Mantle cell lymphoma (MCL) is molecularly characterized by bcl-1 rearrangement and cyclin D1/PRAD-1 gene overexpression. Some aggressive variants have been recognized with a blastic or large cell morphology, higher proliferative activity, and shorter survival. p53 gene mutations in lymphoid neoplasms have been detected mainly in high grade lymphomas and have been associated with tumor progression in follicular and small lymphocytic lymphomas. To determine the role of p53 alterations in MCL, we examined 35 typical and 8 aggressive variants (5 blastic and 3 large cell) of MCLs by a combination of immunohistochemistry, single-strand conformational polymorphism analysis of genomic DNA and/or cDNA obtained by reverse transcriptase-polymerase chain reaction, denaturing gradient gel electrophoresis, and sequencing. Of the 8 aggressive MCLs, 3 (38%) contained missense point mutations in exon 8 codon 278 (Pro → Leu), exon 8 codon 273 (Arg → His), and exon 5 codon 151 (Pro → Ser), respectively. A diffuse p53 protein overexpression was observed in more than 50% of the tumor cells in these 3 cases. A fourth blastic MCL also showed strong p53 immunoreactivity. However, no mutations were detected in exons 5-9 in this case. p53 expression was also detected in 10% of the cells in an additional large cell type of MCL and in less than 1% of the cells in 6 typical cases. No mutations were detected in any of these cases or in the remaining cases with no expression of the protein. Four nucleotide changes were observed by single-strand conformational polymorphism analysis in 4 typical MCLs with no overexpression of the protein. Direct sequencing showed that these nucleotide changes were located at exon 6 (1 case), intron 7 (2 cases), and intron 8 (1 case). The changes in exon 6 and intron 7 were known polymorphisms. The nucleotide change in intron 8 was outside splicing sites of the neighboring exons. The overall survival of the 3 patients with p53 mutations (median, 18.3 months) was significantly shorter than that of patients with the nonmutated MCLs (median, 49 months; P < .01). These findings indicate that p53 gene mutations are an infrequent phenomenon in MCLs and are associated with a subset of aggressive variants.

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participates in a G1 arrest checkpoint that would allow the cell to repair DNA damage before progression in the cell cycle.21,22 Somatic allelic deletions and point mutations of this gene are considered to be the most frequent genetic alterations in human neoplasms.23 p53 mutations have been associated with progression to more aggressive forms of the disease in several hematologic malignancies. Particularly, in non-Hodgkin’s lymphoid neoplasms, p53 gene mutations have been mainly detected in high grade lymphomas.24-26 In addition, they are associated with the histological and clinical progression of follicular27,28 and small lymphocytic lymphomas.24-26,27

The aim of this study was to determine the role of p53 alterations in MCL and their possible involvement in the pathogenesis of the more aggressive variants. For this purpose, we have examined a series of classic and aggressive MCLs by a combination of immunohistochemistry, single-strand conformational polymorphism (SSCP) analysis of genomic DNA and cDNA obtained by reverse transcriptase-polymerase chain reaction (RT-PCR), denaturing gradient gel electrophoresis (DGGE), and sequencing. The findings indicate that p53 mutations and protein expression are rare in typical MCLs but may be involved in the pathogenesis of a subset of aggressive variants of this lymphoma.

