THE ROLE OF THE GENETIC ABNORMALITIES, EPIGENETIC AND microRNA IN THE PROGNOSIS OF CHRONIC LYMPHOCYTIC LEUKEMIA

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Chronic lymphocytic leukemia (CLL) is increased proliferation of B-cells with peripheral blood and bone marrow involvement, which is usually observed in older people. Genetic mutations, epigenetic changes and miRs play a role in CLL pathogenesis. Del 11q, del 17q, del 6q, trisomy 12, p53 and IgVH mutations are the most important genetic changes in CLL. Deletion of miR-15a and miR-16a can increase bcl2 gene expression, miR-29 and miR-181 deletions decrease the expression of TCL1, and miR-146a deletion prevents tumor metastasis. Epigenetic changes such as hypo- and hypermethylation, ubiquitination, hypo- and hyperacetylation of gene promoters involved in CLL pathogenesis can also play a role in CLL. Expression of CD38 and ZAP70, presence or absence of mutation in IgVH and P53 mutation are among the factors involved in CLL prognosis. Use of monoclonal antibodies against surface markers of B-cells like anti-CD20 as well as tyrosine kinase inhibitors are the most important therapeutic approaches for CLL.

Key Words: chronic lymphocytic leukemia, genetic mutation, epigenetics.

INTRODUCTION

Chronic lymphocytic leukemia (CLL) involves increased proliferation of monoclonal B-cells that express CD19, CD5 and CD22 antigens and show reduced expression of CD23, CD79b as well as surface IgM and IgD. The most important genetic abnormalities in CLL include 11q22-23, 17p13 and 6p21. Although the genes involved in these abnormalities have not been detected yet, p53 and ATM appear to be involved in 17p13 and 11q22-23 deletion, respectively [1, 2]. In fact, CLL is a common neoplasm of CD5 B-cells involving peripheral blood, bone marrow, lymph nodes and other lymphoid tissues. It is the most common leukemia in man and accounts for approximately 30% of leukemia types. It is mainly observed in older people with variable prognosis and an average age of 70 years upon diagnosis. Unlike many hematologic malignancies that are characterized by chromosomal translocations, B-cell chronic lymphocytic leukemia (B-CLL) typically involves chromosomal deletions. Studies show that over 80% of patients have genetic abnormalities: 14–40% have del 11q, 11–18% trisomy 12, 3–27% del 17q and 2–9% del 6q [3, 4]. 70–80% of CLL patients are diagnosed by complete blood count [5]. Both French and Spanish groups studying CLL have determined normal hemoglobin, low lymphocyte count, local bone marrow infiltration and lymphocyte doubling time longer than one year as good risk factors [6]. In another study, presence of non-mutated IgVH genes, overexpression of CD38, atypical lymphocyte morphology, trisomy 12, del 11p23 and loss or mutation of p53 gene were reported as poor prognostic factors [7]. Among these anomalies, deletion of the long arm of chromosome 17, which involves TP53 deletion, is associated with the worst prognosis, and the patients are subject to rapid progression of disease and do not respond to standard drugs [8]. The symptoms are variable but some markers like mutations in the variable heavy chain gene, the expression of surface ZAP70 (zeta associated protein 70) and various chromosomal changes can help in management of CLL patients. High and low levels of ZAP70 cause aggressive and indolent CLL, respectively. In a recent classification based on chromosomal changes, CLL has been divided to low risk in patients with a normal karyotype or 13q deletion, intermediate risk in pa-
patients with del 11q, trisomy 12 or del 6q and high risk in patients with del 17p or complex karyotype [9].

