Brief report

Distinct gene expression patterns in chronic lymphocytic leukemia defined by usage of specific VH genes

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The quantitative expression of 26 genes with central roles in BCR signaling/activation, cell cycle, and apoptosis were involved in B-cell receptor (BCR) signaling/activation, cell cycle, and apoptosis control in CLL using specific VH genes compared to VH mutated (VH-MUT) and VH unmutated (VH-UM) CLL not using these VH genes. V3-21 cases showed characteristic expression differences compared to VH-MUT (up: ZAP70 [or ZAP-70]; down: CCND2, P27) and VH-UM (down: PI3K, CCND2, P27, CDK4, BAX) involving several BCR-related genes. Similarly, there was a marked difference between VH-unmutated cases using the V1-69 gene and VH-UM (up: FOS; down: BLNK, SYK, CDK4, TP53).

Introduction

Chronic lymphocytic leukemia (CLL) with unmutated immunoglobulin variable heavy chain (VH) gene (VH-UM) displays a worse prognosis compared to VH-mutated CLL (VH-MUT). Higher expression of ZAP70 (or ZAP-70), a receptor-associated tyrosine kinase, was identified in VH-UM compared to VH-MUT CLL. B-cell receptor (BCR) cross-linking on VH-UM compared to VH-MUT CLL led to increased tyrosine phosphorylation of p72 (SYK), indicating an increased activation after BCR stimulation as a potential mechanism accounting for the clinical differences of the VH mutation subgroups.

CLL subsets with highly restricted BCR structure were identified, indicating a role for specific antigens in CLL pathogenesis. The V1-69 gene is the most common unmutated VH gene in CLL and is associated with a restricted VDJ structure that is distinct from the repertoire of normal B cells. Similarly, V3-21 gene usage comprises cases with a highly specific BCR structure as evidenced by homologous CDR3 sequences and a restricted VL gene usage. Moreover, these cases show a poor clinical outcome regardless of the VH mutation status, indicating an independent pathogenic and prognostic role of specific VH gene rearrangements.

To investigate the role of antigenic stimulation in the pathogenesis of CLL subgroups defined by specific VH genes, we analyzed the quantitative expression of 26 genes with central roles in BCR signaling, B-cell activation, cell cycle, and apoptosis control in cases defined by V3-21 and V1-69 usage as compared to VH-MUT and VH-UM CLL not using these VH genes.

Study design

Peripheral blood samples from patients with untreated CLL diagnosed according to established criteria were included after informed consent was obtained in accordance with the Declaration of Helsinki. These studies have been approved by the institutional review board of the University of Ulm. A non–CD19-purified cohort of 69 cases constituted the following subgroups: V3-21 usage, 16 cases (9 mutated, 7 unmutated); V1-69, 17 (all unmutated); VH-MUT (not using V3-21), 17; VH-UM (not using V3-21 or V1-69), 19. A CD19-purified cohort included 53 cases (30 overlapping with the nonpurified cohort): 8 using V3-21 (5 mutated, 3 unmutated); 12 V1-69, 12 were VH-MUT (not using V3-21); 21 VH-UM (not using V3-21 or V1-69). Distribution of age, sex, Binet stages, and the high-risk aberrations del11q22-23 and del17p13 within the cohorts and subgroups is detailed in Supplemental Table S1 (see the Supplemental Tables link at the top of the online article, at the Blood website). Preliminary data of our group implicate a specific biology for CLL using V3-21,15 However, these results were not reproduced independently and no specific VDJ configuration has been observed in these cases. Because of these uncertainties and to avoid potential interferences, we excluded V3-23 cases from the study. Fluorescence in situ hybridization (FISH) analysis and VDJ sequencing were performed as described.16,17 VH homology cut-off was 98%. CD19 purification was carried out using clonal antibodies recognizing the CD19 and CD20 antigens.
magnetically activated cell sorting (MACS) CD19 MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany). Twenty-six candidate genes were analyzed (Table 1), of which ZAP70, LCK, FOS, E2F1, BCL2, and BCLXL showed an overexpression in the CD19+ as compared to the CD19- fraction of patient (n = 3) or healthy donor (n = 3) samples. These genes were therefore analyzed in the CD19-purified cohort. RNA extraction, cDNA synthesis, and real-time quantitative polymerase chain reaction (RQ-PCR) were performed as described18,19 with 3 housekeeping genes for expression normalization (PGK1, LMBN1, ACTB). Primers for SYBR Green detection were used or designed as described19 and as shown in Table S2. We used a closed testing procedure20 together with the step-down maxT method21 using nonparametric test statistics with bootstrap estimated null distributions to analyze gene expression differences between VH-MUT, VH-UM, and V3-21. For the analysis of VH unmutated CLL with versus without usage of the V1-69 gene adjusted P values were computed by the step-down maxT procedure using Wilcoxon rank-sum statistics. For both analyses, a global significance level of .025 was used to control an overall significance level of 5%. Hodges-Lehmann estimates of log2-transformed expression data served to compute fold-change estimates and their 95% confidence intervals (CIs). The purified and nonpurified cohorts were analyzed separately. All statistical computations were done using R, version 2.1.1.22

