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Novel mechanisms of regulation of miRNAs in CLL

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Abstract

B-cell chronic lymphocytic leukemia (CLL) is the most common adult human leukemia. Although, the molecular alterations leading to CLL onset and progression are still under investigation (specifically, the interplay and exact role of oncogenes and tumor suppressors in CLL pathogenesis). MicroRNAs are small non-coding RNAs that regulate gene expression and are expressed in a tissue specific manner. Deregulation of microRNAs can alter expression levels of genes involved in the development and/or progression of tumors. In CLL, microRNAs can function as oncogenes or tumor suppressors. Here, we review the most recent findings on the role of microRNAs in the onset/progression of CLL, and how this knowledge can be used to identify new biomarkers and targets to treat this leukemia.

Keywords

CLL; microRNA; miR-15/16

Characteristics and outcomes of chronic lymphocytic leukemia

Chronic lymphocytic leukemia (CLL) is the most frequent human leukemia occurring as an aggressive or indolent disease, both characterized by the accumulation of CD5+ B lymphocytes [1]. Patients with indolent CLL usually do not require treatment for several years (or even decades) while most of aggressive CLL patients require immediate treatment. Moreover, indolent CLL can progress to the aggressive form, thus it is essential to find new markers for a more specific and early diagnosis of CLL onset and staging [2]. Several prognostic markers have been already identified such as the expression of non-mutated immunoglobulin heavy variable genes (UM-IgV_H) and high level of 70 kD zeta-associated protein (*ZAP70*), both associated with unfavorable prognosis [3, 4]. Also, chromosomal alterations are detected in >80% of all CLL cases and can discriminate patients with different outcomes: (i) low risk patients have a normal karyotype or 13q deletion; (ii) intermediate risk patients have 11q deletion or trisomy 12; and (iii) high risk patients have 17p deletion or a complex karyotype [5]. Since many CLL cases show discordant prognostic factors, the identification of new parameters able to relate disease stage and clinical outcome is important for patient management. Recently, the presence of single polymorphisms,

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mutations, defects in processing, allele specific loss of transcription and epigenetic inactivation were shown to influence the expression of key microRNAs involved in CLL pathogenesis and affect the clinical course of disease [6–10]. Furthermore, recent studies suggest that the ability of malignant cells to (i) react to microenvironmental stimuli via B-cell receptor (BCR) signaling, (ii) interact with exosomes and accessory cells, and (iii) respond to the presence of viral microRNAs, can have a pivotal role in CLL onset/progression [11–13].

In this review, we discuss how microRNAs affect initiation and progression of CLLs and how these molecules can represent new diagnostic/prognostic markers and possible drugs for therapy.

Signatures of microRNAs in CLLs

MicroRNAs (miRNAs), a new class of endogenous small noncoding RNAs, have been associated with several types of cancer [14] and involved in various cellular processes, including DNA methylation, cellular growth, differentiation and apoptosis [15, 16]. Expression profiling revealed that microRNA signatures can distinguish normal B cells from malignant CLL cells and are associated with prognosis, progression, drug resistance, and BCR stimulation [11, 17, 18]. For instance, aggressive and indolent CLLs exhibit a different microRNAs profile [18] and a greater mortality rate is associated with high levels of *miR-21* and *miR-155* [19, 20], while *miR-181b* levels are decreased during CLL progression acting as a biomarker able to predict time to treatment [2]. Specific microRNA signatures can predict refractoriness to treatment, [17]. Indeed *miR-148a*, *miR-222*, and *miR-21* exhibit a higher expression in fludarabine non-responder patients and lower levels of *miR-34a* were observed in patients resistant to therapy [21]. Finally, a signature of 39 differentially expressed miRNAs was described upon BCR activation [22]. In this study the expression of *miR-155*, which plays a role during T- and B-cell development [20, 23] was increased while the expression of *miR-29c*, *miR-150*, *miR-181b*, and *miR-223* was reduced, as commonly observed in patients with shorter survival and/or time to treatment [22].

