REVIEWS

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Somatic Mutation in Immunoglobulin Gene Variable Region in Patients with Chronic Lymphocytic Leukemia and Prognostic Impact

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ABSTRACT

Chronic lymphocyte leukemia (CLL) is the most common leukemia in elderly individuals that is accompanied by the presence of CD5/CD19/CD20/CD23 positive, FMC7 negative and reduced levels of surface membrane Immunoglobuline (IgM & IgD) and CD79b on B lymphocytes in the blood, bone marrow and lymph nodes. Remarkably, based on the mutational IgVH status, B-CLL cases can be subdivided into two prognostic groups: the first one with presence mutation (>2%) in VH gene and second one with absence mutation (≤2%) in VH gene that is the mutation of IgVH status, affected on progression and overall survival of CLL patients. Correspondingly, also the expression level of several markers is correlated with the mutation of IgVH status and prognosis of CLL disease. In this review we focused on relation between mutation IgVH status and CLL prognosis and overall survival and also some cellular markers which may affect CLL clinical status.

Keywords: CLL, mutation IgVH status, prognosis factor, overall survival

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Introduction

B-cell chronic lymphocytic leukemia (CLL) is characterized by progressive accumulation of malignant B cells in the bone marrow, lymphoid tissue and blood circulation which have suppressed the anti-tumor immune responses and escaped from programmed cell death (apoptosis) in vivo[1,2]. It is one of the most common blood malignancies in elderly individuals in the United States, and it is estimated 14,620 new cases which were diagnosed and estimated 4,650 deaths which is reported by the American Cancer Society in 2015. Although its prevalence in Asia and the Middle East in the past decade was not impressive, but the recent studies have indicated and confirmed the increasing prevalence of CLL[3,4]. Because of the heterogeneity of this disease, especially in clinical manifestation, it is classified into two subtypes: indolent CLL and aggressive CLL. However, one-third of patients diagnosed with CLL can be expected to survive more than 10 years without treatment, whereas some patients will die less than 1 year after diagnosis[5,6]. In this way, the prognosis of CLL is different in indolent and aggressive form and the overall survival in indolent form is more than the aggressive form. Furthermore, disease progression will be impacted on clinical approaches. Nowadays CLL diagnosis is based on the peripheral blood mononuclear cells (PBMC) phenotype and physician's examinations. The several factors such as molecular, genomic variables and cell phenotyping markers have been implicated in the prognosis of CLL disease. Correspondingly, advances in the identification the genomic and molecular researches have led to better understanding prognostic factors for this disease[7, 8]. One of the most important differences between malignant and healthy cells is the mutation status of variable region genes of immunoglobulin's, especially heavy chain variable region which is known as IGVH region. The mutation in IgVH genes is a substantial prognostic factor in CLL patients for classification and survival rate prediction[9, 10]. Relatively, in normal individuals the IgVH genes have used of hyper mutated, but this pattern of mutation status in variable heavy regions in B lymphocytes in CLL is very different, it is unmutated or the clones of malignant cells have one variant of mutation which is known malignant B cells stereotyped[11]. Mutations of IgVH genes are found by comparing DNA sequences of the genes in B cells with corresponding genes in the germ line. DNA sequences of the genes in B cells differing by >2% from its germ line counterpart are defined as mutated form[12]. Therefore, the CLL patients is divided into two main groups: the first group is "mutated" CLL that B cells have rearranged VH genes with >2% mutations; second group is "unmutated" CLL that there are no mutation (or ≤2%) in VH genes[13, 14]. In this review, the most recent achievements have been discussed and events have discussed and challenged focusing on the prognostic role of IGVH mutation status.

B cell development and origin of B-CLL:

In this account, the onset of B cell maturation has occurred in hematopoietic stem cells as lymphoid progenitor in bone marrow. In CLL patients, the HSCs are susceptible to produce a higher number of pro-B cells. The immunoglobulin variable heavy chain rearrangement has begun in pro-B cells and to the ongoing in pre-B cells stage. The recombinase-activation gene (RAG1 and RAG2) and terminal deoxytransferase are necessary enzymes for the rearrangement. In addition, several transcriptional factors such as E2A and EBF is required for RAG1 and RAG2 expression[15].

