Recent advances in microRNA-mediated gene regulation in chronic lymphocytic leukemia

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Abstract

Chronic lymphocytic leukemia (CLL) is the most common leukemia in the western world and is a very clinically heterogeneous disease for which better prognostic biomarkers are needed. Current prognostic markers exhibit both biological and technical limitations. MicroRNAs (miRNAs) are small endogenous, non-coding 22-nucleotide regulatory RNAs that have been shown to modulate hematopoietic lineage differentiation and play important gene-regulatory roles in disease processes. In this manuscript, we review miRNA biology and the association of specific miRNAs with CLL.

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Contents

Introduction .................................................. 901
MicroRNAs and their biology ............................ 902
miRBase and miRNA registry ............................ 903
miRNA and CLL ............................................ 903
miR-15a and miR-16 and 13q14 deletion .......... 904
miR-29, miR-181 ........................................... 904
Signature and prognostic miRNAs in CLL .......... 905
17p deletion, p53 and miRNA .......................... 905
miRNA and target genes in CLL ....................... 906
Discussion and future directions ....................... 906
References .................................................. 906

Introduction

Chronic lymphocytic leukemia (CLL) is the disease of neoplastic B cells derived from antigen experienced B cells [1]. The diagnostic criteria as laid out by the International Workshop on Chronic Lymphocytic Leukemia (IWCLL-2008) requires the presence of at least $5 \times 10^9$ B lymphocytes/L (5000/μL) in the peripheral blood with characteristic morphology of CLL cells that are clonal by flowcytometric studies (Fig. 1). B-CLL cells in the peripheral blood smear are mature lymphocytes, characteristically small with a narrow border of cytoplasm and a dense nucleus lacking discernible nucleoli and having partially aggregated chromatin [2]. These cells co-express the T-cell antigen CD5 and B-cell surface antigens CD19, CD20, and CD23. The levels of surface immunoglobulin, CD20, and CD79b are characteristically low compared with those found on normal B cells. Each clone of leukemia cells is restricted to expression of either kappa or lambda immunoglobulin light chains [2]. Data from the SEER stat fact sheet indicate that from 2004 to 2008, the median age at diagnosis for CLL was 72 years of age and the median age at death for CLL was 79 years of age. Overall median survival in cases of CLL is 10 years [3]. Approximately 70–80% of cases of CLL get diagnosed in early stages. Of these early diagnosed cases some may have an indolent clinical course with no effect on overall life expectancy; however a fraction of these cases may show rapidly aggressive disease with poor outcome. Due to wide clinical heterogeneity,
long term overall survival period and absence of a curative treatment for CLL, management plans for CLL are determined in conjunction with prognostic factors [4]. Clinical staging (Rai and Binet) is the mainstay for prognostication and prediction of the clinical course and this is enhanced by the use of a wide variety of biological markers. Some of the extensively used markers include serological markers such as thymidine-kinase, sCD23 Beta-2 microglobulin, FISH, cytogenetics, IgVH mutational status, CD38 expression, and ZAP-70 expression [4] (Fig. 2).

There are certain limitations to such biomarkers, for example, there are variations in the predictive cut-off levels of the serological markers amongst different laboratories; studies show that CD38 correlates with the IgVH status but the correlation is not absolute and also the expression status changes over time. Immunoglobulin mutation studies and FISH are laborious and expensive [4]. Alternative prognostic markers that are more reproducible with better predictive outcomes are desired.

**MicroRNAs and their biology**

MicroRNAs (miRNA) are short (~22nt in humans), endogenous non-coding ssRNA (single stranded) molecules that regulate gene expression via translational repression or transcript degradation. The first identified miRNA was lin-4, which Lee et al. associated with the regulation of timing of *C. elegans* development. An RNA–RNA antisense interaction between lin-4 miRNA and lin-14 mRNA resulted in downregulation of lin-14 mRNA [5,91]. This interaction between miRNA and mRNA in animals is rather imprecise and indiscriminate. A single metazoan miRNA is capable of regulating, translation of 100 or more miRNAs. In addition, multiple miRNAs may bind to the 3′-UTR of a single mRNA, forming a highly complex and precise mechanism for translational repression [6,7].