Role of specific microRNAs in CLL

MicroRNA-15a/16-1

The *miR-15a/16-1* cluster was discovered in 2002 within the 13q14.3 deleted region in CLL [24] and several studies have demonstrated the central role of *miR-15a/16-1* in CLL [25, 26]. In ~66% of CLL cases, *miR-15a/16-1* expression is downregulated [24] and associates with the longest treatment-free period [27]. Loss of *miR-15a/16-1* expression promotes mature B-cell expansion by deregulating the transition from G1 to S phase [28] and induces higher levels of the antiapoptotic proteins Bcl2 and Mcl1 [24, 29–32]. Indeed, an inverse correlation between m*iR-15a/16-1* and *BCL2* expression levels was found in CLL, and m*iR-15a/16-1* downregulation in leukemic cell lines lead to an increased Bcl2 expression inhibiting apoptosis [29]. *MCL1*, an antiapoptotic *BCL-2* family member associated with CLL cell survival and chemotherapy resistance, was also identified as a deregulated gene in CLL when comparing patients with high or low levels of *miR-15a/16-1* [30].

The role of the second *miR-15b/16-2* cluster located at chromosome 3q25 has been studied by Lovat et al [33]. *MiR-15a* is highly similar to *miR-15b*, and *miR-16-1* is identical to *miR-16-2*; thus, both clusters could control a similar set of target genes and may have overlapping functions. To clarify the role of *miR-15b/16-2 in vivo*, knockout mouse was generated. *MiR-15b/16-2* KO mice developed B-cell malignancy by age 15–18 mo with a penetrance of 60%. Mice showed enlarged spleens with abnormal B cell-derived white pulp enlargement. Flow cytometric analysis demonstrated an expanded CD19+ CD5+ population in the spleen of 40% knockout mice, a characteristic of the CLL phenotype in humans. This phenotype is comparable to that observed in the *miR-15a/16-1* knockout mouse model [34] suggesting an important role of *miR-15b/16-2* loss in CLL pathogenesis.

MicroRNA-29 and microRNA-181

In both indolent and aggressive CLLs, *miR-29* is overexpressed when compared to normal B cells, suggesting a possible role as an oncogene in CLL. On the other hand, *miR-29* expression is down-regulated in aggressive versus indolent CLLs [35, 36]. To clarify the role of *miR-29* in CLL, we designed a transgenic mouse model overexpressing *miR-29* in mouse B-cells. These mice developed a disease resembling the indolent form of human CLL. We observed an increase in CD5+ CD19+ IgM+ B-cell populations (a hallmark of CLL) and the percentage of leukemic cells increased with age, indicating a gradual progression of indolent CLL [36]. In aggressive CLL, *miR-29* down-regulation appears to be involved in Tcl1 over-expression, along with *miR-181* [35]. Activation of the *TCL1* oncogene is a central initiating event in the pathogenesis of aggressive CLL, and high Tcl1 expression correlates with aggressive phenotype [37]. *TCL1* functions as a coactivator of the cell survival kinase *AKT* and inhibits de novo DNA methylthansferases Dnmt3A and Dnmt3B leading to a decrease methylation of DNA in CLL with higher Tcl1 expression [38]. *TCL1* is a predicted target of *miR-29* and expression levels of *TCL1* and *miR-29* are inversely correlated in CLL. *MiR-181b* is also down-regulated in aggressive CLLs and predicted to target *TCL1*. Pekarsky et al demonstrated that co-expression of *TCL1* with *miR-29* and *miR-181* significantly decreased Tcl1 expression [35]. Thus, the role of *miR-29* in CLLs can be explained according to its effect on Tcl1. *MiR-29* up-regulation in indolent CLLs has no effect on *TCL1* expression since *TCL1* is not expressed in indolent CLLs and *miR-29* is not sufficient to cause aggressive CLL [36], In contrast, up-regulation of Tcl1 is required for the initiation of the aggressive form of CLL and down-regulation of *miR-29* in aggressive CLL (compared to the indolent form) contributes to up-regulation of Tcl1 [39]. In both indolent and aggressive CLLs, *miR-181* is downregulated compared to normal B-cells and shows higher expression levels in indolent vs aggressive cases [35, 40] Furthermore, *miR-181b* expression diminishes during CLL progression when evaluated in sequential samples from the same patients, suggesting that this microRNA could be used as markers to track disease progression [2].