MATERIALS AND METHODS

Case selection. Tumor specimens from 43 MCLs were included in the study. A total of 25 cases were obtained from the Anatomic Pathology Department of the Hospital Clinic Provincial (HCP; University of Barcelona, Barcelona, Spain), and 18 were obtained from the Laboratory of Pathology, National Cancer Institute (NCI; National Institutes of Health, Bethesda, MD). A total of 35 cases were classified as typical MCLs (20 from HCP, 15 from NCI),31,32 and 8 cases were classified as aggressive variants of MCLs (5 from HCP, 3 from NCI). These aggressive variants included 5 blastic MCLs and 3 large cell (‘‘anaplastic’’) type of MCLs defined according to criteria previously described.16,29 All the cases were reviewed and classified by two pathologists (E.C. and E.S.J.) together. Immunophenotype was analyzed in all cases using immunohistochemistry on frozen tissue sections and/or cell suspensions by flow cytometry. These studies included Ig light and heavy chains, several of the B-cell (CD19, CD20, CD22, CD45RA) and T-cell (CD2, CD3, CD5, CD7, CD4, CD8, CD45RO, CD43) markers, and CD10 and CD23. Cyclin D1 expression was examined in 25 cases, including 5 aggressive variants, by Northern blot analysis, and it was overexpressed in all of them.16 All patients had advanced stage disease (III or IV) and were treated with systemic chemotherapy according to protocols at the respective institutions. Actuarial survival analysis was performed according to the method described by Kaplan & Meier, and the curves were compared by the log-rank test.

RNA and DNA extraction. Total RNA was isolated from frozen tissues in the 25 cases from HCP by guanidine isothiocyanate extraction and cesium chloride gradient centrifugation.21 High molecular weight DNA was extracted from the 18 cases from NCI and from additional frozen material available in 19 cases from HCP, using Proteinase K/RNAse treatment and phenol-chloroform extraction. DNA and RNA were also extracted from the following cell lines and used as positive controls for p53 mutations during the SSCP, DGGE, and sequencing analysis: MDA-MB-468 breast carcinoma cell line (mutated at codon 273), obtained from the American Tissue Culture Collection (Rockville, MD); the KM12SM colorectal cancer cell line (mutated at codon 179), kindly provided by Dr J.I. Fidler (M.D. Anderson Cancer Center, Houston, TX); and the T-cell line CEM (mutated at codons 175 and 248), obtained from American Tissue Culture Collection.

RT-PCR of p53 gene. p53 cDNA was obtained in 25 cases using RT and the antisense primer 10D (Table 1) located at the 5’ end of the 11th exon. The RT reaction was performed with 1.5 pg of total RNA, 200 U of Moloney murine leukemia virus RT (GIBCO-BRL, Gaithesburg, MD), 0.5 mmol/L of primer, 0.5 mmol/L of each deoxyribonucleotide triphosphate (dNTP), 20 U of RNASin, 10 mmol/L dithiothreitol, and RT buffer (50 mmol/L Tris HCl, pH 8.3; 75 mmol/L KC1, 3 mmol/L MgCl2) in a final volume of 20 pL. The reaction was incubated for 1 hour at 37°C and for 5 minutes at 95°C.

Amplification of the p53 gene. For the RNA samples, a nested PCR was used to amplify a fragment of p53 gene including exons 5 through 9. The primers used in PCR procedures are described in Table 1. The first PCR reaction was performed by mixing 5 pL of the RT reaction product with 1 U of Taq polymerase (GIBCO-BRL), 0.4 mmol/L each primer (1UR and 10DN), and PCR buffer (10 mmol/L Tris HCl, pH 7.8, 50 mmol/L KC1, 1.5 mmol/L MgCl2, and 0.01% gelatin) in a final volume of 25 mL. The reaction was performed for 15 cycles in a thermal cycler (Perkin-Elmer Cetus, Norwalk, CT) at 94°C for 1 minute, at 53°C for 45 seconds, and at 72°C for 1 minute. The second PCR reaction was performed with 0.5 pL of the first reaction in the same conditions but with 120 mmol/L dNTPs and 2 mmol/L of each primer (126U and 331DB2), at an annealing temperature of 55°C, and for 35 cycles.

For the DNA samples, 0.5 pg of DNA was added to 1 U of Taq, 2 mmol/L each primer (12979U and 14875D), 120 mmol/L dNTPs, and PCR buffer in a final volume of 25 pL. The reaction was performed for 25 cycles at 94°C for 45 seconds, at 63°C for 15 seconds, and at 72°C for 1 minute and 15 seconds. Two nested PCR reactions were performed on 1 pL of 1/1,000 dilution of the first PCR product, with two pairs of primers (126U/331D3 for exons 5 to 6, and 13966U/331DB2 for exons 7 to 9). The reactions were performed for 35 cycles at 94°C for 45 seconds, at 63°C for 35 seconds, and at 72°C for 1 minute.

