (on chromosome 17p), the miR-34b/34c cluster (on chromosome 11q) and ZAP-70, a surrogate prognostic marker for CLL, are the molecular effectors involved in this network. In particular, miR-15a/16-1 directly target TP53 and are also trans-activated by TP53. TP53 also trans-activates the miR-34b/34c cluster, which in turn directly targets ZAP-70. Overall, we have outlined a new model of CLL pathogenesis that accounts for the prognostic implications of three of the most frequent recurrent chromosomal abnormalities in B-CLL (25) (Figure 1).

Recently, Klein et al. investigated the causal involvement of 13q14-MDR-encoded tumor suppressor genes in CLL pathogenesis. They generated two groups of transgenic mice models: one mimicking the MDR, the other containing a specific deletion of the miR-15a/16-1 cluster. Both groups developed indolent B-clonal lymphoproliferations, with low penetrance (40% MDR, 25% miR-15a/16-1 deletion) (31). Although the same spectrum of clonal lymphoproliferative disorders was observed in both animal models, the disease was more aggressive in the MDR group than in the miR-15a/16-1 group, suggesting that additional genetic elements in the 13q14 region may affect the severity of the disease. Another well known CLL mouse model is the New Zealand black (NZB) strain that spontaneously develops a CD5-positive lymphoproliferation at an advanced age. This strain carries a point mutation close to the stem-loop of miR-16-1 (32). This mutation leads to abnormal processing of miR-16-1 and reduced expression of the mature miR-16. Although NZB mice develop CLL later than miR-15a/16-1 deleted mice (31, 32), suggesting a milder phenotype, a germline mutation that has been described in a small fraction of human CLL cases (9), has been associated with cases of familial CLL.

The oncogene TCL1 (T-cell leukemia/lymphoma 1A) is a marker of aggressive CLL and associates with other markers of poor prognosis such as high levels of ZAP-70 and unmutated IgV_H status (33, 34). Pekarsky et al., have demonstrated that TCL1 is also regulated by two miRNAs, namely miR-29b and miR-181b (35). Furthermore miR-181a directly targets BCL2 (36), suggesting a molecular concerted targeting action of members of the miR-181 family and of the miR-15a/16-1 cluster in the regulation of BCL2 expression. In a cohort of 110 CLL patients, with a median follow up of 72 months,

Stamatopoulos et al., found that downregulation of miR-29c and miR-223 are predictive of treatment free survival (TFS) and overall survival (OS) (37). Low expression of miR-223, -29b, -29c, and miR-181 family are associated with disease progression in CLL cases harboring the 17p deletion, whereas patients carrying the trisomy 12 abnormality and high expression of miR-181a experience a more aggressive variant of CLL (38). Interestingly, miR-29 family has been demonstrated to control key epigenetic mechanisms (such as the expression of all three main DNA methyltransferases) both in solid tumors and in hematological malignancies (39, 40). Therefore further studies to interpret the prognostic significance of miR-29 de-regulation based on the epigenetic modifications induced by this miRNA family are warranted. Another miRNA frequently up-regulated in several types of cancer is miR-155, expression of which is higher in aggressive CLL versus normal CD19+ B cells and indolent CLLs (9, 41). Recently, it has been demonstrated that the miR-155 host gene in the long non-coding RNA BIC (B-cell integration cluster) is regulated by MYB (42), providing new evidence for a potential role for this transcription factor in aggressive CLLs.

miRNAs in Lymphomas

De-regulation of miRNAs has been described in both Hodgkin (HL) and non-Hodgkin lymphomas (NHL).