MicroRNA-34a and microRNA-34b/c

In CLL, 11q deleted region includes the *miR-34b/c* cluster [41], deletion of 17p contains the *TP53* tumor suppressor [42], and 13q deletion causes *miR15a/16-1* downregulation [30]. Thus, we investigated weather, *miR-34b/c* cluster, tumor protein p53, and *miR-15a/16-1* cluster, share a molecular pathway that could clarify the prognostic implications of 11q, 17p,

and 13q deletions in CLL [41]. The regions upstream *miR-15a/16-1*, *miR-34b/c*, and *miR-34a* contain several *TP53* binding sites. Thus, *TP53* can induce the expression of these microRNAs. On the other hand, *miR-15a/16-1* target *TP53 and BCL2*, while *miR-34* members target *ZAP70* [41]. Loss of *miR-15a/16-1* in 13q deleted patients, leads to higher levels of both Bcl2 and p53 [29]. In this scenario, high levels of Bcl2 cause a decrease in the number of apoptotic cells; however high levels of p53 keep the tumor burden relatively low, explaining the indolent course of 13q deleted CLL patients, and boost the transactivation of *miR-34b/c* leading to lower levels of *ZAP70* [4]. In CLL patients with 11q deletion, since *miR-15a/16-1* are not deleted, *TP53* is not upregulated thus offering a lower control of apoptosis. Furthermore, *TP53* transactivation of *miR-34b/c* is ineffective, since this microRNA is deleted [41] leading to a higher expression of *ZAP70* [4]. Lastly, deletion of 17p highly correlates with unfavorable outcomes and response to treatment is often poor. Indeed, the majority of chemotherapy-resistant patients show 17p deletions and *TP53* mutations. However, the lack of p53 expression alone cannot explain almost half of the refractory cases. In order to clarify this observation Zenz et al. studied *miR-34a* expression in chemotherapy-resistant CLL with and without 17p deletion or *TP53* mutation and observed a low expression of *miR-34a* in all cases [21, 43]. Thus, *miR-34a* is associated with chemotherapy-refractory regardless of 17p deletion/*TP53* mutation.

MicroRNA-155

MiR-155 is increasingly overexpressed as normal B-cells progress toward a monoclonal B-cell lymphocytosis and to CLL, thus it represents a biomarker for the risk of progression [20, 44]. Furthermore, relative expression levels of *miR-155* in plasma collected from patients before therapy were significantly lower in patients who achieved complete remission after treatment than in those who had poorer treatment responses. Hence *miR-155* expression can identify CLL patients who may not respond well to therapy [44]. A transgenic mouse expressing *mmu-miR-155* in B-cells was generated to study its role in B-cell development and lymphomagenesis [45]. Eμ-*mmu-miR-155* mice showed an initial preleukemic pre-B-cell proliferation followed by a frank B-cell malignancy, indicating that *miR-155* is able to induce polyclonal expansion and suggesting that *miR-155* is directly implicated in the initiation/progression of B-cell lymphomas [45].