Approximately 100 genes encoding for miRNA are known in *C. elegans* and over 250 genes encoding for miRNAs are known in the vertebral genome [9]. miRNA genes may be present as individual genes with independent promoters for transcription as well as in clusters [9]. miRNAs are typically transcribed from genes which are far removed from their respective mRNA targets, indicating that they are likely transcribed independently [10]. Several miRNAs in mammals are found within the introns of mRNA coding genes and are referred to as “mirtrons”. These are most often found in a sense orientation relative to their host genes, suggesting that genes and their associated mirtrons can form transcription units [11–13].

miRNAs are synthesized as primary miRNAs (pri-miRNAs), in the nucleus by the polymerase activity of Pol II [14,15]. Pri-miRNAs demonstrate a highly-folded secondary structure, with many hairpin and stem-loop features. Pri-miRNA transcripts are processed by the “microprocessor complex”; a Class 2 R Nase III endonuclease (Drosha) bound to the accessory protein DGC8R (Pasha) [16,17]. The microprocessor complex selectively acts and cleaves the hairpins with a large terminal loop, removes two helical RNA turns into the stem, and converts...
pri-miRNAs into “precursor miRNAs” (pre-miRNAs) [18]. Pre-miRNAs are ~60 nucleotides long, dsRNA (double stranded) with characteristic hairpin structures and imperfect base pairing [12,13]. A pre-miRNA is exported out of the nucleus by Exportin5 into the cytoplasm [19]. In the cytoplasm, pre-miRNAs are further processed by RNase III endonuclease enzyme (Dicer). Dicer removes the terminal loop of the hairpin loop from pre-miRNA, yielding a ~22nt dsRNA molecule consisting of the mature miRNA base-paired to a roughly complementary fragment (miRNA*). The miRNA strand from the miRNA:miRNA* duplex binds to the RNA-induced silencing complex (RISC), and miRNA* is released and rapidly degraded in the cytoplasm [20]. The newly-formed RISC is a ribonucleoprotein complex consisting of an RNase endonuclease—a member of the Argonaute family—bound to the mature miRNA; which acts as a guide template [21]. Once a miRNA is incorporated into a RISC, it can act upon potential miRNA targets to down-regulate gene expression [21].

mRNA degradation occurs occasionally in animals when the miRNA guide template binds to the miRNA transcript and activates the RNase activity of its associated Argonaute protein. Translational repression is seen more frequently in animals, and arises from the miRNA guide molecule in a RISC binding imperfectly with a sequence in the 3′-UTR of a target mRNA. In this case, the RISC will simply remain bound to the transcript, inhibiting ribosome activity [92].

More recent studies in miRNA biology is on the structure of the Dicer molecule. The initially suggested “L”-shaped structure of the human dicer, is now proposed to have accessory domains (with N-terminal helicase activity) from the arm towards the base of dicer, enhancing the “L”-shape of dicer with a clamp shape structure at the base. Lau et al. propose that a single continuous channel runs through the clamps of helicases, via a RNAase III active site, and ends with a cRNA binding pocket of the PAZ domain (head of Dicer contains PAZ domain and recognizes the dsRNA of a miRNA) as the major surface used by Dicer enzyme for processing the dsRNA molecules [22-24].

miRBase and miRNA registry

A myriad of miRNAs have been identified by using bioinformatics and reverse genetics approaches. Use of recent algorithms that rely upon sequence conservation between orthologous sites and place emphasis upon “seed” regions, have improved target detection and have reduced false-positive associations. These and other increasingly sophisticated bioinformatics approaches have now raised estimates of the total number of human miRNAs to approximately 1048 [8].

miRBase is a widely used database that broadly consists of a “miRBase registry” that provides independent assignments of names to novel miRNA genes; “miRBase Database” that provides a predicted sequence data of hairpin miRNA along with published sequence data of mature miRNA; and “miRBase target database” that provides a prediction of target genes of miRNA [8]. The most recent release is “miBase 18” that contains 18,226 entries of hairpin precursor miRNAs, and 21,643 mature miRNA products, in 168 species. miRBase 18 contains 1488 new hairpin sequences and 1929 novel mature products [8,27-30,93].