SSCP analysis. SSCP analysis was used to screen for p53 mutations according to a modified protocol of a previously described method.16,22 Nested PCRs were performed as described above in the presence of 2 pCi 32P deoxycytidine triphosphate per PCR. For the 37 genomic DNA samples and for the 25 cDNA samples, 5 pL and 9 pL, respectively, of the radioactive PCR product were digested with Hpa II. Samples were diluted 20-fold in formamide-dye loading buffer,16 incubated for 3 minutes at 95°C, and immediately cooled on ice; 2 pL were loaded on a 6% polyacrylamide nondenaturing gel with or without 10% glycerol. Electrophoresis was performed at room temperature at 30 W for 12 minutes followed by 6 W for 14 hours for 10% glycerol gels and at 30 W for 12 minutes followed by 6 W for 6 hours for the gels without glycerol. The gels were

Table 1. Primer Sets Used for the RT-PCR, PCR, SSCP Analysis, and Sequencing Analysis of p53 Gene

<table>
<thead>
<tr>
<th>Primer Set</th>
<th>5’ Primer</th>
<th>3’ Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>10D</td>
<td>5’ TGTCACTGTGAATCGCAATCTG 3’</td>
<td></td>
</tr>
<tr>
<td>1UR</td>
<td>5’ AGACTGCATTCCGGTGACT 3’</td>
<td></td>
</tr>
<tr>
<td>10DN</td>
<td>5’ ATGGCGGGAAGTTACTACGACTG 3’</td>
<td></td>
</tr>
<tr>
<td>128U</td>
<td>5’ TACTCCCTCGCTCAACAA 3’</td>
<td></td>
</tr>
<tr>
<td>331DB2</td>
<td>5’ ACAGACGTGAAAGGTGAAATATCTTCC 3’</td>
<td></td>
</tr>
<tr>
<td>12979U</td>
<td>5’ GCTGGCCGTGGTCATGGTCTG 3’</td>
<td></td>
</tr>
<tr>
<td>14875D</td>
<td>5’ AGGCATACCTCGCCCTCGATG 3’</td>
<td></td>
</tr>
<tr>
<td>13963D</td>
<td>5’ CTCTGCCAGACGACCCCGATG 3’</td>
<td></td>
</tr>
<tr>
<td>13966U</td>
<td>5’ CTGCCCTAGCTTGGGCGCTG 3’</td>
<td></td>
</tr>
<tr>
<td>225U</td>
<td>5’ TGCGCTCGACTGGACCAACCA 3’</td>
<td></td>
</tr>
<tr>
<td>5DN</td>
<td>5’ CATAGGGCACCACACCACTA 3’</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Primer Sets Used for the PCR Reaction and Conditions for DGGE for p53 Mutations

<table>
<thead>
<tr>
<th>p53 Exon</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Fragment Size</th>
<th>DGGE Conditions*</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>5'(GC)TTCCCTCCCTGCTAGACTC-3'</td>
<td>5'(GC)CATGGGCGGCCGC-3'</td>
<td>282</td>
<td>50%-65% for 5 h</td>
</tr>
<tr>
<td>6</td>
<td>5'(GC)CATGGGCGGCCGC-3'</td>
<td>5'(GC)CATGGGCGGCCGC-3'</td>
<td>227</td>
<td>35%-60% for 8 h</td>
</tr>
<tr>
<td>7</td>
<td>5'(GC)TTCCCTCCCTGCTAGACTC-3'</td>
<td>5'(GC)TTCCCTCCCTGCTAGACTC-3'</td>
<td>174</td>
<td>35%-65% for 7 h</td>
</tr>
<tr>
<td>8</td>
<td>5'(GC)TTCCCTCCCTGCTAGACTC-3'</td>
<td>5'(GC)TTCCCTCCCTGCTAGACTC-3'</td>
<td>260</td>
<td>35%-65% for 8 h</td>
</tr>
<tr>
<td>8 &amp; 9</td>
<td>5'(GC)TTCCCTCCCTGCTAGACTC-3'</td>
<td>5'(GC)TTCCCTCCCTGCTAGACTC-3'</td>
<td>375</td>
<td>35%-65% for 7 h</td>
</tr>
<tr>
<td>9</td>
<td>5'(GC)TTCCCTCCCTGCTAGACTC-3'</td>
<td>5'(GC)TTCCCTCCCTGCTAGACTC-3'</td>
<td>156</td>
<td>35%-65% for 8 h</td>
</tr>
</tbody>
</table>