MiR-155 also interferes with the BCR induced signaling pathways by modulating the expression of *SHIP1* in CLL. *SHIP1* encodes for Src homology-2 domain containing inositol 5-phosphatase 1, a phosphatase that may suppress BCR signaling. *MiR-155* targets *SHIP1* and, by reducing its expression, enhances the sensitivity of B-cells to BCR stimulation [20]. Furthermore T-cells or accessory cells of the lymphoid tissue can augment *miR-155* expression by stimulating the BCR response via CD154/CD40 or *BAFF/APRIL* interaction. Indeed, a reduced expression of *SHIP1* and enhanced responsiveness to BCR ligation was observed *in vitro* in CLL cells stimulated with CD154 or *BAFF/APRIL.* Similar effects were also observed in normal B cells, suggesting that *miR-155* could play a physiological role in the regulation of B-cell response to BCR ligation [20].

MicroRNA-17/92 cluster

MiR-17/92 is a polycistronic microRNA cluster able to inhibit the expression of the tumor suppressor *PTEN* and the proapoptotic protein Bim [46]. This cluster is overexpressed in many lymphoid malignancies including CLL. To study the role of this microRNA cluster in lymphomagenesis, a mouse model expressing high levels of *miR-17/92* in lymphocytes was generated [46]. These mice died prematurely as a consequence of a developed autoimmunity followed by a lymphoproliferative disorder characterized by lymphocytes with a high proliferation rate and decreased apoptosis. Later, a transgenic mouse overexpressing *miR-17/92* specifically in B-cells was generated [47] to investigate the effect of *miR-17/92* in B cells malignancies. Eighty percent of *mir-17/92* transgenics developed a B-cell malignancy characterized by expansion of CD19+ B cells. Forty-four microRNAs and 680 genes were differentially expressed in malignant B-cells compared to controls. Eleven downregulated miRs were correlated with 66 upregulated target mRNAs and 8 upregulated miRs were correlated with 101 downregulated target mRNAs [47]. A very recent study showed that in aggressive UM-IgV_H CLL, BCR response is achieved through upregulation of *miR-17/92*. Accordingly to this study, the induction of *miR-17/92* in UM-IgV_H CLLs is driven by BCR activation [48].

Role of SNPs and mutations in the expression of microRNAs

MicroRNA expression can be modulated by single nucleotide polymorphisms (SNPs). For instance, the regulation of *miR-34a* expression is subjected to the presence of a single nucleotide polymorphism in the intronic region of the promoter of ubiquitin ligase *MDM2* (SNP 309) [6]. *TP53* transactivates *miR-34a* enhancing apoptosis and cycle arrest [49–51] and *miR-34a* has been implicated in the CLL response to DNA damage through a p53-mediated induction [42, 43, 52]. In patients with intact p53, the presence of SNP 309 in *MDM2* promoter induces down-regulation of *miR-34a* [6]. Indeed, SNP 309 leads to increased expression of *MDM2*, which binds p53 inhibiting its transactivation effects on *miR-34a* [6]. The presence of SNPs in pre-miRNAs and/or miRNA processing genes also contributes to predisposition for CLL [53]. Indeed, 57 out of 91 SNPs genotyped in 107 CLL patients and 350 cancer-free controls were located in miRNA processing genes (62.6%) and 34 in pre-miRNAs (37.4%) sequences. Nine of these SNPs were significantly associated with CLL risk; seven of them were located in six miRNA processing genes and two of them in pre-miRNAs. Thus, SNPs located in genes involved in miRNAs biogenesis pathway or in pre-miRNAs contribute to CLL [53].

We recently showed that that *miR-3676*, a potent inhibitor of *TCL1* expression, is mutated in CLL [7]. DNA sequencing of 545 CLL samples and 146 samples from healthy individuals revealed that six CLL samples (~1%) contained five different mutations within the *miR-3676* gene. Interestingly, two of these mutations significantly inhibited expression of *miR-3676*, confirming that these are loss-of-function mutations [7]. Since mature *miR-3676* starts exactly at the end tRNA-Thr and ends at a transcription termination stop for RNA polymerase III, it cannot be excluded the possibility that *miR-3676* represents a member of a new class of small RNAs, tRNA-derived small RNAs (tsRNAs), generated during tRNA processing [54, 55].