Some miRNAs remained undiscovered since they are expressed in undetectable quantities and or secreted in a time and tissue specific manner. In that case the hairpin loop structure of pre-miRNA can be used as a mode of identifying undiscovered miRNAs. Some predictive techniques that take advantage of the hairpin loop structure of pre-miRNA include support vector machine (SVM), random forest (RF), hidden Markov model (HMM) and naïve Bayes classifier (NBC). Genetic Algorithm and Support Vector Machine (GA-SVM) and SVM Classifier (miR-SF) have used features of RNA folding in the secondary structure as an effective manner of identifying pre-miRNAs [30].

A number of computational programs have been designed that predict the target genes of miRNAs. Some of the target prediction programs available for public use include DIANA-microT, MiRanda, PicTar I, TargetScanS and TargetScan. Sethupathy et al. provides a guide to the computational approaches for the identification of mammalian miRNA targets. The guide provides a comparison of the above five widely used target prediction programs based on certain selected features such as the sequence, thermodynamics and conservation of sequence between species. It also provides a practical guide and stepwise approach towards the use of these programs for experimental purposes to search for targets for a particular miRNA or to refer to a precompiled list of targets for miRNA [31,32]. An example of gene targets for specific miRNAs associated with CLL is shown in Fig. 3. The National Center for Biotechnology Information is another widely available database system [32].

miRNA and CLL

miRNAs were first associated with cancer and CLL in 2002 [33] and now genome wide miRNA expression profiles have shown a dysregulation of miRNAs in almost all cancers of various origins including hematological, epithelial and mesenchymal [25]. Data supports the role of miRNAs in cancer as either tumor-suppressors or oncogenes (referred to as oncomirs) [25]. They may also be regulatory in acquisition of invasive abilities of the tumor cells [26]. Data support the widespread action and regulatory function of miRNA in cancer and its pathogenesis, for these reasons and many more miRNA’s are actively being explored as therapeutic targets [26].

<table>
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<th>TP53</th>
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Fig. 3. Gene targets of specific miRNAs.
miR-15a and miR-16 and 13q14 deletion

Calin et al. published the first evidence of the involvement of a miRNA in CLL pathogenesis, as part of the 13q14 deletion present in roughly 50% of CLL cases [33]. Hemizygous and/or homozygous loss at 13q14 is the most frequent chromosomal abnormality in CLL and is also seen in mantle cell lymphoma, multiple myeloma, DLBCL, mature T-cell lymphoma, and solid tumors, suggesting the presence of tumor suppressor genes at 13q14 [34]. The 13q14 region contains the deleted in leukemia (DLEU) 2 gene and the miR-15a/miR-16-1 cluster [33,35–37]. In SNP array studies, Pfeifer et al. validated that the del(13q14) region contained the miR-15a/miR-16-1 cluster [38]. Calin et al. identified germline mutations in the primary precursors of miR-15a and miR-16-1 in 2% of CLL cases [39]. Point mutations in the 3′ flanking region of miR-16-1 in the New Zealand Black Mouse strain has been associated with reduced miR-16-1 expression, and mice harboring this mutation developed a B-cell lymphoproliferative disorder that is a model for human CLL [40]. These studies emphasize that miR-15a and miR-16-1 are critical in CLL pathogenesis, functioning as micromanagers that fine tune gene expression [6].

Smonsky et al. used miRNA qRT-PCR along with FISH data in 23 cases of CLL and compared levels of expression of miR-15a and miR-16-1 in monoallelic versus biallelic deletions of 13q14, using cases with trisomy 12 as a control group. The monoallelic loss group had a significantly lower miRNA expression than the control group, whereas the biallelic loss group displayed even lower levels of miR-15a when compared to the control group. Their data showed a significant difference in the level of expression [41] and that the differences in miRNA expression levels in monoallelic and biallelic 13q14.3 deletion CLL samples are detectable using qRT-PCR [41].

Cimmino et al. proposed that miR-15a and miR-16-1 function as tumor suppressor genes by suppressing BCL2 (B-cell CLL/lymphoma 2), an anti-apoptotic protein that is highly expressed in CLL; and showed homology of the first nine nucleotides between the 5′ ends of both miRNAs and the BCL2 mRNA sequence. Whereas in normal CD5+ tonsils the levels of both miRNAs were high and the BCL2 protein was expressed in low levels, the opposite was true in leukemic cells. These findings supported a role for miR-15a and miR-16-1 as having an antimutagenic effect by targeting the BCL2 gene [42]. Cimmino et al. validated their work by observing highly reduced BCL2 levels in a tumor model [43].