**Genetic abnormalities in CLL** (Table 1).

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Prognosis</th>
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<tr>
<td>IgVH mutation</td>
<td>Poor</td>
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<tr>
<td>13q deletions</td>
<td>Good</td>
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<tr>
<td>6q deletion and 11q deletion</td>
<td>Poor</td>
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<tr>
<td>CD38 overexpression</td>
<td>Poor</td>
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<td>Increased ZAP70 expression</td>
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**IgVH mutation.** Nearly 50–70% of CLL patients show evidence of hypersomatic mutations in variable region genes of immunoglobulin heavy chain (IgVH). In these patients, leukemic cells originate from cells in post germinal centers in which IgVH hypermutation has occurred [10, 11]. IgVH mutation is a common mutation associated with poor prognosis, and can play a role in determining the clinical status of patient and disease progression in addition to other factors. The disease is usually more malignant and in a progressed stage, the survival is shorter and the lymphocytes are more atypical in patients in whom IgVH is unmutated [12]. Patients with unmutated IgVH and increased CD38 expression show worse symptoms and clinical presentations relative to patients with mutated IgVH and a low expression level of CD38. Therefore, CD38 expression level and IgVH mutation status can be helpful prognostic factors in CLL patients [13]. Studies have shown that CD38 expression can also specify IgVH mutation status. IgVH is unmutated in cases with CD38 overexpression, and IgVH is mutated in cases with decreased CD38 expression [14]; however, lack of correlation between the two has also been reported [15]. Because some of studies showed correlation and other studies did not confirm this correlation. We can not say this is a definite correlation.

**13q deletion.** Deletion of 13q14.3 is the most frequent chromosomal abnormality in B-cell CLL. Deletions at 13q14.3 are associated with the longest survival. It involves the deletion of several genes like BCMS, ALT1, CAR, CLLD6, KPN13, CLLD7, CLLD8 and LOC51131. Therefore, deletion of these genes could be involved in pathogenesis of this disease [16]. Moreover, a small gene called deleted in leukemia gene has been detected in this region and adjusts the proliferation of B-cells via BCL2 regulation [17]. 13q deletion is monoallelic in 76% and biallelic in 24% of cases [17]. Del 13q14 status is usually determined by FISH while other methods such as array comparative genomic hybridization, single nucleotide polymorphism analysis, Northern blot or quantitative polymerase chain reaction can be used to determine the deletion status [18]. 13q deletion occurring in CLL is heterogeneous in size; therefore, probably more than one tumor suppressor gene is located in del 13q14 locus, which is involved in biological and clinical characterization of CLL with del 13q14 deletion [19]. Correlation between the percentage of deleted 13q in the nucleus and CLL prognosis has been demonstrated. Clinical symptoms are mild and disease progression is slower in small 13q deletions when RB1 locus is not involved while the symptoms are severe and disease progression is higher in cases with higher 13q deletion involving RB1 locus [20].

**6q deletion and 11q deletion.** Deletion of the long arm of chromosome 6 (6q) is one of the chromosomal aberrations in CLL, which is observed in 6–9% of cases [21–23]. This disorder most often presents as 6q21 and is usually associated with t(14–18) q32p21, and is observed in some prolymphocytic leukemia and acute lymphoblastic leukemia cases [21, 24]. Clinical analysis shows high white blood cells count, large lymphocytes in peripheral blood as well as extensive lymphadenopathy in this type of disorder [25]. The results also show better response to treatment relative to other mutations [25, 26]. Deletion of the long arm of chromosome 11 (11q) is observed in 8–19% of CLL cases. Lack of 11q suggests the presence of a tumor suppressor gene in this locus, which is associated with extensive nodular progress, rapid disease progression and shorter survival [9, 22, 24, 27–30].

**P53 mutation.** P53 is located on chromosome 17 next to p13.1. It is a transcription factor involved in cell cycle arrest and induction of apoptosis in damaged cells [31]. P53 mutation tends to decrease P53 expression in several lymphoid disorders. P53 gene is expressed on average in 15% of CLL cases, mainly B-CLL. These mutations mostly represent a single allelic loss of the variable region in short arm of chromosome 17. Evidence suggests that the loss of or change in this protein may disrupt the regulation of growth features in cancer cells [31, 32]. The risk of death in patients with P53 mutation is thirteen times that of patients lacking it, because of possible involvement of this protein in the pathogenesis of the more aggressive forms of CLL. There were no significant differences in age, sex, absolute lymphocyte count, or lymphocyte doubling time between P53-positive and -negative patients. By contrast, P53-positive patients had a significantly higher percentage of prolymphocytes and a significantly lower percentage of residual CD3-positive T lymphocytes [31].