Several studies showed that the marginal zone B cells as the origin of CLL, because of the marginal zone B lymphocytes have cell surface markers similar to B-CLL (CD5+, CD23+, CD27+, low Ig) and marginal zone B cell usually express VH1-69 and VH4-34[16, 17].

Also human B1 cells have a phenotype same as B

cells of CLL patient. Normal human B1 cells express CD5+ as a surface marker and ZAP-70 and ILT-3. In addition, B1 cells use different IgVH mutation status that has seen in CLL disease. Thus, these are the reasons for transitions of normal B1 cells to leukemic B cells with umutated IgVH in CLL[18].

Immunoglobulin genes in CLL:

In the first line of CLL studies, the researchers have believed naïve B lymphocytes transformation to leukemic B cells and had not exposure to antigen and mutation somatic. Since the 1990s, the first studies had done on the immunoglobulin (IG) gene usage restriction in chronic lymphocytic leukemia. However, it was later found that about fifty percent of the CLL patients showed somatic mutation in the variable region of their Ig genes[19, 20]. In 1998, Chiorazzi et.al, reported that the genomic content of IGVH loci in CLL patients is different in comparison with normal CD5+ B cells. In B cells of CLL mostly used of VH1, VH3 and VH4 genes family; however, the VH1 gene family is most frequently express than VH3 gene family in CD5+ B cells. Certain genes of these families such as IgVH1-69, IgVH4-34, IgVH3-23 and IgVH3-07 is over selected in CLL[20]. The presence and severity of clinical courses is associated with the use of these genes family. In 1999, Hamblin and colleagues[10] and Damle and colleagues[9] demonstrated that the IgVH gene somatic mutation status on CLL prognosis has played important role. Transparently, CLL patients without somatic mutation in IgVH genes had more malignant disease and aggressive clinical course and resistance to the therapy, also shorter overall survival than those with somatic mutations. Thus, the CLL disease based on the presence (M-CLL) or absence (U-CLL) of somatic hypermutation on VH genes can be divided two subsets[21].

In U-CLL patients, VH1 family especially VH1-69 subfamily is most frequent and the 40% of cases are IgVH1-69, IgVH4-39 and IgVH1-2 genes were used. The remarkable note for this variation of VH1 family is more CMV and HIV susceptibility in CLL patients, in contrast that the IgVH3 (VH3-21) family genes almost 60% of all M-CLL observed[22-24].

The IgVL genes repertoire such as Vκ O12/2 and A27 and Vλ3h and 3r are most frequently expressed in B cells of CLL, while the expression of these VL genes repertoire in B-CLL cases is similar normal B cells and had not play a major role in CLL prognosis[25].

BCR stereotypy:

The novel and recent studies have determined that the CLL subgroup using the IgVH gene and VH3-21 with identical variable heavy chain particularly in CDR3 region, causes the production of the distinct BCR without or little variation in patients with CLL[26, 27]. Relatively, these unique features of BCRs were referred to as "stereotyped" and other definition is "something conforming to a fixed or general pattern"[28]. More recent studies that indicated several significant features of stereotyped BCRs includes:

1. Frequency of BCR stereotypes in CLL patients is more than 25% cases or ¼ in cohort studies. Also BCR stereotypes is more frequently in unmutated CLL (more than 40%), however in the mutated CLL (only ~10%) also observed[29, 30].
2. The most common BCR stereotypes in CLL are recognized in IgVH1, IgVH4 and IgVH3 subset genes. On the other hand, the BCR stereotypes is frequent exceed 30% in CLL cases using certain IgVH gene: IgVH3-21, IgVH1-69, IgVH1-2, IgVH1-3, IgVH4-39 and IgVH3-48, whereas BCR stereotypes is low for other IgVH genes (<5%) such as IgVH3-7, IgVH3-74 and IgVH2-5[29].
3. The comparison of CLL heavy CDR3 sequences to a large panel of public database sequences from non-CLL B cells of diverse sources demonstrated that <1% non-CLL clones carry HCDR3s homologous to one of the reported 'CLL-biased' subgroup[29].
4. Recent study in Mediterranean-Scandinavian