While some studies support the BCL2 mechanism of tumorgenesis in leukemia (DLEU) 2 gene and the miR-15a/miR-16-1 cluster [44]. Further, TUNEL assays showed that BCL2 down-regulation by miR-15 and miR-16-1 triggers apoptosis [42].

miR-29, miR-181

miR-29 has been described as a tumor-suppressor miRNA which targets several oncoproteins including TCL1, MCH1, and CDK2 [50–52] or conversely, as an oncopgenic miRNA in AML [53]. The T cell leukemia gene 1 (TCL1) oncogene was first discovered in T-cell prolymphocytic leukemia (T-PLL) [54]. To investigate the role of TCL1 in B-cell malignancies, Bichi et al. created Eμ-TCL1 transgenic mice with TCL1 expression up-regulated by a VH promoter and an IgH-Eμ enhancer. The Eμ-TCL1 mice developed disease closely resembling human CLL with hepatosplenomegaly, lymphadenopathy and clonal CD5 + B lymphocytes [55]. In a subsequent microarray analysis, Pekarsky et al. showed that TCL1 protein expression in CLL patients was inversely correlated with miR-29 and miR-181 expression [50]. TCL1 is highly expressed in CLL and correlates with an aggressive phenotype often displaying unmutated IgVH, high ZAP-70 expression and del(11q22-23) [56]. Pekarsky et al. postulated that a down-regulation of miR-29 in CLL could contribute to the development of a more aggressive phenotype [50]. To show a more direct pathogenic mechanism of miR-29 in CLL, Santanam et al. created an Eμ-miR-29 mouse model with miR-29 expression controlled by the VH promoter/IgH-Eμ enhancer combination. The transgenic mice went on to develop CLL at a higher rate than wild-type controls. 85% of transgenic mice developed an expanded CD5 + lymphocyte population starting at 2 months and by 2 years greater than 65% of B cells were CD5+. These findings were correlated with peripheral blood smears which showed a progressive increase in malignant lymphoid cells including smudge cells and a disrupted spleen architecture showing advanced diffuse disease. They also noted Cyclin D1 protein expression in the abnormal B-cells [57]. Albeit not characteristic of CLL, weak Cyclin D1 upregulation has been seen in CLL and mouse models sometimes may not recapitulate human disease [58]. Gaudio et al. have proposed a possible mechanism of action of TCL1 in cases with ATM deletion. The TCL1 oncogene is located on chromosome 14q31.2. A biallelic loss of ATM in cases of ataxia telangiectasia (AT), leads to chromosomal translocation and inversion at 14q31.2. Preleukemic and leukemic T cells in AT patients with 14q31.2 chromosomal rearrangements, overexpress TCL1 [60–62]. Gaudio et al. by using comminoprecipitation experiments identified that TCL1–ATM proteins potentially interact with each other by forming a complex [59]. As a result of this cooperation between TCL1 and ATM the physiological inhibitor of NF-κB pathway is phosphorylated and degraded leading to activation of NF-κB pathway.
Visone et al. studied expression of miR-181b in 23 patients with progressive CLL disease by analyzing and comparing miR 181b in two sequential samples from the same patient with clinically progressive disease. A sequential decrease in the levels of expression of miR-181b was documented in patients with progressive disease whereas the levels were unchanged in patients with stable disease. The study suggests evaluation of miR-181b as a potential tool to monitor disease course [63].

**Signature and prognostic miRNAs in CLL**

Microarray studies have analyzed hundreds of miRNAs to determine which may help identify the leukemic cells involved in CLL. Using miRNA-specific microarrays, Calin et al. found at least 25 miRNAs that were differentially expressed in CLL cells versus CD5+ “normal” cells and validated their findings for four miRNAs including miR-16-1, miR-26a, miR-206, and miR-223 by northern blot analysis [64]. In 2007, Flici et al. also identified a set of miRNAs including miR-155, miR-21, miR-150, miR-92 and miR-222 that was differentially expressed between CLL lymphocytes and their “normal” counterparts via qRT-PCR and molecular cloning techniques [44]. Marton et al. used qRT-PCR and northern blot analysis to identify several miRNAs including miR-181, miR-30d and let-7a that are differentially expressed between CLL lymphocytes and CD19+ control cells [65].