**ZAP70 expression.** ZAP70 is a member of syk-ZAP70 tyrosine kinases family normally expressed in T-cells and NK cells, which plays an important role in triggering signal transduction in T-cells [33]. Studies have indicated increased expression of ZAP70 in CLL patients, which is associated with IgVH mutations. When IgVH is not mutated, ZAP70 is increased but it is decreased when IgVH is mutated [34]. Therefore, increased ZAP70 can be considered as a factor of poor prognosis.

**CD38 expression.** CD38 expression varies during the development of B-cells, so that its expression is highly increased in bone marrow precursor cells, is decreased in resting mature B-cells and is then re-expressed in plasmocytes [35]. CD38 is a multifunctional protein member of growing number of molecules acting independently as an ectoenzyme and...
receptor [36]. CD38 overexpression can worsen CLL prognosis and cause progression of disease course, resulting in severe symptoms.

The role of miRs in CLL (Table 2). miR-15a and miR-16a are located on chromosome 13q14 and are deleted in over 50% of CLL cases. Therefore, 13q14 deletion, which is observed in 68% of CLL cases as well as decreased expression of miR-15a and miR-16a can be involved in pathogenesis of B-CLL [16]. Decreased expression of miR-15a and miR-16a as well as increased expression BCL2 seems to be the main selective mechanism involved in pathogenesis of several B-CLL types [37, 38]. It has also been shown that miR-15a and miR-16a cause apoptosis of ME6.01 cells via activating intrinsic pathway of apoptosis [39, 40]. Additionally, in many cases of CLL, miR-15a and miR-16a, the tumor suppressor role of which has been indicated in vivo, are decreased [41]. miR-29 and miR-181 target the TCL1 gene and reduce its expression [42–44]. TCL1 is a molecule that plays an important role in pathogenesis of CLL; it has been shown that increased TCL1 expression can lead to progressive CLL [45, 46]. Studies have shown that miR-29 expression is 4.0 to 4.5 times higher in indolent CLL patients than normal B-cells. In addition, miR-29 level has been decreased in cases with 11p deletion, which usually indicates progressive CLL [47, 48]. Increased expression of miR-29 has been demonstrated not to be sufficient to develop progressive CLL but overexpression of TCL1 is positively required to start the progressive form of CLL [49]. miR-146a is mainly involved in negative regulation of acute responses during the activation of innate immune system. Changing expression of miR-146a has been observed in inflammatory diseases and cancer. miR-146a can function as a tumor suppressor, and inhibition of its function can cause tumor growth and metastasis. A wide range of miRs were used in search for diagnostic markers in CLL, and observed that miR-146a is increased in CLL with trisomy 12 compared to other CLL varieties. This suggests that increased expression of miR-146a is associated with tumorigenesis via dysregulation of inflammatory responses. miR-146a as well as several other molecules plays a role in cell migration, motility and adhesion in CLL with trisomy 12. miR-146a over expression is associated with increasing encoding of integrin genes ITGA4 and ITGB2. These two genes show heterodimer subunits of αLβC and α4β1, which is involved in TEM and mobility of malignant cells during tissue invasion in CLL [50–52]. Downregulation of miR-34a and miR-17-29 is observed in CLL with tp53 disorder. Low level of miR-34a has been observed in CLL similar to complete absence of tp53. miR-34a has been shown to function as a regulator of p53 gene. A strong correlation has been observed between 17p mutation in tp53 and decreased miR-34a levels. A strong correlation has also been observed between disrupted DNA damage responses, apoptosis and presence of disease associated with Fludarabine resistance. Finally, it has been observed that miR-34a plays an important role in response to DNA damage and thus CLL therapy [53, 54].

