Polymorphism in the CD5 Gene Promoter in B-Cell Chronic Lymphocytic Leukemia and Mantle Cell Lymphoma

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Abstract

Despite the low incidence of microsatellite instability (MSI) in lymphoid malignant neoplasms, it has been reported that the CD5 promoter MSI was relatively frequent among B-cell chronic lymphoproliferative disorders. We studied the presence of MSI in the CD5 promoter in 134 cases of B-cell chronic lymphocytic leukemia (B-CLL) and 47 of mantle cell lymphoma (MCL) by comparing the pattern of microsatellite repeats on autologous germline and tumor DNA samples. Microsatellite alterations were not observed in any case. However, the allele distribution of this polymorphism showed a higher frequency of the 18 CA allele (0.585) in MCL cases (P = .026; odds ratio [OR], 1.75; 95% confidence interval [CI], 1.07-2.87) and of the 19 CA allele (0.179) in B-CLL cases (P = .005; OR, 2.26; 95% CI, 1.27-4.01) compared with control cases (0.442 and 0.087, respectively). This suggests that although MSI seems not to be involved in the pathogenesis of these 2 lymphoid malignant neoplasms, the polymorphic CD5 promoter is associated with increased susceptibility to these disorders.

Microsatellites are short tandem nucleotide repeats located in noncoding areas and remain stable from one generation to another. Generally they show length polymorphisms and seem to be involved in transcription and chromatin organization.1,2 Microsatellite instability (MSI) is a situation in which a germline microsatellite allele has gained or lost repeated units and, thus, has undergone a somatic change in length. Several lines of evidence suggest that genomic instability (in combination with other cytogenetic changes) might be involved in the development and progression of myeloid leukemias, but its possible role in the progression of lymphoproliferative disorders is not well known.3

B-cell chronic lymphocytic leukemia (B-CLL) and mantle cell lymphoma (MCL) are 2 lymphoproliferative disorders derived from CD5+ B lymphocytes. In their typical forms, B-CLL and MCL are characterized by distinctive histologic and cytologic morphologic features, immunophenotypes, and cytogenetic abnormalities. Although MCL is associated with higher complexity of the karyotype, there are striking similarities between common genetic aberrations in MCL and B-CLL, such as 11q aberrations. Genomic instability analyses of markers at chromosome 11q might be especially informative for both clinical entities.3 The gene coding for the human CD5 lymphocyte surface receptor maps to the 11q12.2 region, which is in the vicinity of a region commonly affected by multiple somatic mutations in human cancers. It has been reported that a polymorphic (CA)n microsatellite located in the promoter of CD5 might represent a candidate marker of genetic instability.4
Materials and Methods

DNA Samples

After informed consent, paired normal and tumor DNA samples were obtained from 134 patients with B-CLL and 47 patients with MCL diagnosed by established morphologic and immunophenotypic criteria. According to the Binet staging system, 94.0% of patients with B-CLL had stage A disease and 6.0% had stage B disease. These cases were obtained from the hematology units of the San Carlos, La Paz, and Puerta de Hierro University Hospitals of Madrid, Spain. Samples from 102 unrelated white, volunteer blood donors were used as control samples.

Briefly, peripheral blood mononuclear cells were separated from heparinized venous blood samples by density gradient centrifugation, and B lymphocytes were purified by negative selection using immunomagnetic methods (Miltenyi-Biotech, Bergisch Gladbach, Germany). Tumor and normal DNA samples were isolated from B lymphocytes and granulocytes, respectively, using the standard Proteinase K/RNase treatment and phenol-chloroform extraction.

Microsatellite Analysis

For the analysis of the human CD5 microsatellite, the sequences of the primers were as follows: forward, 5'-CTC TAC ATG GAG CTC ACA CAT A-3' and reverse, 5'-CAT GAA TGC TGG GCT TGT GC-3' (TIB MOLBIOL, Berlin, Germany). Polymerase chain reaction was performed with 100 ng of genomic DNA, a 10-mmol/L concentration of tris(hydroxymethyl)aminomethane hydrochloride, pH 9, a 1.5-mmol/L concentration of magnesium chloride, 50-mmol/L concentration of potassium chloride, 200 µmol/L each deoxynucleoside triphosphate, 5-µmol/L concentration of unlabeled primers, and 1.25 U of Taq DNA polymerase (Amersham-Biotech, Uppsala, Sweden) in a total volume of 25 µL.