Several studies also attempted to identify miRNAs that could act as prognostic indicators in CLL, typically by correlating expression levels of these miRNAs with previously established prognostic indicators such as IgVH mutation status or ZAP-70 expression. Based on microarray studies, Calin et al. published a signature of 13 miRNAs that were differentially expressed between unmutated IgVH/ZAP70+ and mutated IgVH/ZAP70-CLL patients and could predict the time from diagnosis to initial treatment. This signature included miR-15a, miR-195, miR-221, miR-23h, miR-155, miR-223, miR29a-2, miR-24-1, miR-146, miR-16-1, miR-16-2, and miR-29c [39]. A study using quantitative RT-PCR of mature RNAs correlated the expression of three miRNAs—miR-150, miR-223 and miR-29b/c—with IgVH mutation status [44]. However, they also noted that while these miRNAs were on average over-expressed in patients with CLL with IgVH mutations, they were not perfect surrogate markers for IgVH mutation status—i.e., not all patients with mutated IgVH demonstrated over-expression of these three miRNAs. Marton et al. correlated expression levels of several miRNAs, including miR-15a, miR-16 and miR-29a, with IgVH mutation status [65]. Stamatopoulos et al. found decreased expression of miR-29c and miR-223 correlated with progression of disease from Binet stage A to C, poor prognostic sub-groups defined by IgVH unmuted status, positive ZAP-70 expression and LPL expression and with treatment-free survival (TFS) and overall survival (OS). They also developed a quantitative RT-PCR score combining miR-29c, miR-223, ZAP70 and lipoprotein lipase [66]. Over-expression of miR-21 and low miR-181b expression have been reported as unfavorable prognostic factors independent of other clinical–pathologic factors [67]. Based on high miR-21 expression and del(17p) analysis by either karyotype or FISH, Rossi et al. developed a “21FK” score (1 point each for high miR-21 and 17p deletion; 0 points each for low miR-21 and normal karyotype/normal FISH) and found that the survival of patients with a score of 0/2 was significantly higher than those with 0/1 and 2/2, even in low Rai stage patients. In multivariate analysis including beta-2-microglobulin, ZAP-70, IgVH, CD19+, and CD38+, the “21FK” score was associated with the lowest P value [67].

**17p deletion, p53 and miRNA**

TP53, located at the 17p13.1 locus, is the gene that encodes the tumor-suppressor protein p53, which is a stress-response protein activated by genetic instabilities or cytotoxic signals. p53 plays a central role in regulating the cell cycle and apoptosis by up-regulating a number of genes which generally arrest the cell cycle, including p21 (which causes G1-arrest) and PUMA (which induces apoptosis), or down-regulating cyclin dependent kinases (CDK4) and cyclins (Cyclin E2) [68]. CLL patients with loss of TP53 via 17p deletions are resistant to treatment with fludarabine and have poor clinical outcomes [69]. Zenz et al. showed that monoallelic p53 inactivation is associated with poor prognosis, and survival was equally poor for patients with 17p deletion only, 17p deletion plus TP53 mutation, and TP53 mutation only [70]. In 2007 several groups demonstrated that members of the miR-34 family are direct p53 targets. miR-34 genes are up-regulated by p53, and their over-expression in turn causes senescence, apoptosis, or cell cycle arrest by regulating proteins such as BCL2, Cyclin D1, Cyclin E2, CDK4, and c-MYC Sirt-1, depending on the cell type [71–77]. Two independent studies suggested that p53 mutations are equivalent to p53 deletions in that both lead to low miR-34a expression and hence are associated with impaired DNA damage response, apoptosis, and fludarabine refractory CLL disease [67,70].