Results and discussion

In line with previous studies,3,4 a higher ZAP70 expression was identified in VH-UM as compared to VH-MUT cases (Table 1; Figure 1). In addition, VH-UM showed a characteristic overexpression of PI3K and CCND2, for both of which a BCR-dependent up-regulation has been demonstrated experimentally.23,24 This finding is in line with Chen et al3 demonstrating that ZAP70 expression is associated with increased BCR signaling and reinforces the concept of ongoing BCR stimulation as a pathomechanism in VH-UM CLL.5,7

The V3-21 cases under study showed the characteristic distribution into VH mutated and VH unmutated cases with a median VH homology of 97.86%; half of them exhibited the characteristic CDR3 region of 7 amino acids; Table 1, Figure 1). In VH-UM cases, significant CDR3 region homology was observed in 97.86% of cases; half of them exhibited the characteristic CDR3 region of 7 amino acids; Table 1, Figure 1). ZAP70 levels as compared to VH-MUT with similar expression levels in the different V3-21 subsets (VH mutated/unmutated/7-amino acid CDR3; Table 1, Figure 1). ZAP70 expression in V3-21 cases was comparable to VH-UM cases, strengthening the role of ZAP70 as a prognostic marker recognizing unfavorable CLL subgroups and pointing to a common pathomechanism involving differential BCR signaling in these subgroups as recently proposed for VH unmutated CLL.6,7 However, marked differences occurred when comparing V3-21 cases with VH-UM CLL including a characteristic down-regulation of several genes in V3-21 cases (Table 1). The lower expression of PI3K and CCND2 implicates a down-regulation of these BCR target genes despite antigenic stimulation and points to alternative pathways. Indeed, the down-regulation of candidate genes such as BAX and P27 (Table 1) suggests apoptosis impairment and reduced cell cycle
inhibition as additional pathomechanisms in V3-21 CLL, which is supported by a recent report.\textsuperscript{25} When comparing the mutated versus unmutated V3-21 subset, although restricted by low case numbers, no highly characteristic gene expression differences occurred (Table S4). Therefore, cases with V3-21 usage appear to represent a rather homogeneous biologic group independently of the VH mutation status.

V1-69 cases under study showed the previously described CLL-specific VDJ configuration,\textsuperscript{3,14} such as unmutated VH, long CDR3 lengths, and a biased usage of JH6 (Table S5) indicating antigen-specificity of the BCR. When comparing these cases with VH unmutated cases not using V1-69, a number of differentially expressed genes was detected despite the concordant VH mutation status including the BCR-related genes BLNK, SYK, and FOS (Table 1). This finding points to a distinct biology of the V1-69 subset among VH unmutated CLL, which might be explained by the involvement of a distinct antigen leading to antigen-specific BCR responses in V1-69 CLL cells. Additionally, V1-69 cases exhibited reduced levels of TP53, which was not due to a biased distribution of 17p cases (Table S1), and of CDK4 implicating cell-cycle deregulation and apoptosis impairment in these cases.

The concept of antigen-specific BCR stimulation is also supported by the characteristic gene expression differences that occurred between the V3-21 and V1-69 subgroups (Table S6).

Generally, the VH gene-specific gene expression pattern identified supports the concept of antigen selection, as already implicated by the highly restricted VDJ features of these cells, and a persisting antigen-dependence of the CLL cells with ongoing BCR-specific stimulation as a pathomechanism in these CLL subgroups.\textsuperscript{7,12} These findings strengthen the impact of VH gene usage on CLL pathogenesis and suggest a future role for epitope-specific therapeutic approaches in CLL.

Note added in proof. While this manuscript was under review, the results of high ZAP70 expression in V3-21 CLL could be confirmed by Krober et al.\textsuperscript{25}

References


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