Amplifications were carried out for 30 cycles of 30 seconds at 95°C, 30 seconds at 60°C, and 30 seconds at 72°C, with a final extension of 10 minutes at 72°C. We analyzed 1 µL of each reaction on denaturing 6% polyacrylamide gels (Amersham), and bands were visualized by silver staining (Amersham), following the manufacturer’s instructions. For quality control to ensure reproducibility of allele assignments between gels, 1 lane in each gel was loaded with a sample that had been genotyped previously. Each lane of the sequencing gel was loaded with an internal marker consisting of 4 DNA fragments (130, 150, 200, and 220 bases) from a 10-base-pair DNA ladder (Life Technologies, Rockville, MD) isolated using a DNA extraction kit (Qiagen, Hilden, Germany). Allele assignment was performed by densitometry using Quantity One software (Bio-Rad Laboratories, Hercules, CA).

CD5 Surface Expression

Initially, fluorescein isothiocyanate–conjugated anti-CD5 (Becton Dickinson Biosciences, San Jose, CA) was titrated to determine the amount that identified positive cells with the optimal mean fluorescence intensity; this amount then was used in subsequent studies. B lymphocytes were stained by standard methods and analyzed in a FACSort cytometer using CellQuest software (Becton Dickinson Immunocytometry Systems, San Jose, CA).

Statistical Analysis

Allelic and genotypic frequencies were calculated by simple counting, and tests for Hardy-Weinberg equilibrium and heterozygosity were performed with software for population genetics data analysis. Probability values for 2 × 2 contingency tables comparing genotype frequencies were calculated by using the \( \chi^2 \) test using SPSS, version 10 for Windows (SPSS, Chicago, IL). The Bonferroni correction was applied for multiple comparisons, and corrected \( P \) values are shown. Individuals with an allele occurring fewer than 4 times in the population were excluded. For each significant \( P \) value (\( P < .05 \)), strength of association was assessed by odds ratios (OR) and the exact limits for 95% confidence intervals (CIs). CD5 surface expression levels were compared by using the non-parametric Kruskal-Wallis test using SPSS, version 10.

Results

Analyses of CD5 microsatellites in control samples showed 8 different alleles, corresponding to sequences containing from 11 to 20 CA repeats. The heterozygosity value was 0.68, showing the usefulness of this polymorphic marker in our population.

When normal and tumor DNA from 134 patients with B-CLL and 47 patients with MCL were compared, none of the cases showed MSI or loss of heterozygosity in the CD5 promoter. Demonstrating a low level of CD5 microsatellite alterations in B-cell lymphoproliferative disorders. We then examined whether this polymorphism could be of interest as a genetic marker for both disorders. We found differences between the allelic distribution of the CD5 microsatellite in control samples and in samples from patients with MCL (\( P = .008 \) but not in samples from patients with B-CLL. When we compared each allele frequency with all others by using the Yates continuity correction, the allele containing 17 CA repeats was less common in MCL samples than in control samples (\( P = .002 \)) and B-CLL samples (\( P = .021 \)), and the 18 CA allele was overrepresented in MCL samples compared with control (\( P = .026 \) and B-CLL (\( P = .011 \)) samples. In addition, the 19 CA allele was represented more in B-CLL than in control samples.
Genotype analysis showed that 16.4% of patients with B-CLL (22/134) and 11 (23%) of 47 patients with MCL had the 19/18 genotype compared with 6 (5.9%) of 102 control subjects (B-CLL vs control subjects, \( P = .013 \); OR, 3.14; 95% CI, 1.22-8.07; MCL vs control subjects, \( P = .002 \); OR, 4.89; 95% CI, 1.68-14.20, respectively), whereas the 18/17 genotype was underrepresented in MCL (4/47 [9%]) compared with control subjects (24/102 [23.5%]) \( (P = .029; \text{OR}, 0.30; 95\% \text{CI}, 0.10-0.93) \). There was no statistical difference in the remainder of genotypes analyzed; however, we noted that the 19/19 genotype appeared only in patients with B-CLL and the 20 CA allele only in patients with B-CLL or MCL.