Asslaber et al. found that miR-34a expression in transgenic Eµ-TCL1 mice in the preleukemic phase was comparable to that of wild-type littermate controls. However, miR-34a expression increased more than 20-fold in the leukemic phase, suggesting that p53 expression was highly up-regulated during this transition. This was further confirmed by increased p21 and PUMA miRNA levels; the downstream effectors of p53 [78]. Human B-CLL blood samples showed a 4.6-fold increase in miR-34a expression compared to controls [78]. These increases in miR-34a expression were mostly in patients with wild-type TP53 while those patients with either TP53 mutations or 17p deletions consistently showed low miR-34a levels [78]. Asslaber et al. also showed that low miR-34a levels predicted shorter time to treatment. In further transfection experiments with synthetic oligonucleotides that mimic mature miR-34 transcripts in B-CLL cell lines, they found that miR-34a could induce apoptosis only in patients without “p53 attenuation” indicating that the apoptosis-inducing effects of miR-34a require p53 activation and suggests a positive feedback loop between miR-34a and p53. In some patients decreased miR-34a is seen without p53 aberrations. These patients were homoygous for the single nucleotide polymorphism 309 (SNP309) in the intronic promoter of MDM2, a negative regulator of p53. These findings led Asslaber et al. to recommend miR-34a as an easily detected surrogate marker for any deregulation in the p53 pathway, including mutations or deletions of TP53 or its upstream regulators [78].

Fabbri et al. showed a relationship between TP53 and other miRNA (miR-15a, miR-16-1, miR-34b and miR-34c). In patients with a homoygous 13q deletion(13q−/−) and a heterozygous 13q deletion (13q+/-), there is a significant downregulation of miR-15a and miR61-1, and also significantly higher TP53 expression levels at the mRNA level and protein level as compared to cases of CLL with a normal cytogenetic profile. Also, levels of TP53 was higher in (13q−/-) when compared with (13q+/−). There was an inverse relationship between miR-15a and miR-16-1 expression with TP53 and its downstream effectors CDKN1A, BBC3, BCL2. Further, demonstrations showed cleavage of caspase3, in cell lines that stably express miR-15a, and miR-16-1, suggesting a caspase dependent proapoptotic role of miR-15a/ miR-16-1 cluster in TP53 positive cell lines only. The targeting effects of miR-15a and miR-16-1 on BCL2 persisted in TP-53 null cells [79] (indicating that BCL2 may be the direct target of the miR-15a/ miR-16-1 cluster as was seen by in silico target genes). By using sequenc er software, a binding site for miR-15a and miR-16-1 inside the 3’ UTR region was identified and conversely, TP53 binding sites were found upstream of the 2 homologous miR-15a/miR-16-1 loci on chromosome 13 and chromosome 3. The binding of TP53 was confirmed by chromatin immunoprecipitation. These experiments show a 17p−13q molecular interaction in patients with B-CLL. Chromatin immunoprecipitation studies also showed direct interaction of TP53 with a pre-miR-34b/ miR-34c site on chromosome 11; TP53 being a positive transcriptional regulator of the miR-34b/miR-34c cluster in leukemia cells. Patients with 11q+/− CLL cells had lower levels of miR-34b and
miR-34c and higher levels of ZAP-70; a binding site for miR-34 family members was detected in ZAP70 open reading frame [79].

The findings in this study proposed a novel mechanism in the pathogenesis of CLL that connects the well-known cytogenetic abnormalities (−11q, −13q), in cases of CLL, with abnormally expressed miRNA’s and their interaction with TP53, which is a tumor suppressor gene and affects the genes/proteins mediating the apoptotic pathway [79].

**Discussion**

SNP seen in the promoter region of Bcl-2 [88]. In our study in silico CLl, GWAS studies explain the overexpression of Bcl-2 based on the breast cancer [90].

The immunoglobulin heavy chain gene locus [94]. In familial cases of (14; 18) translocation that brings the Bcl-2 gene under the control of higher levels of expression with advanced stage of disease (stage C) [81].

**miRNA and target genes in CLl**

Genome Wide Association Studies (GWAS) in familial CLl cases have implicated several genes in the pathophysiology of CLl [83]. Some of the candidate genes from GWAS studies include P2X7, TNF-alpha-308 SNP, IL10 promoter SNP, Bcl6, Caspase and Bcl-2 [84–88]. Nuckel et al. showed that a single nucleotide polymorphism in the promoter region of the Bcl-2 gene has a significant effect on disease progression and survival in cases of familial CLl [88].