Role of defects in microRNA processing and allele specific transcription of microRNAs

Primary transcripts (pri-miRNAs) of *miR-15a/-16/-15b* are elevated and the processing intermediates (precursor miRNAs) are reduced in cells from CLL patients compared with non-malignant B-cells, indicating a block of miRNA maturation at the DROSHA processing step [8]. A defect in the post-transcriptional processing of tumor suppressor pri-miRNA transcripts is an interesting novel miRNA-related tumor escape mechanism and a possible underlying cause for miRNAs downregulation whenever levels of mature miRNAs are reduced.

Recently, Veronese et al. identified a novel allele-specific mechanism that involve RNA polymerase III (RNAPIII) for *miR-15a/16-1* transcription [9]. This mechanism is independent of the *DLEU2* host gene, which is typically transcribed monoallellically by RNA Polymerase II (RNAPII). Usually, one allele of *miR-15a/16-1* is transcribed by RNAPII along with *DLEU2* host gene, the other one by RNAPIII. In a subset of 13q14 deleted CLL patients characterized by high expression of *ZAP70*, *miR-15a/16-1* is transcribed exclusivelly by RNAPIII. Thus, a double allele-specific transcriptional regulation of the *miR-15a/16-1* locus represents a mechanisms that may distinguish at onset aggressive from indolent forms of CLL and provides a basis for the clinical heterogeneity of the CLL patients carrying 13q14 deletions [9]. Indeed, in a CLL case of monozygotic twins that differed in *ZAP70* status and clinical features, transcription of *primiR-15a/16-1* was driven by RPIII in the aggressive *ZAP70*-positive patient and by RPII in the indolent *ZAP70*-negative case [9].

Epigenetic regulation of microRNAs

Deregulation of epigenetic processes can modify microRNA expression, and microRNAs can be involved in epigenetic regulation of genes [56]. The histone deacetylases (HDACs) promote chromatin compaction, epigenetic gene silencing [57] and affect microRNAs expression. Indeed, *miR-15a/16-1* is silenced by epigenetic mechanisms in 30%-35% of CLL samples and in samples with monoallelic 13q14 deletion *HDACs* repressed *miR-15a/16-1* expression of the residual allele. Moreover, HDAC1-3 are over-expressed in CLL but not in normal lymphocytes [56]. The two copies of the 13q14.3 critical region replicate asynchronously, suggesting differential chromatin packaging. Monoallelic expression originates from either the maternal or paternal copy, which excludes an imprinting mechanism, and that one CpG island of the region is methylated. DNA demethylation of this CpG island and global histone hyperacetylation induced biallelic expression, whereas replication timing was not affected. Thus, differential replication timing represents an early epigenetic mark that distinguishes the two copies of 13q14.3, resulting in differential chromatin packaging and monoallelic expression [10].

NF-kB signaling is enhanced in CLL as a consequence of epigenetic inactivation of *NF-kB* targeting microRNAs. Baer et al. found that *miR-708* strongly represses *NF-kB* pathway and identified an enhancer region downstream of the *miR-708* promoter that displays a distinct DNA methylation status in CLL. High enhancer methylation significantly correlates with

lower *miR-708* expression and is predominantly found in patients with poor prognosis and shorter time to treatment [58]. *MiR-9-3* was also revealed as a hypermethylated tumor suppressor miRNA in CLL, able to down-regulate the *NF-κB1*pathway. *MiR-9-3* methylation is tumor-specific in CLL cell lines, and tumor suppressor activity of *miR-9-3* was demonstrated in CLL [59]. Indeed, restoration of *miR-9-3* in I83-E95 cells (cell line harboring complete methylation of *miR-9-3*) led to reduced cellular proliferation and enhanced apoptosis together with downregulation of *NF-kB*. Moreover, *miR-9-3* methylation was associated with advanced disease stage. Therefore, *miR-708* and *miR-9-3* methylation may account for constitutive upregulation of NF-kB signaling pathway in CLL [58, 59]. Lastly, the *miR-34b/c* cluster located within the commonly deleted region at 11q is also epigenetically regulated [60]. The expression and methylation status of these miRs was investigated in CLLs with or without 11q-deletion. The *MiR-34b/c* promoter was found aberrantly hypermethylated in 48% of CLL cases and *miR-34b/c* expression was inversely correlated to DNA methylation. Furthermore, increased *miR-34b/c* methylation inversely correlated with the presence of 11q-deletion, indicating that methylation and del(11q) independently silence these miRs [60].