which was performed on CLL patients with 2.7% CLL cases that used certain IgVH genes such as IgVH1-2, IgVH1-3, IgVH1-18 and IgVH1-8 and all of these cases used certain stereotyped of kappa light chain. Thus, using the certain VH CDR3 and VL CDR3 is highly frequent in CLL repertoire[30]. CLL immunophenotype and IGVH mutation status: The CLL immunophenotypes are necessary aspects in CLL diagnosis; there are essential markers for malignant cells detection and CLL staging. The malignant B cells in CLL patients express CD5, CD19, CD20, CD22, CD23 and a weak expression of surface immunoglobulins, CD79b and FMC7[12]. CD5+ and CD23+ marker has diagnostic role in CLL assessment. CD5+ is T and B lymphocytes marker and normal CD5+ B lymphocytes are localized in germinal center or in the mantle zone of secondary follicles. The analysis of mutation IgVH gene in normal CD5+ positive cells, cord blood isolated B cells and malignant CLL B cells showed that malignant B cells gene expression is highly similar to human naïve CD5+ B cells and prenatal B cells population[31]. In addition, CD27 expression level is related to IGVH mutation status. The mutation IgVH status in normal CD5+/CD27+ is better than CD27-/CD5+ cells. Recent studies showed that the CD27+ B cells of CLL patients have used mutated IgVH genes according to one stereotype but CD27- B cells of CLL patients have unmutated IgVH genes. CD27 is one of the most important elements in lymphoid progenitor's development has led to an increasing diversity of B cells and T cells receptors and expressed in pre-B and pre-T cells[32].

Biological & mulecular markers and mutation IgVH status:

Several biological parameters such as serum thymidine kinase (s-TK), lipoprotein lipase (LPL), β2-microglobulin (β2M) and soluble CD23 (sCD23) have an important role in the prognosis and survival of CLL disease[33]. In addition to routine CLL diagnostic methods, some molecular markers can be important in disease prognosis determination. There are some molecular markers which have correlation between IgVH mutation status[34].

The serum thymidine kinase, thymidine kinase is a cellular enzyme that has a functional role in cell division and DNA synthesis. The high level of serum thymidine kinas is associated with poor prognosis in patients with CLL. Several studies reported the relationship between IgVH mutation status and level of serum thymidine kinase in CLL progression. These findings showed that the level of s-TK detected by chemiluminescence that the level of s-TK was >15 U/L[6], these result from CLL patients was higher than normal subjects ($P<0.05$). Moreover, patients with unmutated IgVH genes had higher level of serum thymidine kinase ($P=0.030$), thus the high level of s-TK in CLL patients is correlate with unmutated IgVH genes[35].

The lipoprotein lipase is water soluble enzyme that hydrolyzes triglycerides to one fatty acid in lipoprotein. This enzyme is produced in many tissues. The high expression of LPL significantly associated with disease progressive that is strongly accompanied with unmutated IgVH gene ($P=0.010$) in patients with CLL[35].

The CD23 is a glycoprotein receptor for IgE on surface B lymphocyte. This is unstable and shedding to serum, thus the soluble form is created (sCD23). In recent study, the sCD23 doubling time (sCD23DT) is a prognostic factor for CLL patients. Patients with unmutated IgVH that sCD23DT is less than 1 year, the overall survival is short (83 months) than CLL patients with mutated IgVH and sCD23DT is more than 1 year (overall survival: >177 months)[36].