It is well known that B lymphocytes in B-CLL and MCL express CD5 in their surface, but the density per cell of this marker is higher in B-CLL. It may be that differences in this polymorphic promoter could be related to the protein expression level. However, no significant differences in the density of CD5 surface expression were observed when patients were stratified according to microsatellite genotypes. For this comparison, we considered genotypes with 2, 1, or none of the disease-associated allele, the 19 CA allele in B-CLL and the 18 CA allele in MCL. **Figure 1.** Moreover, in B-CLL, we also compared only homozygous individuals (3 cases of 19/19 vs 15 cases of 18/18 vs 8 cases of 17/17), and there were no differences in CD5 expression (mean fluorescence intensity ratio values = 15.3, 24.4, and 24.2, respectively). Taken together, these data indicate an association of the 19 CA allele of the CD5 microsatellite with B-CLL and the 18 CA allele with MCL, and the association seems unrelated to protein surface expression.

**Table 1**

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<th>Allele Frequencies of the Polymorphic CD5 Microsatellite</th>
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**P**; Odds Ratio (95% Confidence Interval)

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<th><strong>Control vs B-CLL Cases</strong></th>
<th><strong>Control vs MCL Cases</strong></th>
<th><strong>B-CLL vs MCL Cases</strong></th>
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B-CLL, B-cell chronic lymphocytic leukemia; MCL, mantle cell lymphoma; n, total number of alleles; ND, not determined.
Discussion

Microsatellite markers represent a valuable tool for detecting loss of heterozygosity in regions with candidate tumor suppressor genes and chromosomal alterations present in several tumors. Also, polymorphic microsatellites are excellent genetic markers for disease. It seems that the 11q region might be of relevance when analyzing genome instability in lymphoproliferative disorders because this region contains some growth regulatory and tumor-suppressor genes (ATM, RDX) supposedly implicated in tumorigenesis. The CD5 promoter microsatellite, owing to its location at 11q, has been proposed as a potential valuable marker with a probable role in the pathogenesis of B-CLL and MCL.

In the present study, we analyzed the incidence of CD5 MSI in B-CLL and MCL and, in contrast with data reported by Lopez-de la Iglesia et al., no alterations were observed, indicating a modest or null involvement of CD5 MSI in the pathogenesis of these lymphoproliferations. It has been suggested that microsatellite instabilities are present more frequently in patients with B-CLL with an advanced Binet stage. It could be postulated that differences in the clinical stage of patients might explain the discrepancy in the incidence of CD5 MSI between our results and previously published data. Accordingly, none of our patients had an advanced disease stage, compared with 28% of patients with Binet C stage disease in the study by Lopez-de la Iglesia et al. No comparison between stages and CD5 MSI appearance was done in their work; however, they observed no correlation with progression to Richter syndrome in accordance with a previous MSI study performed in B-CLL, suggesting that other mechanisms could be implicated. It is possible that CD5 MSI, as with other MSIs and sporadic genetic alterations, appears with a more advanced stage of disease, although these changes are not associated with clinical transformation of the disease.

It is interesting that when we studied allele frequencies of the polymorphic CD5 promoter in patients and control subjects, we found that the 18 CA allele was associated with increased susceptibility to MCL, whereas the 19 CA allele was associated with B-CLL. These findings were concordant with other studies that found several molecular differences between these entities. Because the density per cell of CD5 is higher in B-CLL and this polymorphism is located at the CD5 promoter region, we postulated that the observed allele association could be related to the protein expression level. However, we could not find a correlation between genotypes and CD5 surface expression, suggesting that other mechanisms support this association. This microsatellite might be a marker of a functional polymorphism in the CD5 molecule related to tumoral cell physiology.

Nevertheless, no functional polymorphism in CD5 has been described to date, raising the possibility that it could be a marker of another gene segment involved in the pathogenesis of lymphoproliferative disorders.

We demonstrated that B-CLL and MCL present different allelic distributions of the CD5 promoter microsatellite. Thus, this polymorphic marker might contribute to better discrimination between these hematologic malignant neoplasms. Furthermore, it also might correlate with clinical features such as disease staging or survival to test its potential prognostic value. However, further studies in a larger series of patients are needed to confirm the biologic role and significance of this marker in CD5+ lymphoproliferative disorders.

References