TargetScan predicts biological targets of miRNAs. miRNA targets could be predicted by finding perfect Watson–Crick (W→C) seed pairing. A refined analysis, “TargetScan”, requires a whole genome alignment followed by matching conserved seed regions of miRNA to the conserved 3’ UTRs of mRNA [89].

Our in-house studies using in silico miRNA target prediction, independently showed Bcl2 to be a target of miR-15a and miR-181a. Similar findings were shown by Cimmino et al. where a negative regulatory effect of the mir-15/16 family on Bcl2 expression was identified. Approximately 22 different miRNAs target Bcl2, which makes it likely that a deregulation of more than one miRNA is involved in Bcl2 pathogenicity. Conversely, one miRNA may have a global effect in deregulating a group of genes belonging to a single oncogenic pathway. For example, miR-29a targets BCC3, Bak1 and Mcl-1 and miR-181a targets Bcl-2, Bmf and Mcl-1 members of the intrinsic pathway of apoptosis [82] (Fig. 3).

B-CLl cells have increased survival as they do not undergo programmed cell death and are arrested in the G0 phase of the cell cycle. There are more than 20 members in the BCL-2 family that act as pro-apoptotic and anti-apoptotic [80]. Chronic lymphoproliferative disorders in general and CLl in particular express high levels of antiapoptotic proteins, particularly Bcl-2 [82]. Bcl-2 has been shown to have relatively higher levels of expression with advanced stage of disease (stage C) [81].

Whereas in the case of follicular lymphoma, this is a result of the (14; 18) translocation that brings the Bcl-2 gene under the control of the immunoglobulin heavy chain gene locus [94]. In familial cases of CLl, GWAS studies explain the overexpression of Bcl-2 based on the SNP seen in the promoter region of Bcl-2 [88]. In our study in silico prediction shows that miRNAs deregulated in CLl, target Bcl-2 and other genes with a negative effect on apoptosis, thus providing a means of survival to the leukemia cells. Target-prediction algorithms can be used to identify protein targets, but experimental validations of such targets are still necessary.

**Discussion and future directions**

Research has increasingly shown a role for miRNAs in the development, progression and metastatic potential of various cancers. More recent work focuses on various oncogenic pathways deregulated by miRNA. miRNAs finely balance a myriad of biological functions and pathways. Their expression profiles are shown to change significantly under various circumstances, such as, normal versus cancer tissue, benign versus malignant, and indolent versus aggressive disease course. This provides a fundamental reason for their use as diagnostic and prognostic markers [90]. Some of the markers under elaborate studies include miR-21 for glioblastoma; miR-135 and let-7a-2 for lung adenocarcinoma; miR-205 for squamous lung cancer; and miR-21 for breast cancer [90].

Distinct actions of miRNA in cancer suggest that they may emerge as novel therapeutic targets. Use of miRNAs singly or in combinations may be used to target one or multiple genes [26]. The two approaches currently being tested for miRNA inhibition include, use of a “Antagomir”-oligonucleotide complimentary to miRNAs and “miRNA sponges” which are synthetic miRNAs that may competitively sequester the endogenous miRNAs [90]. Chemically modified antagonims have been tested in cancer cell lines derived from glioblastoma, colon cancers, breast cancers and lung cancers [90]. Delivery strategies being tested for synthetic miRNA or antagonist include use of plasmids, transposons, and cationic liposomes coupled with monoclonal antibodies [90].

miRNA research also has an increasing influence in the diagnosis, prognosis and treatment of human cancers, including CLl. In CLl, miRNAs such as miR-21c, miR-223, miR-21 and miR-34a, may prove to be novel surrogate markers for common cytogenetic lesions and prognostic indicators such as IgVH mutation status [39,44,67]. In addition, the discovery of the oncogenic and tumor-suppressive properties of various miRNAs raises the possibility of miRNA therapy in the future. The oncogenic pathways involving miRNAs are highly complex, subtle, and multifaceted, raising questions as to the practical limitations and unintended consequences of miRNA-based therapies. Though caution is warranted, miRNAs remain one of the most exciting new avenues for cancer research.

**References**


Srivastava et al. / Clinical Biochemistry 46 (2013) 901–908


