Analysis of NOTCH1 mutations in monoclonal B cell lymphocytosis

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Analysis of NOTCH1 mutations in monoclonal B cell lymphocytosis

Monoclonal B-cell lymphocytosis (MBL) represents asymptomatic monoclonal B-cell expansions characterized by a chronic lymphocytic leukemia (CLL)-phenotype, but with less than 5.0 x 10^9/L circulating cells.\(^1\)\(^-\)\(^3\) Clinical MBL (cMBL) are recognized during the diagnostic workup of an asymptomatic lymphocytosis.\(^4\)\(^-\)\(^6\) Although the molecular pathogenesis of MBL is scarcely known, the biological indolence of this condition is documented by the rare occurrence of genetic lesions predicting poor prognosis in CLL, such as TP53 and ATM disruption.\(^4\)\(^-\)\(^6\)

Recently, two independent investigations of the CLL coding genome have revealed that activating mutations of the NOTCH1 proto-oncogene occur in ~10% CLL at diagnosis and their frequency increases in advanced disease phases, exemplified by the case of Richter syndrome.\(^7\)\(^,\)\(^8\) Initial evidence suggests that NOTCH1 alterations might predict an unfavorable clinical outcome in CLL. The prevalence of NOTCH1 mutations in MBL is currently unknown.\(^7\)\(^-\)\(^11\)

Here we investigated the occurrence of NOTCH1 mutations in 63 consecutive cMBL presenting at our clinic for the initial evaluation of an asymptomatic lymphocytosis. The cMBL cohort was provided with prospectively collected peripheral blood mononuclear cell samples drawn at presentation, and with a prospectively maintained clinical database. All cMBL were analyzed for NOTCH1, TP53 and IGHV mutations by DNA Sanger sequencing, and for FISH karyotype using the LSI13 and LSID13S319, CEP12, LSIp53 and LSIATM probes (Abbott, Rome, Italy).\(^5\)\(^,\)\(^7\) A NOTCH1 mutation (c.7544_7545delCT) that is known to be highly recurrent in CLL was also independently investigated by amplification refractory mutation system (ARMS) PCR. Patients provided informed consent in accordance with local IRB requirements and Declaration of Helsinki. The study was approved by the Ethical Committee of the Ospedale Maggiore della Carità di Novara associated with the Amedeo Avogadro University of Eastern Piedmont (Protocol Code 59/CE; Study Number CE 8/11).
The clinical profile of the cMBL cohort was representative of this condition. Median age was 68 years (range: 40-88 years). The male:female ratio was 33:30. Median absolute lymphocyte count was 5.0 x10^9/L (range: 1.7-7.6 x10^9/L), median B-cell count was 2.9 x10^9/L (range: 0.2-4.9 x10^9/L), and median CLL-phenotype lymphocyte count was 2.8 x10^9/L (range: 0.2-4.9 x10^9/L). Median value of Hb was 14.1 g/dL (range: 11.0-16.9 g/dL), and of platelets 220 x10^9/L (range: 148-420). The biological profile of cMBL was consistent with the indolent nature of this condition. CD38 >30% and ZAP70 >20% occurred in 8/60 (13.3%) and in 9/53 (17.0%) cases, respectively. An IGHV identity >98% was present in 9/59 (15.3%) cMBLs, and stereotyped VH CDR3 in 9/59 (15.3%). Deletion of 13q14 was observed in 29/63 (46.0%) cases, trisomy 12 in 0/63, and 11q22-q23 deletion in 2/63 (3.2%). One of 63 (1.5%) cMBLs harbored biallelic TP53 disruption by deletion of one allele and mutation of the second allele.

To assess the prevalence of NOTCH1 mutations in cMBL, the NOTCH1 mutational hotspots identified in CLL (exons 26, 27 and 34; RefSeq NM_017617.2) were initially analyzed by Sanger sequencing of tumor DNA obtained at cMBL presentation.7,8 By this approach, NOTCH1 mutations occurred in only 2/63 (3.2%) cMBL, with a prevalence that was significantly lower than that observed in a large CLL dataset (70/603, 11.6%) (p=.050). In both cases, mutations were represented by a two bp frameshift deletion (c.7544_7545delCT) that represents the most recurrent (~80%) type of NOTCH1 mutation detectable in CLL (Figure 1).7,8 Case 1 presented with an absolute lymphocyte count of 6.3 x10^9/L, a B-cell count of 2.1 x10^9/L, and a CLL-phenotype cell count of 2.0 x10^9/L; was CD38 and ZAP70 negative; rearranged the 3-74/2-21/4 IGHV/D/J genes; expressed mutated IGHV genes (IGHV identity 92.01%); lacked a seterotyped HCDR3; showed a normal FISH karyotype and lacked TP53 mutations. Case 2 presented with an absolute lymphocyte count of 6.5 x10^9/L, a B-cell count of 4.1 x10^9/L, and a CLL-phenotype cell count of 4.1 x10^9/L; was CD38 and ZAP70 negative; rearranged the 1-18/6-13/4 IGHV/D/J genes; expressed mutated IGHV genes (IGHV identity 86.11%); lacked a seterotyped HCDR3; harbored a monoallelic 13q14 deletion and lacked TP53 mutations.
The sensitivity of DNA Sanger sequencing does not allow the identification of a mutation whose allelic representation is <10%. Because NOTCH1 mutations in CLL may be subclonal in a fraction of cases, and considering that the representation of peripheral blood monoclonal B cells in MBL is lower than in CLL, we reasoned that a mutation detection assay with a sensitivity higher than Sanger sequencing might be useful to define the true occurrence of NOTCH1 mutations in MBL. To this purpose, we specifically designed a high sensitivity ARMS PCR assay\textsuperscript{12} for the NOTCH1 c.7544_7545delCT mutation, that accounts for the overwhelming majority of NOTCH1 mutations in CLL. Despite the high sensitivity (1%) of this assay, ARMS did not identify additional c.7544_7545delCT mutations in cMBL.

The low prevalence of NOTCH1 mutations in cMBL is consistent with the low frequency in this condition of genetic lesions that are otherwise associated with high risk CLL, namely TP53 or ATM disruption, and corroborates the notion that cMBL is characterized by an indolent biological phenotype.

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Contributions

SR, SM, and VS performed molecular studies and interpreted data; RF, GG and DR designed the study and wrote the manuscript.
The authors have no conflict of interest to disclose
REFERENCES


FIGURE LEGENDS

Figure 1. *NOTCH1* mutations in clinical monoclonal B-cell lymphocytosis. Sequencing traces of the two clinical monoclonal B-cell lymphocytosis tumor samples (case 1 and case 2) harboring the *NOTCH1* c.7544_7545delCT mutation (RefSeq NM_017617.2); arrows point to the position of the nucleotide change.
Case 1

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CC T T C T C A C C C G T C C C C T G A C T C O N G T G A C C A G T G G T C A C A N P
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c.7544_7545delCT

Figure 1

Case 2

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c.7544_7545delCT

Figure 1