* The percentage of denaturant range and electrophoresis run time at 150 V and 60°C.
† (GC) is 5'-CGCCGCCGCCGC-3'.

DGGE analysis. DGGE analysis was performed in the 37 cases in which genomic DNA was available. Primers flanking p53 exons 5-9 were the same as those described by Beck et al. except for the exon 8 and 9 primers, and are listed in Table 2. One primer of each pair was synthesized with an added 40-bp GC-rich sequence (GC clamp) at the 5' end. This results in a high temperature melting domain at one end of each PCR product and renders the remainder of the exon sequence accessible to analysis by DGGE. PCR reactions were performed in a previously reported protocol with minor modifications. Briefly, 200 ng of genomic DNA was mixed with 40 pmol of each primer and 75 nmol of each dNTP in 50 µL of PCR buffer (10 mmol/L Tris HCl, pH 8.3; 50 mmol/L KCl; 1.5 mmol/L MgCl2; 0.01% gelatin). A total of 1.5 U of Taq was added to each sample, and PCR was performed at 94°C for 1 minute and 15 seconds, at 58°C for 1 minute and 15 seconds, and at 72°C for 35 seconds plus a 1-second extension per cycle for 40 cycles. The PCR products were analyzed using D-gene gel electrophoresis apparatus (Bio-Rad Laboratories, Hercules, CA). Gels were cast using the Model 475 Gradient delivery System (Bio-Rad). Electrophoresis of 40 µL of the PCR product was performed at 150 V at 60°C in 7.5% polyacrylamide gel under the appropriate predetermined denaturing conditions. Except for the gradient conditions for exon 5 (see Table 2), all other p53 exons were analyzed under the same conditions as described by Beck et al.37

Sequencing of the SSCP* and DGGE** fragments. To confirm the possible p53 mutations the samples of DNA with altered migration by SSCP or DGGE were sequenced. Sequences were performed using a commercial cycle sequencing kit (Perkin-Elmer Cetus) and 33P dideoxynucleotide triphosphate. A total of 0.5 µL of the nested PCR product was used as template for sequencing. Several internal primers (126U, 331DB2, 13463D, 13966U, 225U, and 5DN) were used for the sequencing reaction at a final concentration of 14 mmol/L. The reaction was performed according to the instructions of the manufacturer. The reaction was performed for 30 cycles at 94°C for 45 seconds, at 60°C for 30 seconds, and at 72°C for 1 minute and 30 seconds. The final product was diluted twofold in formamide-loading buffer. Samples were denatured for 3 minutes at 95°C, and 2 µL was analyzed in a denaturing 6% polyacrylamide/8 mol/L urea sequencing gel for 2 or 3 hours at 55 W. The gels were dried under vacuum at 85°C and exposed to an x-ray film at room temperature for 3 days.

The presence of a mutation was confirmed by sequencing both the genomic DNA and cDNA of each mutated case, when available, and by sequencing the other DNA strand.

