NOTCH1 mutations in CLL associated with trisomy 12

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Two recent studies reported whole-genome sequencing of chronic lymphocytic leukemia (CLL) samples and found repeated mutations in the XPO1 and NOTCH1 genes. XPO1 was found mutated in 2.4% of cases, while NOTCH1 was found mutated in 12.2% or 15.1% of CLL samples. Here we report the results of sequencing of XPO1 and NOTCH1 in 186 CLL cases. Our results confirmed frequency of XPO1 mutations. However, we found only 5 NOTCH1 mutations in 127 IGVH unmutated/ZAP70+ CLL samples (4%), and one mutation was found in IGVH mutated/ZAP70+ CLL for a total percentage of 1.5%. Because 4 of 6 mutated samples also showed trisomy 12, we sequenced NOTCH1 in an additional 77 cases with trisomy 12 CLLS, including 47 IGVH unmutated/ZAP70+ cases. Importantly, we found 41.9% NOTCH1 mutation frequency in aggressive trisomy 12 CLL cases. Our data suggest that activation of NOTCH1 plays a critical role in IGVH unmutated/ZAP70+ trisomy 12 CLL. (Blood. 2012;119(2): 329-331)

Introduction

B-cell chronic lymphocytic leukemia (CLL) is the most common adult leukemia in Western societies.1 Genetic aberrations can be identified in the CLL samples of more than 80% of patients.2 CLL cases can subgroup into 2 major types, aggressive or indolent, which we define here as cases that express high levels of ZAP70 and unmutated IgH V region genes (IGVH), or low-to-negligible ZAP70 and mutated IGVH. The most frequent recurrent genetic alterations include deletion/inactivation of 13q14 (>50%), deletion of 11q22-23 (18%), trisomy of 12 (15%-18%), and deletion 17p (7%-10%).2 Two recent studies reported whole-genome sequencing of CLL samples and found 40 somatic mutations in 5 samples and 46 somatic mutations in 4 samples, respectively.3,4 Subsequent sequencing of larger numbers of CLL samples revealed NOTCH1 mutations in 18%-20% of IGVH unmutated/ZAP70+ CLL samples, but only in 4%-7% of IGVH mutated/ZAP70− CLln samples.3,4 One of these 2 reports also showed recurrent mutations in the XPO1 gene.4 These mutations were found in 4 of 165 CLL samples or in 2.4% of cases. All these mutations were found in IGVH unmutated/ZAP70+ CLL samples, and the percentage in this cohort was 4.6%.5 This gene encodes a member of the importin-β/ karyopherin-β family of nuclear transport factors, namely Xpo1, which mediates nuclear export of proteins and ribonucleoprotein.5 Xpo1 also is involved in the control of several cellular processes by controlling the localization of cyclin B and members of the MAPK pathway.6 NOTCH1 encodes a class I transmembrane protein functioning as a ligand-activated transcription factor.7,8 On ligand binding, Notch1 undergoes several proteolytic cleavages resulting in translocation of the Notch1 intracellular domain (ICN) to the nucleus where it plays an important role in cell differentiation, proliferation, and apoptosis leading to transcriptional activation of multiple target genes, including c-Myc.9 ICN contains PEST domain targeting ICN for ubiquitinylation and degradation.7,8 Almost all NOTCH1 mutations in CLL are represented by the 2 base deletion frameshift resulting in a truncated constantly active protein, lacking the C-terminal PEST degradation domain.3,4 In addition, one frameshift insertion and 2 nonsense mutations were observed, each resulting in truncated Notch1.

Methods

Sequencing

The study was carried out in accordance with the institutional review board protocol approved by The Ohio State University. CLL samples were obtained from 186 CLL patients enrolled in the CLL Research Consortium on written informed consent in accordance with the Declaration of Helsinki, including 127 IGVH unmutated/ZAP70+ and 65 IGVH mutated/ZAP70− CLL samples. For 6 of these patients, 2 time points were provided, for a total of 192 samples analyzed. The 2 time points represent different stages of the disease: the first time point was provided in a clinically indolent stage while the last time point was provided during the aggressive stage. Progression was determined by clinical parameters such as increase in spleen size, white blood count, and overall Rai stage. Aggressive status was defined as unmutated IGVH (>98% of homology to the germline), and >20% of ZAP70-positive cells. Indolent status was defined as mutated IGVH, and <20% of ZAP70-positive cells.10 DNA was extracted with the DNeasy Blood & Tissue Kit (QIAGEN). XPO1 and NOTCH1 mutations were determined by PCR amplification and sequencing of the coding XPO1 exons 15 and 16, and the last coding NOTCH1 exon which encodes the portion of the PEST domain. For amplification, we used high-fidelity advantage 2 polymerase master mix (Clontech). The primer sequences were: xpo15-16dir2: ttagaatgcttgaagtttct, xpo15-16rev2: gggtctctaaacaagagaaat; notch33dir: ttagaatgcttgaagtttct, xpo15-16rev2: gggtctctaaacaagagaaat; notch33dir:


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accagacctatgtgcaga, notch33rev: tcgccccgcatcaccagac. If mutated peak(s) on chromatograms were as high as the wild-type (WT) peak, we concluded that mutations were in 100% of cells. Otherwise, mutations were found in 50% and 25% of cells accordingly (supplemental Table 1, available on the Blood Web site; see the Supplemental Materials link at the top of the online article).