One of the most commonly up-regulated miRNAs in both lymphoma groups is miR-155. In a transgenic mouse model with conditional miR-155 over-expression in B-lymphocytes, a polyclonal pre-leukemic pre-B-cell proliferation followed by full blown B-cell malignancy was described (43). The chain of events leading to the accumulation of large pre-B cells and acute lymphoblastic leukemia/high-grade lymphoma in these mice is initiated by miR-155 targeting of SHIP (Src homology 2 domain-containing inositol-5-phosphatase), and C/EBPbeta (CCAAT enhancer-binding protein beta), two key regulators of the interleukin-6 signaling pathway (44). In miR-155 knock-out mice models, the observed phenotype consisted mainly in a defect in cytokine production that interfered with TH2 lymphocyte differentiation, and compromised dendritic cell function (45, 46). In a subtype of NHL, the diffuse large B-cell lymphoma (DLBCL), miR-155 also carries prognostic implications. The 5-year survival rates for the activated B-cell variant (ABC-DLBCL), and the germinal center B-cell-like variant (GBC-DLBCL) are 30% and 59% respectively (47), and high levels of miR-155 are characteristic of ABC-DLBCL (48, 49). This suggests that miRNA is a biomarker of poor prognosis for DLBCL. Similarly, higher levels of miR-21, and miR-221 are associated with the variant of DLBCL with the worst outcome (49).

Recently, Medina et al. have provided the first in vivo evidence that miR-21 acts as a key oncogene in B-lymphomagenesis (50). The authors generated mice conditionally expressing miR-21 and observed that this miRNA drives a pre-B malignant lymphoid-like phenotype, which completely regresses when miR-21 is inactivated. Interestingly, this study also demonstrates that tumors can become addicted to oncogenic miRNAs, and supports efforts to target these miRNAs pharmacologically as a strategy for cancer treatment.

In addition to prognostic biomarkers, miRNAs can also be used effectively for differential diagnosis of NHLs. A group of four miRNAs (composed of miR-330, -17-5p, -106a, and -210) is able to distinguish DLBCL, follicular lymphoma and reactive lymph nodes, with an accuracy of 98% (51). By investigating genome-wide miRNA expression and copy number in 86 DLBCLs (59 primary tumors and 27 cell lines), Li et al identified a signature of miRNAs that segregated DLBCLs into three groups with different prognosis and markedly different MYC transcriptional activity (52). These groups were not related to the cell of origin, or to the extent of T-cell infiltrate or to the tumor site, but represent newly identified, miRNA-driven prognostic groups. Up-regulation of the miR-17-92 cluster occurs in about

65% of B-cell lymphomas (53). When this cluster is over-expressed in murine multipotent progenitor cells of MYC-transgenic mice, an increased lymphomagenesis was observed (53). Moreover, miR-17-92 transgenic mice models have a higher rate of lymphoproliferative disorders, autoimmunity and premature death (54) and these effects are mediated by the direct targeting of two tumor suppressor genes: PTEN and BIM (54). Interestingly, two members of the miR-17-92 cluster (miR-17-5p and miR-20a) are transactivated by the transcription factor c-MYC (55), and in turn the cluster directly targets E2F1, a c-MYC transactivated transcription factor promoting cell-cycle progression (55). Therefore, the miR-17-92 cluster functions as a fine tuner of c-MYC regulatory function on cell-cycle progression. Recently, it has been demonstrated that the miR-17-92 cluster also significantly enhances resistance to radiotherapy in human mantle cell lymphoma cells (56), revealing a role for this cluster as a predictive biomarker of response to treatment. Another effect of c-MYC on the miRNome is miR-34a downregulation (57). Recently, Sotillo et al., showed that in B-lymphoid cells over-expressing Myc, miR-34a confers drug resistance, by inhibiting Tp53-dependent bortezomib-induced apoptosis (58). This result was unexpected, since upregulation of miR-34a by Tp53 is thought to enhance its acetylation and contribute to the pro-apoptotic effects of this tumor suppressor (59). This study casts a new light on the role of miR-34a in B-malignancies and confirms that a miRNA can act both as an OG and as a TSG, depending on the cellular environment. Reduced expression of miR-143 and miR-145 has also been documented in B-cell malignancies (60). Re-expression of these miRNAs in a Burkitt lymphoma cell line demonstrated a dose-dependent growth inhibitory effect, mediated in part by miRNA induced down-regulation of the oncogene ERK5 (60).