Immunohistochemical analysis. p53 protein expression was immunohistochemically assessed in all cases on formalin-fixed-paraffin-embedded material using the DO-7 (Dako Corp, Carpinteria, CA). In addition, the 25 cases from the HCP were assessed on frozen sections using the anti-p53 monoclonal antibodies (MoAbs) Pab 1801 and Pab 240 (Oncogene Science, Cambridge, MA). The results with the paraffin-embedded material and with the frozen sections in these cases were concordant. Before the application of the primary antibodies on the fixed and paraffin-embedded sections, an antigen retrieval technique was performed. The deparaffinized and rehydrated slides were placed in 10 mmol/L citrate buffer (pH 6) and were heated in the microwave oven for 15 minutes at 700 W. The frozen sections were air-dried, fixed in cold (4°C) acetone for 10 minutes, hydrated, and incubated with the MoAbs. The MoAbs were incubated overnight at 4°C. The immunoreaction was detected by means of the streptavidin-biotin-alkaline phosphatase (Biogenex, San Ramon, CA) technique using Fast-Red as chromogen and levamisole to inhibit endogenous alkaline phosphatase or, alternatively, using the avidin-biotin-peroxidase complex technique (Vectastain ABC kit; Vector Laboratories, Burlingame, CA) and 3'3'-diaminobenzidine tetrahydrochloride (Sigma Chemical Co, St Louis, MO) dissolved in 10 mL of Tris buffer 0.05 mol/L, pH 7.6, and 0.03% of H2O2 as developer. The slides were counterstained with hematoxylin. The cases were evaluated as negative when no positive cells were observed, and p53 was scored as 1+ (weak), 2+ (moderate), and 3+ (strong), when less than 1%, less than 20%, or greater than 20% of the tumor cells, respectively, showed nuclear immunoreactivity.

RESULTS

SSCP and DGGE analysis. A series of 35 typical and 8 aggressive variants of MCLs were analyzed for the presence of p53 mutations using SSCP of genomic DNA and/or cDNA samples and DGGE of genomic DNA. The results are summarized in Tables 3 and 4 and in Figs 1, 2, and 3. Only 4 of the 35 typical MCLs and 3 of the 8 aggressive cases showed altered electrophoretic mobility. Two of these aggressive variants (cases no. 10 and 31) had a blastic morphology (Fig 4), and the third case (case no. 15) was a large cell MCL variant (Fig 5). In cases no. 10 and 15, the anomalous mobility was found within the fragments encompassing exon 8 using all three techniques, ie, SSCP and DGGE of genomic DNA and SSCP of cDNA. In case no. 31, an abnormally migrating band was identified in exon 5 by SSCP and DGGE.

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of genomic DNA. SSCP analysis of cDNA was not performed in this case because of the unavailability of RNA.

Of the 4 abnormalities found in typical MCLs, 3 occurred only in the SSCP analysis of genomic DNA (cases no. 2, 9, and 38). The anomalous mobility was observed in the fragment containing exons 7-9. This genomic SSCP fragment also contains introns 7 and 8; whereas the other methods used, ie, DGGE and SSCP of cDNA, do not include intronic sequences, suggesting that the abnormalities present were caused by changes in introns 7 or 8. One typical MCL (case no. 4) showed an anomalous band in the exon 6 fragment using all three techniques in a pattern suggestive of the known polymorphism at codon 213.

DNA sequencing. All the fragments with anomalous SSCP and DGGE were subsequently sequenced. The results are summarized in Table 4 and in Figs 2 and 3. The 3 high grade MCLs showed a missense mutation in exon 8 codon 278 (Pro → Leu; case no. 10), exon 8 codon 273 (Arg → His; case no. 15), and exon 5 codon 151 (Pro → Ser; case no. 31), respectively. In cases no. 10 and 15, the mutation was confirmed in both genomic DNA and cDNA.

The altered mobility detected in exon 6 in 1 typical MCL (case no. 4) by SSCP and DGGE was shown to be the result of the known polymorphism at codon 213, with the neutral change CGA (Arg) → CGG (Arg). This nucleotide change was confirmed in both genomic DNA and cDNA. The anomalous SSCP detected at the genomic level in 2 typical MCLs (cases no. 2 and 38) were caused by a known polymorphism in intron 7 characterized by C → T and T → G changes at 71-bp and 91-bp downstream of exon 7, respectively.*

The fourth typical MCL (case no. 9) with an altered mobility detected at genomic level was a nucleotide change in intron 8 (G → A), 32-bp downstream of exon 8 (Table 4 and Fig 2). This nucleotide change was outside splicing sites of the neighboring exons. No DNA from normal tissues of this case was available to rule out the possibility that this change was an intronic polymorphism.