Microenvironment, exosomes and viral microRNAs

MicroRNAs can interact with BCR signaling and microenvironment. BCR stimulation can alter the expression of certain microRNAs, contributing to B cell proliferation and/or apoptosis, and certain microRNAs can influence BCR signaling and immunoglobulin production [20, 23]. A signature of 39 differentially expressed miRNAs was found upon BCR stimulation [11] and BCR activation lead to reduced levels of *miR-29c*, *miR-181b*, or *miR-223*, frequently observed in patients with shorter survival and/or time to treatment [22, 61]. *MiR-155* is instead upregulated in response to BCR ligation and plays a role in T- and B-cell development [20, 23].

BCR activation in CLL also induces the secretion of exosomes, whereas inhibition of BCR reduces CLL exosome release. Many reports showed that miRNAs are released by donor cells through circulating exosomes and that plasma of CLL patients presents a different amount of extracellular miRNAs when compared to healthy controls [62, 63]. Expression of *miR-150* and *miR-155* in exosomes was elevated in CLL-derived exosomes vs normal B cells and further increased in response to BCR activation, indicating that the BCR signaling is involved in CLL exosome secretion [63]. Furthermore, CLL-derived exosomes are incorporated by endothelial and mesenchymal stem cells contributing to a tumor-supportive microenvironment [12]. Since therapy often reduced the pool of malignant cells but does not affect plasma features, it is likely that exosomes continue to induce abnormal gene profiles. Therefore, miRNA profiling in plasma could reflect another mechanism of malignant B-cell proliferation [12]. Additionally, in CLL derived exosome, packaging of *miR-202-3p* results in enhanced expression a Hedgehog signaling intermediate in the parental CLL cells thus altering the transcriptome and behavior of recipient cells [64].

Lastly, several studies investigated the role of Epstein-Barr Virus (EBV) in CLL [65]. EBV is a human herpes virus associated with a subclinical latent infection of B cells in healthy individuals and a variety of B-cell lymphomas [66]. EBV infection may influence the

expression of cellular miRNAs and in lymphoblastoid B-cells is able to induce *miR-155* expression [67]. Furthermore, EBV encodes for two viral miRNAs: *BHRF1* and *BART* [68]. *BHRF1-1* expression levels in plasma from CLL patients are higher than in plasma of healthy donors, associated with shorter survival and correlated with tumor burden, markers, and outcome of patients with relapsed CLL, suggesting a role of this viral miRNA in CLL onset/progression. Moreover, in primary malignant B-cells, exogenous expression of *BHRF1-1* lead to reduced levels of TP53, indicating that EBV viral miRNAs can target cellular genes. Lastly, the expression levels of EBV miRNAs in plasma vs B-cell from CLL patients showed a discrepancy. The exosome phenomenon is likely to explain this finding. Indeed, malignant B-cells are actively secreting exosomes containing viral miRNAs and EBV infects not only B lymphocytes, but also epithelial cells, smooth muscle cells, T- and NK cells which can release *BHRF1-1* into the plasma via exosomes. Thus, latent EBV-infected cells communicate with the surrounding cells by releasing viral miRNAs embedded in exosomes [69].