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<th>miR</th>
<th>Target</th>
<th>Prognosis</th>
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<tr>
<td>miR-15a</td>
<td>Decreased expression BCL2</td>
<td>Good</td>
</tr>
<tr>
<td>miR-16a</td>
<td>Decreased TCL1</td>
<td>Good</td>
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<tr>
<td>miR-146a</td>
<td>Tumorigenesis via dysregulation of inflammatory responses</td>
<td>Good</td>
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<tr>
<td>miR-34a</td>
<td>Function as a regulator of p53 gene</td>
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The role of epigenetics in CLL. Epigenetic changes include DNA methylation, nucleosome remodeling and histone modifications (acetylation, methylation, ubiquitination), which alter the level of translation in a number of genes. Some of these changes are heritable and reversible, changing the pattern of gene expression in certain genes without changing the DNA sequence [55, 56]. These changes play an important role in embryonic development, stem cell biology, cell growth and differentiation [57–59]. Dysregulation of these changes can lead to several disorders and diseases [60]. Genetic and epigenetic studies on CLL have boosted our understanding of CLL and have provided further outlooks for diagnostic and therapeutic strategies [61].

DNA methylation. DNA methylation in mammalian cells is limited to CPG dinucleotides with multiple clusters called CPG islands [62]. In general, promoter methylation prevents the translation of gene promoter via promoter inhibition, and different diagnostic subgroups of CLL have been detected by methylation profiles [63]. CPG methylation in DNA is mediated by DNA methyltransferase 1 (DNMT1). In cancer cells, DNMT1 expression is increased, and methylation of genes, including tumor suppressor genes, is increased [61]. Activation of proto-oncogenes through hypomethylation of promoters is a common change in CLL, and in contrast, hypermethylation of promoter region plays a key role in silencing of tumor suppressor genes [64]. Hypermethylation of P16 INK4A and P15INK4B gene promoters has been reported in a number of CLL patients [65]. Recently, it was found that epigenetic changes such as CPG methylation play an important role in the formation of progenitor stem cells, cancer progression and metastasis [66]. Therefore, regulation of DNMT1 in cancer cells is essential for a better understanding of carcinogenesis. Melki et al. [67] compared CLL with a deficiency disease in maturation and proliferation of lymphocytes. They showed that the expression level of DNMT1, DNMT3a and DNMT3b is higher in leukemic cells compared to normal cells [67]. BCL2 gene, which is an anti-apoptotic gene subject to increased expression in CLL, is one of the molecules undergoing hypomethylation in CLL patients [68]. In addition, drug resistance gene of MDR1 [69] and NF-κB activator of TCF1 are subject to upregulation and hypomethylation in CLL [70]. E-cadherin is among the first promoters undergoing hypomethylation in CLL [71],
which has been recognized as a metastasis suppressor of solid tumors. BTERT promoter methylation of telomerase enzyme reduces the activity of telomere and shortens the telomere length [72]. TWIST2 transcription factor gene and P53 suppressor are also methylated in CLL patients [73]. ZAP70, an intracellular tyrosine kinase and a prognostic factor in CLL, is methylated in CLL [74]. HOXA4 is another molecule methylated in CLL, which expresses HOXA Gene. HOXA4 is a component of HOXA gene complex, which is considered a transcription factor for cell growth altered in lymphocytic malignancies [75, 76]. In 2007, the importance of DAPK1 (Death-associated protein kinase 1) methylation was specified in CLL patients. It was shown that DAPK1 methylation results in DAPK1 silencing, which is a pro-apoptotic gene detected in nearly all cases of sporadic CLL [77, 78]. Activation of WNT signaling pathway, the main pathway in B-cell development with continuous activity in CLL, has been indicated to be associated with hypermethylation of WNT inhibitor genes. Methylation in seven WNT inhibitor genes has been studied, including WIF1, DKK3, APC, SFRP1, SFRP2, SFRP4 and SFRP5 [79–81].

Global patterns of aberrant methylation in CLL. The character of DNA methylation in a CLL gene is clinically and biologically important by affecting different functional genes. Methylation patterns are determined by capillary electrophoresis-laser and HPLC. It was demonstrated that CLL patients with high methylation index need more extensive treatment compared to patients with lower methylation index [82]. Aberrant methylation downregulates non-coding miRs in CLL. For example, 13q deletion, in which tumor suppressor miR-15a and miR-16-1 are involved, leads to upregulation of BCL2 and reduced apoptosis [16]. Pallasch et al. [83] showed that downregulation of miR promoter is associated with decreased expression of miR, which is methylated in several acute lymphoblastic leukemia cases in comparison with normal B-cells. miR-139 and miR-582 promoters are methylated in CLL promoters. Hypomethylation of miR-21, miR-29a, miR-34a, miR-155, miR-574 and miR-1204 leads to increased expression, and hypermethylation of miR-708, miR-551, miR-9, miR-124 results in a decreased expression [84]. miR-29a family, including miR-29a, miR-29b-1, miR-29b-2 and miR-29c as well as miR-181 family are subject to decreased expression in CLL [43, 85].