Immunohistochemical analysis of p53 protein. Expression of p53 protein was examined in the 43 cases by immunohistochemistry. The 3 aggressive cases with a missense p53 mutation showed strong nuclear immunostaining in a high proportion of the tumor cells (>50%; see Figs 4 and 5). The positive cells were diffusely distributed throughout the tumor. In 1 of these cases, a paraffin block of a previous biopsy performed 2 years before was also available. This sample was obtained at the time of presentation of the tumor and was interpreted as a large-cell type of MCL with a nodular growth pattern. Comparing both samples, the second specimen showed a progression to a more aggressive morphology with a diffuse growth pattern and larger and more irregular cells that contained nuclei with blastic chromatin and occasional nucleoli. The mitotic index in the first biopsy specimen was lower (2 mitoses × high power field [HPF]) than that in the second specimen (6.5 mitoses × HPF). The patient died 2 months after the second biopsy was performed. In spite of the morphological progression between the first and second biopsy specimens, the p53 expression was similar in both samples, with a strong immunoreactivity in more than 70% of the tumor cells.

In addition to the 3 mutated cases described above, 1 blastic MCL (case no. 29) showed strong (3+) p53 overexpression, with nuclear immunoreactivity in more than 50% of cells. However, no gene mutations were detected in exons 5-9 in this case. An additional large-cell type of MCL (case no. 42) showed p53 immunostaining in 10% of the cells. No gene alterations were detected in this case.

None of the remaining nonmutated cases showed overexpression of the protein, including the case with the codon-213 polymorphism and the 3 cases with nucleotide changes in intronic regions. In 6 typical MCLs with no DNA alter-

<table>
<thead>
<tr>
<th>Case No.</th>
<th>MCL Variant</th>
<th>IHC</th>
<th>RNA/cDNA SSCP</th>
<th>SSCP</th>
<th>DGGE</th>
<th>Mutation</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Blastic</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Codon 278 CCT → CTT</td>
<td>Pro → Leu</td>
</tr>
<tr>
<td>15</td>
<td>Large cell</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Codon 273 CGT → CAT</td>
<td>Arg → His</td>
</tr>
<tr>
<td>31</td>
<td>Blastic</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Codon 151 CCC → TCC</td>
<td>Pro → Ser</td>
</tr>
<tr>
<td>4</td>
<td>Typical</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>Codon 213 CGA → CGG</td>
<td>Arg → Arg</td>
</tr>
<tr>
<td>2</td>
<td>Typical</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>Intron 8</td>
<td>−</td>
</tr>
<tr>
<td>9</td>
<td>Typical</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>Intron 7</td>
<td>−</td>
</tr>
<tr>
<td>38</td>
<td>Typical</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>Intron 7</td>
<td>−</td>
</tr>
</tbody>
</table>

Abbreviations: IHC, immunohistochemistry; AA, amino acid.

* SSCP analysis of the genomic DNA included exonic and intronic regions, whereas DGGE examined only exonic areas.
Fig 1. SSCP analysis of p53 cDNA (A, B, and C) and genomic DNA (D) in MCLs. cDNA fragments corresponding to exons 5-6 (A) and exons 7-8 (B and C) of p53 gene run in a nondenaturing 6% acrylamide gel containing 10% (A and B) or no (C) glycerol. Genomic DNA fragment corresponding to exon 5 (D) of p53 gene run in a nondenaturing 6% acrylamide gel with no glycerol. Lane numbers indicate the different MCLs. Lane C1 in (A) contains cDNA from the positive control cell line KM12SM mutated at codon 179. Three aggressive MCLs (cases no. 10, 15, and 31) showed an abnormal migration in exon 8 (B and C) and exon 5 (D), respectively. Case no. 4 was a typical MCL, with a polymorphism in exon 6 codon 213 (A).

