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 subgrouped 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 (*IGHV*), or low-to-negligible ZAP-70 and mutated *IGHV*. 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$^-$ CLL 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%.[4] 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-teminal 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/ZAP-70$^+$ CLL and 65 *IGVH* mutated/ZAP-70$^-$ 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: ttaggaaatgtacttgtagtttcta, xpo15-16rev2: gggtctctaacaagacaaaaacat; notch33dir:

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Table 1. *NOTCH1* mutation frequency in CLL

Sample description	Total mutation frequency (%)	Aggressive (%)	Indolent (%)
Total samples, first set	6/192 (3.1)	5/127 (4.0)	1/65 (1.5)
Trisomy 12 samples in the first set	4/19 (21.1)	4/15 (26.7)	0/4 (0)
Trisomy 12 set	23/77 (29.9)	22/47 (46.8)	1/30 (3.3)
Total trisomy 12 samples	27/96 (28.1)	26/62 (41.9)	1/34 (2.9)

acccagcctcacctggtgcaga, notch33rev: tcggccctggcatccacagag. 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.[4] 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* unmutated/ZAP70$^+$ cohort, with a frequency of 4 of 127 samples (3.1%). In addition, we found the V565I (ex16-61719490 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.[4]

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.[3,4] 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[3,4] (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 also had 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,[3,4] 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,[3,4] 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.[3,4] 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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