Recently, two studies have investigated the miRNA profile in cutaneous lymphomas. In a recent study, van der Fits et al. have demonstrated high levels of miR-21 in Sézary Syndrome (SS), a cutaneous T-cell lymphoma with CD4+ tumor cells (Sézary cells) present in the skin, lymph nodes, and peripheral blood (61). They also showed that STAT3, a transcription factor induced by IL-21, directly activates miR-21, and that this mechanism is involved in miR-21 de-regulation in SS. The same group also published the first profile of de-regulated miRNA in mycosis fungoides (MF), the most common type of cutaneous T-cell lymphoma. High levels of miR-155, miR-92a, and miR-93 were associated specifically with MF, and not with SS (62), suggesting a possible role for these three miRNAs in the differential diagnosis of these two cutaneous lymphomas.

In Hodgkin Lymphoma (HL), high levels of miR-9 and let-7a were described, paralleling low expression of their target PRDM1/Blimp-1 (63). In a study conducted in 49 HL primary samples, Navarro et al. demonstrated that a signature of 25 miRNAs could distinguish HL from reactive lymph nodes, and another 36 miRNAs were able to differentiate between nodular sclerosis and the mixed cellularity subtype of HL (64). Interestingly, miR-96, -128a and -128b are selectively downregulated in HL cells with Epstein Barr virus (EBV) infection, but only one of these miRNAs is part of the signature of 25 de-regulated miRNAs in HL versus reactive lymph nodes, suggesting that EBV might not be relevant in driving HL pathogenesis. In HL cell lines, down-regulation of miR-150 and up-regulation of miR-155 are frequent (65). Since HL has its origin in the germinal center (GC), and high levels of miR-155 have also been described in GC during normal lymphopoiesis, this suggests that the observed up-regulation of miR-155 in HL might be the result of an abnormal block to lymphocyte differentiation at the GC level. In another study, Van Vlierberghe et al., compared the miRNome of microdissected Reed Sternberg cells and Hodgkin cell lines versus CD77+ B-cells, and found a signature of 12 up- and 3 down-regulated miRNAs (66). This signature overlaps only in part with the one observed in HL patients (64), and this may be due to the fact that a microdissection procedure was used in only one of the two studies. Finally, miRNAs can also have prognostic significance in HL. Low expression of miR-135a is associated with a higher relapse risk and a shorter disease

free survival (67). These effects are in part mediated by miR-135a direct targeting of JAK2, a cytoplasmic tyrosine kinase involved in a specific subset of cytokine receptor signaling pathways. By targeting JAK2, miR-135a induces down-regulation of the antiapoptotic BCL-X_L, therefore leading to apoptosis and decreased cell growth (67).

Conclusion

The involvement of miRNAs in the pathogenesis of lymphoid malignancies is now well established. The identification of the molecular effectors mediating the effects of the miRNome de-regulation on the development of malignancy is undergoing, and suggesting potential new strategies to overcome such aberrations. In addition to new promising therapeutic implications, these discoveries have clearly demonstrated that miRNAs can also serve as molecular biomarkers of cancer with prognostic implications and as predictive biomarkers of response to treatment. It is likely that in the next few years, new miRNA-based diagnostic, prognostic and predictive kits will be available to clinicians and that miRNA-derived treatments will begin their clinical trial journey towards the development of new clinical agents. In addition it is likely that in the next few years miRNAs and anti-miRNAs will themselves be used as drugs.

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Key Points

- miRNA de-regulation is implicated in the pathogenesis of lymphoid malignancies.
- Restoration of normal expression levels of miRNAs can lead to anti-cancer effects.
- miRNAs can effectively be used as tumoral biomarkers with diagnostic, prognostic implications, and may also predict response to therapy.