Fig 2. Sequence analysis of SSCP cases. The p53 gene regions amplified by PCR were sequenced by a direct cycle reaction. (A), (B), (C), and (D) show the sequences of cases no. 10, 15, 2, and 9, respectively. Cases no. 10 and 15 were mutated in codon 278 and 273, whereas cases no. 2 and 9 showed a nucleotide change in intron 7 and 8 in distant regions of the splicing sites.
Fig 3. DGGE and sequence analysis of case no. 31. (A) 200 ng of genomic DNA was amplified with the exon 5 primers and was analyzed by DGGE. Lane 1 is a normal placental control showing the normal homoduplex pattern. Lane 2 is case no. 31 showing an abnormal homoduplex. Lane 3 is an equal mixture of control DNA with case no. 31 DNA. The resulting complex band pattern is caused by the formation of normal and mutant heteroduplex species, in addition to the two homoduplex species, allowing a clearer evaluation of the abnormality present. Lane 4 is a positive control cell line (CEM). Bands labeled “1” represent abnormal homoduplexes; bands labeled “2” represent heteroduplexes. (B) Sequence analysis of case no. 31 showing a C → T transition in codon 151 that results in the substitution of serine for the normal proline (CCC → TCC).

**Fig 4.** Histological section of a blastic MCL (case no. 31) with p53 mutation (hematoxylin and eosin [H&E]; original magnification × 630).

*p53 Alterations and survival of the patients.* The median overall survival of this series of MCL patients was 42 months (range, 2 to 132 months). Patients with aggressive variants of MCLs had an overall survival (median, 18.3 months; n = 8) significantly shorter than that of patients with typical mantle cell lymphomas (median, 49.8 months; n = 30; P < .02). No follow-up was available in 5 patients with typical MCL. The survival of the 3 patients with p53 mutations (median, 18.3 months) was significantly shorter than that of the patients with nonmutated MCLs (median, 49 months; 95% confidence interval, 24 to 69 months; P < .01; see Fig 6). However, the survival of 5 aggressive MCLs with no detectable p53 mutations was similar to the survival of the mutated aggressive cases (median, 14 and 18.3 months, respectively). The blastic MCL with strong p53 immunostaining in which no mutations were detected also had a short survival of 18 months.

**DISCUSSION**

In this study we have examined 35 typical and 8 aggressive variants of MCLs for the presence of p53 mutations and protein expression. Only 3 of 43 (7%) MCLs showed p53 gene mutations and strong overexpression of the protein. However, all 3 were in the histologically aggressive subgroups, further classified as either blastic or large-cell variants of MCL. None of the typical MCLs with low proliferative fraction and more indolent behavior showed p53 mutations. Protein expression in these cases was negative or negligible. These findings indicate that p53 gene mutations in MCLs are a relatively infrequent phenomenon (7% of all the MCLs examined). However, the presence of mutations in 3 of the 8 histologically aggressive cases (38%) with shortened survival indicates that p53 may be involved in the pathogenesis of a subset of aggressive MCLs.

One of the two mutations found was a G → A transition in codon 273. This is a known hot-spot codon with a CpG dinucleotide. Most of the mutations occurring in this codon, including the one detected in our study, lead to a loss of the biological functions of p53 protein.40 p53 mutations in leukemias/lymphomas, as in other tumors, occur frequently at CpG sites that are considered to be particularly susceptible to spontaneous mutations.24 The other two mutations were C → T transitions in codon 278 and 151. Mutations in these codons are rare in lymphomas but have been detected in several lymphomas and hematologic disorders.27,29,30,41 Three other nucleotide changes were detected in intronic regions of the gene. Two of them were a known polymorphism,30 and, in the third case, the change was distant from splicing sites.