Therapeutic implications

The contribution of microRNAs to CLL onset/progression suggests the possibility of developing therapeutic approaches based on miRNAs. The Eμ-*TCL1* transgenic mouse model mimicking the aggressive form of human CLL was used to test *miR-181b* as a therapeutic agent. *In vitro* enforced expression of *miR-181b* induced apoptosis in human B-cell lines and in mouse Eμ-*TCL1* leukemic splenocytes. *MiR-181b* affected the expression of Tcl1, Bcl2 and Mcl1, Akt and phospho-Erk1/2 indicating that *miR-181b* exerts a broad range of actions that affect proliferation, survival and apoptosis, and can potentially be used to reduce expansion of CLL cells. Interestingly, an anti-*TCL1* siRNA was not as effective as *miR-181b*. Thus, the anti-leukemic effect of *miR-181b* can be effective also for not *TCL1*-driven CLLs. Furthermore, *in vivo* studies demonstrated that *miR-181b* reduces leukemic cell expansion and increases mice survival [70]. Lastly, a synergic effect of *miR-181b* with fludarabine was observed in human primary CLL cells [71], providing an additional evidence for a potential role of *miR-181b* as a therapeutic agent in CLL.

Finally, Dereani et al designed specific oligonucleotides to target endogenous *miR-17* (antagomiR17). In vitro administration of antagomiR17, reduced *miR-17* expression and the proliferation of CLL-like MEC-1 cells. When injected in vitro in tumor**s genetated by the MEC-1 cells in SCID mice, antagomiR17 reduced tumor growth and increased mice survival,** providing the rationale for the use of antagomiR17 as a novel potential therapeutic tool in CLL [72].

Concluding remarks

The ability of microRNAs to modulate gene expression is essential to provide fine control of several cell processes and deregulation of microRNAs can be involved in CLL development/progression (Fig. 1). Deregulation of microRNAs can originate from chromosomal alteration, epigenetic modulation, allele selection, aberrant precursor processing, or interaction with other gene. *MiR-15a/16-1* deletion, an initializing step in CLL, leads to an increase of Bcl2 expression. *MiR-34* family members are involved in a fine-regulated

feedback circuitry with p53 and *miR-15a/16-1*, suggesting bidirectional interplay between microRNAs and genes. Downregulation of *miR-29 and miR-181b* in aggressive CLL contributes to overexpression of Tcl1. Furthermore, study of *miR-181b* and *miR-17/92* may provide useful insights into drug design, delivery, resistance mechanisms, and microenvironmental responses [70–72]. Given these results, we can conclude that microRNAs have a deep impact on CLL development/progression (Table 1).

As discussed above, numerous studies were carried out to uncover molecular mechanisms related to microRNA expression, regulation and targeting. However the ways to practically utilize this knowledge is still under development. It remains to be seen if microRNAs from CLL cells or exosomes can be used in clinical practices to track CLL. The same applies to the usage of microRNAs as drugs, as high costs and a lack of reliable delivery systems represent significant obstacles in the development of microRNA based therapies.

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Trends

Many microRNAs show disregulated expression in CLL.

MicroRNAs function as oncogenes or tumor suppressors in CLL.

MicroRNAs can serve as markers for CLL classification and progression.

Outstanding questions

Can microRNA signatures predict time to treatment and clinical outcomes of CLL?

Are alterations in microRNA expression initiating events in CLL pathogenesis, or they occur later during the progression of the disease?

Are other classes of small non-coding RNAs (like tsRNAs or piRNAs) also involved in CLL pathogenesis?