Zap70: The lymphocytes receptors related to the adaptors like LCK, SYK, FYN have important role

in signal transduction and function of the cells. Relatively, any dysregulation in expression level of signaling molecules can change the tumor microenvironment. Zeta-chain (TCR)-associated protein kinase 70 kD (Zap70) is one of the SYK related adaptors in T cells which has important role in T cells activation and initiation of responses. Zap70 expression level is raised in CLL especially in poor stages of binet staging (defined as ZAP-70 cut off ≥20%), thus CLL patients with <20% expression of Zap 70 have a good prognosis[37, 38]. The mutation status of malignant cells in comparison with germline in Zap70 positive cells has mostly reported unmutated in cases of CLL[39]. Previous studies indicated that the Zap-70 and CD38 are usually positive in cases of CLL with unmutated immunoglobulin variable region genes (IgVH) and may be used to predict IgVH mutation status and prognosis[38]. In Kern et.al study the expression level of Zap-70 was analyzed with flow cytometry on 1229 patients. The results showed that Zap-70 expression in patients with poor staging increased and IgVH mutation status was poorly prognostic which correlated with chromosomal abnormalities[37].

CD38 expression: CD38 is a glycoprotein from ribose transferase family which has expressed on B, T, NK and other lymphoid and myeloid cells. It has an important regulatory role in proliferation, homing, chemotaxis and cell migration which is can change the surrounding malignant cells microenvironment [40]. In the patients with CLL using the threshold of ≥30% for CD38 is defined as high level of CD38 expression on B cells[41]; moreover, several studies reported that ≥20% as a cut off value for defining CD38 in CLL cases[42]. Considerably, the CD38 expression of 30% or more is a strong predictor for unfavorable prognosis and worse overall survival in CLL patients[43]. In previous study, in unmutated CLL the CD38 expression level (defined as ≥30%) is higher than mutated CLL. The continuous treatment or >2 regimens is necessary for CLL patients with unmutated IgVH and CLL cases with ≥30% CD38, while mutated IgVH and <30% CD38 do not need chemotherapy or very little therapy[9].

AID: Activation-induced cytidine deaminase (AID) is the most responsible molecule in somatic hyper mutation and class switch DNA recombination; It's expression is restricted and limited in CD19+/CD38+ B cells of germinal center and it has an important role in B cell maturation in normal individuals[44, 45]. Frequent studies have shown a strong positive correlation effect of AID over expression on IgVH genes rearrangement. In one study, the twenty two of twenty five unmutated IgVH patients (88%) had AID mRNA expression. But of 32 cases mutated patients, in 15 cases (47%) the AID could be detected. The gene expression analysis results showed that the AID expression level in U-CLL patients was more than M-CLL and it has a correlation between patients staging, so the AID expression level can be a prognostic factor in U-CLL patients[46].

IRF4/MUM1: The interferon regulatory factor 4 (IRF4) also known as MUM1[47] is a transcription factor that expressed in immune cells like lymphocytes, dendritic cells and macrophages, which has variety function such as proliferation, differentiation and apoptosis[48, 49]. In last study, the IRF4 expression was considered as a prognostic factor in B-CLL. However, all CLL patients with the IRF4 mutation have an unmutated IgVH status and have poor prognosis[50].

Prognosis of IgVH mutation status:

Nowadays, the CLL patients classified into two groups; first group is the malignant cells belong to mature B cells which have passed the germinal center maturation. The IgVH mutation status in these cases are mutated and CD38 expression is

<30% and also ZAP-70 expression is <20%, these patients have a good prognosis of disease and known as indolent cases and overall survival is longer than second group; the median overall survival in these cases were 24 years or not reached. In other group (atypical CLL) the malignant cells are immature B cells which have trisomy in chromosome12 and CD38 expression is positive (≥30%), ZAP-70 expression is positive (≥20%), the IgVH mutation status in these type of malignant cells is unmutated and these cases are aggressive and prognosis of disease is poor. This group has median overall survival of 9-10 years. Therefore, the VH mutation status has been shown to be stronger prognostic predictors in multivariate analyses[43, 51, 52].

Conclusion:

Conclusively, one of the most important prognostic factor which is a remarkable strong predictor for CLL disease, is IgVH mutation status. Correspondingly, enough information about IgVH mutation status has led to the progression of the CLL research and also cellular origin of CLL. Deep understanding of correlation between IgVH mutational status and other prognostic factors can cause a new therapeutic strategy.

Conflict of interest:

The authors declare no conflict of interest.

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