Results and discussion

A recent study found mutations at position 571 of Xpo1 (namely E571K and E571G) in 4.6% of CLL IGVH unmutated/ZAP70+ cases. We sequenced the same region (exons 15 and 16) in our set of samples from 186 CLL patients. Six cases had 2 samples collected at 2 different time points, resulting in total of 192 CLL samples analyzed. 127 IGVH unmutated/ZAP70+, and 65 IGVH mutated/ZAP70-. We found the E571K mutation in 4 of 192 patients (2.1%). All the mutated samples were in the IGVH unmuated/ZAP70+ cohort, with a frequency of 4 of 127 samples (3.1%). In addition, we found the V565I (ex15-61719490 G-A) mutation in the first and second sample collected from a patient who first had indolent disease (sample collection 1) that later become progressive (sample collection 2). The other IGVH unmutated/ZAP70+ sample displayed a V520A mutation in exon 15 (ex15-61719700 [T-C], in ~ 25% of cells). In summary, we found XPO1 mutations in 6 of 127 IGVH unmutated/ZAP70+ cases (4.7%), but in only 1 of 65 IGVH mutated/ZAP70- cases (1.5%). These data confirmed previously reported results.

We used the same set of samples to screen for NOTCH1 mutations in the last coding exon of NOTCH1. Interestingly, we found only 5 mutations among 127 IGVH unmutated/ZAP70+ CLL samples (4%). All these changes were previously described 2-bp frameshift deletion P2515fs, resulting in truncated Notch1 protein. One mutation was found in IGVH-mutated/ZAP70+ samples for a total percentage of 3.1% (6 of 192 samples, Table 1). These results show 4- to 5-fold lower NOTCH1 mutation frequency in IGVH unmutated/ZAP70+ CLL compared with previous reports (4% vs 18%-20%), suggesting that NOTCH1 mutations may not be as prevalent as previously reported.

Because 4 of 6 samples with NOTCH1 mutations had trisomy 12, we examined for NOTCH1 mutations in 77 additional cases that had also trisomy 12. This set of samples included 47 IGVH unmutated/ZAP70+ aggressive cases (this set also included 2 patients, 28-Y and 23-V [supplemental Table 1], that were discordant for ZAP70 expression; however, they were characterized as aggressive because they were treated within 1 year of diagnosis), and 30 IGVH mutated/ZAP70- cases. Among these samples, we found NOTCH1 mutations in 22 of 47 (46.8%) IGVH unmutated/ZAP70+ aggressive cases, but only in 1 of 30 (3.3%) IGVH-mutated/ZAP70- cases. Collectively, for all cases examined with trisomy 12, we found NOTCH1 mutations in 26 of 62 (41.9%) IGVH unmutated/ZAP70+ aggressive cases, and in 1 of 34 (2.9%) IGVH mutated/ZAP70- indolent cases (Table 1). Twenty-five cases had mutations in NOTCH1 that were similar to those described, namely a heterozygous 2-bp frameshift deletion P2515fs. Two other cases had mutations resulting in Q2409Stop or L2457V. All mutations were observed in 100% of cells in each sample, except in 2 cases in which the P2515fs mutation was observed in ~ 50% of the cells, and in one case, in ~ 25% of the cells (supplemental Table 1).

Although 2 previous studies reported high mutation frequency for NOTCH1 in IGVH unmutated/ZAP70+ CLL, in our set of samples we only observed 4% frequency. On the other hand, our data suggest that almost half of IGVH unmutated/ZAP70+ trisomy 12 CLL patients (41.9%) harbor NOTCH1 mutations, indicating that NOTCH1 activation is strongly associated with trisomy 12. These differences could be explained, at least in part, by the fact that previous reports did not specifically study NOTCH1 mutations in trisomy 12 CLL, and did not specify how many trisomy 12 samples were present in their sample pools. All NOTCH1 mutations except one resulted in a truncated protein, lacking the C-terminal PEST degradation domain, rendering it constitutively active. Functional significance of L2457V mutation remains to be elucidated.

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Authorship

Contribution: Y.P. and C.M.C. designed research; T.J.K. and L.Z.R. provided patient samples and clinical data; V.B., A.B., Y.P., A.P., H.A. performed research and analyzed data; Y.P., V.B. and C.M.C. wrote the manuscript. All authors critically reviewed and edited the manuscript.

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