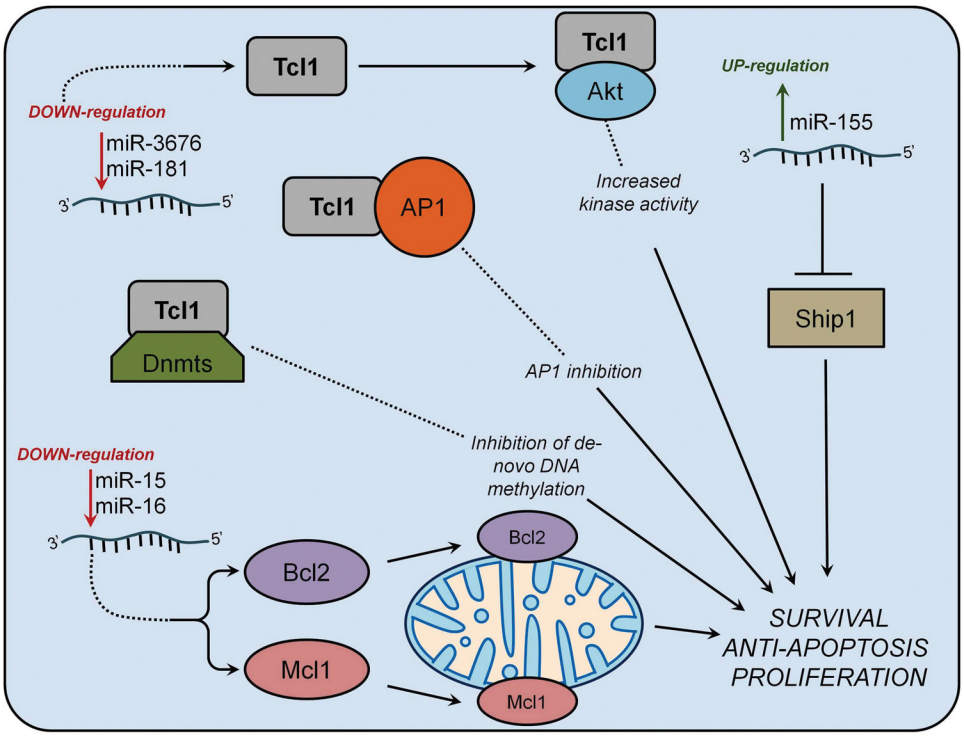

Figure 1. Role of microRNAs in CLL pathogenesis

miR-15/16 deletion results in Bcl2 and Mcl1 overexpression leading to B-cell transformation. Down-regulation of *miR-181* and *miR-3676* causes Tcl1 overexpression, increased Akt activity and inhibition of *de novo* DNA methylation. *miR-155* up-regulation causes B-cell malignancies through the targeting of *SHIP1.*

Table 1

MicroRNAs in CLL can function as oncogenes or tumor suppressors.

miRNA	Function	References
miR-155	Oncogenic function in CLL Activates of BCR response by targeting *SHIP1*	20, 44, 45
miR-17/92	Oncogenic function in CLL Targets tumor suppressor genes *PTEN* and *BIM* Trigged by BCR response in aggressive CLL	46, 47, 48
BHRF1-1	Viral microRNA with oncogenic function Targets still under investigation	68, 69
miR-29	Oncogenic function in indolent CLL (upregulated vs normal B cells) Tumor suppressor function in aggressive CLLs (down regulated vs indolent CLL) Targets *TCL1* oncogene in aggressive CLL	35, 36
miR-15a/16-1	Tumor suppressor function in CLL Targets antiapoptotic genes *BCL2* and *MCL1*	24, 29–32, 41
miR-15b/16-2	Tumor suppressor function in CLL Targets still under investigation (likely same targets as miR-15a/16-1)	33
miR-181b	Tumor suppressor function in CLL Targets *TCL1* oncogene Marker for CLL progression	2, 25, 40
miR-34a	Tumor suppressor function in CLL Low levels associate with chemotherapy-refractory Transactivated by p53	6, 21, 43
miR-34b/c	Tumor suppressor function in CLL Targets *ZAP70* (marker for CLL progression) Transactivated by p53	41, 60
miR-3676	Tumor suppressor function in CLL Targets *TCL1* oncogene	7
miR-708	Tumor suppressor in CLL Targets *IKKβ* (activator of *NF-kB*) Epigenetically inactivated in aggressive CLL patients	58
miR-9-3	Tumor suppressor in CLL Targets *NF-kB* Epigenetically inactivated in aggressive CLL patients	59