Ubiquitination. Ubiquitination is a post-translational modification mediated by UPS (ubiquitin proteasome system), which regulates cellular processes. Ubiquitination involves a cascade transferring ubiquitin to protein substrate [86]. In humans, there are two E1 proteins, almost 30 E2 proteins and thousands of E3 ligase, and E3 transfers the specific substrate to UPS [87]. Many E3 ligases are involved in hematologic malignancies [88] and some act as oncogenes or tumor suppressors [89]. Proteolysis of important regulatory proteins is essential for cell cycle, translation settings, response to DNA damage and apoptosis, and their abnormal function leads to neoplastic disorders. Reduced levels of p27 are frequent in human cancers and have been associated with poor prognosis. Skp2, a component of the Skp1-Cul1-F-box protein (SCF) ubiquitin ligase complex, has been implicated in p27 degradation. Increased Skp2 levels are seen in malignant lymphoma [87]. Mdm2 targets p53 and retinoblastoma protein, two major tumor suppressor gene products, for ubiquitin-dependent degradation. SCF targets other tumor suppressor gene products and CDK inhibitors such as p130, Tob1, p27, p57, and p21. The stabilization of oncogene products and enhanced degradation of tumor suppressor gene products or DNA repair proteins might be associated with carcinogenesis and malignant progression, due to defects or the abnormal expression of their E3 ligases [89].

Acetylation. Acetylation is among post-translational modifications mediated by histone modifying enzymes increasing or decreasing histone acetylation [90]. Increased histone acetylation leads to increased gene translation but hypomethylation results in gene silencing. Epigenetic DNA inhibitors act as anti-cancer agents inhibiting cell cycle and causing apoptosis [91]. Histone deacetylase inhibitors lead to increased transcription factors and gene translation [90].

BAX is a pro-apoptotic gene that is silent in CLL but BCL2 is an anti-apoptotic gene overexpressed in CLL patients. CDK4 is a protein kinase active in G1–S phase, which is inhibited by cyclin-dependent kinase inhibitors. CDKN2A gene is a tumor suppressor, CDKN2A promoter is hypoacetylated in CLL cases but CDK4 promoter is hypermethylated, leading to increased CDK4 expression and decreased CDKN2A expression [92].

New treatments of CLL. CLL treatment is still challenging, and the disease is incurable in many cases. The most important CLL treatments include purine analogues like fludarabine and pentostatine, polychemotherapy such as fludarabine with cyclophosphamide or cyclophosphamide, prednisone and vincristine, treatment with monoclonal antibodies such as alemtuzumab (anti-CD52), rituximab (anti-CD20) or ofatumumab (anti-CD20). Ofatumumab is a human monoclonal antibody detecting a distinct epitope of CD20. It has been evaluated to be used for treatment of CLL patients resistant to fludarabine and alemtuzumab [93].

In another study, CD47 agonists were used to overcome drug resistance. Studies show that targeting CD47 with peptides derived from the second C-terminal thrombospondin domain not only effectively kills malignant cells in CLL, including those with TP53 disorder, but also induces cell death via caspase independent pathways [94]. Dinaciclib is a CDKi of choice for treatment progress in refractory and recurrent CLL cases, and could enable a clinical trial for CLL. In another study, fludarabine, cyclophosphamide and rituximab were used to treat relapsed CLL as chemomunotherapy, in which improvement
CONCLUSION

In pathogenesis of CLL many factors are involved including genetic abnormalities and translocations (IgVH mutation, 13q deletions, 6q deletion, 11q deletion), CD38 overexpression, increased ZAP70 expression, miR and epigenetics.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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