Figure 1. A network involving miRNAs and Protein Coding Genes is involved in B-CLL pathogenesis and can explain the prognostic implications of the most recurrent chromosomal abnormalities in B-CLL

A. In CLL with normal cytogenetics, the miR-15a/16-1 cluster (located at 13q) is transactivated by TP53 (on chromosome 17p) and directly silences this tumor suppressor gene. TP53 can also transactivate the miR-34b/34c cluster (located at 11q), which directly target the surrogate prognostic marker ZAP-70.

B. In CLL carrying the 13q deletion (the most common chromosomal abnormality), the miR-15a/16-1 cluster is absent because of the deletion, which releases TP53 from the inhibitory control by the cluster. High levels of TP53, activate the expression of the miR-34b/34c cluster, which targets ZAP-70 and reduces the expression of this tumoral marker.

Table 1
List of most frequently de-regulated miRNAs in lymphoid malignancies

Disease	miRNA	Expression	Biomarker	Comment	Reference
ALL	miR-128b	High	D		19
	miR-128a and miR-128b	High	D	Differentiate ALL from AML	20
	miR-7, miR-198, miR-663	High	P	Higher risk of CNS relapse	21
	miR-126, miR-345, miR-222, miR-551a	Low	P	Higher risk of CNS relapse	21
	miR-17-92 cluster	High	D		22
	miR-16	Low	P	Better prognosis in childhood ALL	23
CLL	miR-15a/16-1 cluster	Low	D, P	Down-regulated in 13q deleted CLL; Associates with indolent CLL, by targeting TP53	11, 25
	miR-34b/34c cluster	Low	P	In 11q deleted CLL, aggressive disease, high levels of ZAP-70	25
	miR-29b and miR-181b	Low	P	Down-regulated in aggressive CLL, characterized by high levels of their direct target TCL1	35
	miR-29c and miR-223	Low	P	Associates with TFS and OS	37
	miR-155	High	P	Aggressive CLL	9, 41
NHLs	miR-155	High	D, P	Induces ALL/high grade lymphoma in transgenic mouse models; Distinguishes ABC-DLBCL and GBC-DLBCL	43, 48, 49
	miR-21 and miR-221	High	D, P	High expression in ABC-DLBCL	49
	miR-21	High	D	Key miRNA in B-lymphomagenesis	50
	miR-330, miR-17-5p, miR-106a, and miR-210	High/Low	D	Distinguish DLBCL, FL and reactive lymph nodes	51
	miR-17-92 cluster	High	D, PR	High expression in 65% of B-cell lymphomas; Associated with resistance to radiotherapy	53, 56
	miR-34a	High	PR	Confers drug resistance in B-lymphocytes over-expressing Myc	58
	miR-143 and miR-145	Low	D	In BL and other NHL	60
HL	miR-9 and let-7a	High	D		63
	miR-96, miR-128a, and miR-128b	Down	D	Selectively down-regulated in HL cells infected with EBV	64
	miR-150	Low	D		65
	miR-155	High	D		65
	miR-135a	Low	P	Higher relapse risk and shorter DFS	67
SS	miR-21	High	D		61
MF	miR-155, miR-92a and miR-93	High	D	In MF versus SS	62

ALL= Acute Lymphocytic Leukemia; AML= Acute Myeloid Leukemia; CLL= Chronic Lymphocytic Leukemia; NHLs= Non-Hodgkin Lymphomas; HL= Hodgkin Lymphoma; SS= Sézary Syndrome; MF= Mycosis Fungoides. D= Diagnostic Biomarker; P= Prognostic Biomarker; PR= Predictive-of-response-to-treatment Biomarker; CNS= Central Nervous System; TFS= Treatment Free Survival; OS=

Overall Survival; DLBCL= Diffuse large B-cell lymphoma; ABC-DLBCL= Activated B-cell variant of DLBCL; GBC-DLBCL= Germinal center B-cell-like variant of DLBCL; FL= Follicular Lymphoma; BL= Burkitt Lymphoma; EBV= Epstein Barr Virus; DFS= Disease Free Survival.