The immunohistochemical detection of p53 protein in a high number of cells (>50%) was relatively concordant with the presence of gene mutations in this series of MCLs because strong p53 immunostaining was observed in the 3 cases with p53 gene mutations, whereas all typical MCLs...
were negative or weakly positive. However, no mutations were detected in exons 5-9 in 2 blastic MCLs with strong (case no. 29) and moderate (case no. 42) p53 overexpression, respectively. Immunohistochemical detection of p53 protein in human tumors has been considered to be a consequence of the gene mutation and protein stabilization with a longer half-life. However, several studies have now shown that in some high grade non-Hodgkin’s lymphomas (NHLs), p53 overexpression is not always associated with detectable gene mutations. Although we can not completely rule out the existence of mutations in our 2 blastic MCLs overexpressing the protein, mutations in regions outside of exons 5 to 9 in NHL seem to be rare. The increased expression of the protein in these cases may be caused by nondetected mutations or by the stabilization of the protein by other mechanisms, or, alternatively, it may be related to the high proliferative activity of tumor cells. Overexpression of wild-type p53 has been observed in highly proliferating cells and reactive tissues in which it may represent a normal expression related to its function in the control of cell proliferation.

The presence of p53 mutations in aggressive MCLs is consistent with the observations in other hematopoietic disorders and NHLs in which p53 mutations mainly occur in high grade tumors and in association with progression of the disease. In particular, p53 alterations are rarely found in low grade lymphomas and indolent chronic lymphocytic leukemias. However, mutations are detected in high grade lymphomas evolving from B-cell chronic lymphocytic leukemia (Richter’s syndrome) and in transformed follicular lymphomas, suggesting that p53 mutations may be implicated in the histological progression of these tumors. Similar to those in follicular lymphomas, p53 mutations were only present in less than half of the blastic and large-cell types of MCLs, indicating that other mechanisms may also be implicated in the pathogenesis of these aggressive variants.

MCLs are considered indolent lymphomas with frequent relapses and an intermediate overall survival (median, 3 to 5 years) between low and high grade NHL. Histological progression from typical MCLs to more aggressive variants may occur in tumor relapses, with an increase in the mitotic

Fig 5. Histological section and p53 immunohistochemical staining of case no. 15. (A) Biopsy specimen obtained at the time of diagnosis shows a large-cell (anaplastic) MCL. (H&E; original magnification × 630). (B) Histological section of the biopsy specimen obtained 2 years later in the same patient. The cells are larger with irregular nuclei, disperse chromatin, and occasional nucleoli (H&E; original magnification × 630). (C) p53 immunostaining shows nuclear positivity in more than 50% of the cells. Sequencing analysis showed a p53 gene mutation in codon 273 (p53 immunostaining; original magnification × 400).
index, nuclear size, and chromatin dispersal. However, most aggressive variants of MCL are recognized at diagnosis, indicating that they may arise de novo as primary aggressive MCLs. In our series, the 8 aggressive cases were diagnosed at presentation. In 1 of these cases, a further morphological progression was observed at relapse after an interval of 24 months. In this tumor, the p53 mutation was found in the second biopsy specimen. Although no molecular studies could be performed in the first biopsy specimen of this patient, the strong overexpression of the protein with an intensity similar to that of the second sample suggests that p53 was already mutated at presentation. Similarly, the p53 mutations observed in the other 2 blastic MCLs were also detected during biopsies performed at diagnosis. These findings suggest that p53 mutations may occur in an early stage of the development of these aggressive variants of the tumors. This model seems to be different from that observed in follicular lymphomas, in which p53 mutations appear during the evolution of the tumor and are closely associated with its histological transformation to a high grade lymphoma.

The mechanisms by which p53 mutations may lead to the higher proliferative activity and more aggressive behavior of this subset of MCLs are not clear. Wild-type p53 participates in the control of cell cycle progression, particularly in a G1 checkpoint that allows the cell to repair DNA damage before progression in the cell cycle. Inactivation of p53 may favor the accumulation of other genetic lesions that would confer to the cells a selective growth advantage.

In conclusion, our findings indicate that p53 gene alterations are an infrequent phenomenon in MCLs, but that they are associated with a subset of aggressive variants and short survival. Further studies are needed to clarify other possible molecular mechanisms implicated in the pathogenesis of the aggressive MCLs in which no p53 mutations are detected.

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