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Expression of Retinoblastoma Gene Product (pRb) in Mantle Cell Lymphomas

Correlation with Cyclin D1 (PRAD1/CCND1) mRNA Levels and Proliferative Activity

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Mantle cell lymphomas (MCLs) are molecularly characterized by bcl-1 rearrangement and constant cyclin D1 (PRAD-1/CCND1) gene overexpression. Cyclin D1 is a G1 cyclin that participates in the control of the cell cycle progression by interacting with the retinoblastoma gene product (pRb). Inactivation of the Rb tumor suppressor gene has been implicated in the development of different types of human tumors including some high grade non-Hodgkin's lymphomas. To determine the role of the retinoblastoma gene in the pathogenesis of MCLs and its possible interaction with cyclin D1, pRb expression was examined in 23 MCLs including 17 typical and 6 blastic variants by immunohistochemistry and Western blot. Rb gene structure was studied in 13 cases by Southern blot. Cytogenetic analysis was performed in 5 cases. The results were compared with the cyclin D1 mRNA levels examined by Northern analysis, and the proliferative activity of the tumors was measured by Ki-67 growth fraction and flow cytometry. pRb was expressed in all MCLs. The expression varied from case to case (mean, 14.1% of positive cells; range, 1.3 to 42%) with a significant correlation with the proliferative activity of the tumors (mitotic index r = 0.85; Ki-67 r = 0.7; S phase = 0.73). Blastic variants showed higher numbers of pRb-positive cells (mean, 29%) than the typical cases (10%; P < 0.005) by immunohistochemistry and, concordantly, higher levels of expression by Western blot. In addition, the blastic cases also had an increased expression of the phosphorylated protein. No alterations in Rb gene structure were observed by Southern blot analysis. Cyclin D1 mRNA levels were independent of pRb expression and the proliferative activity of the tumors. These findings suggest that pRb in MCLs is normally regulated in relation to the proliferative activity of the tumors. Cyclin D1 overexpression may play a role in the maintenance of cell proliferation by overcoming the suppressive growth control of pRb. (Am J Pathol 1996, 148:1591–1600)

Chromosomal translocations and deregulation of genes rearranged in these processes are important mechanisms in the pathogenesis of several lymphomas. The t(11;14)(q13;q32) translocation is mainly associated with mantle cell lymphomas (MCLs),[1–4] although it may also occur in a minority of other B cell

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lymphoproliferative disorders.[5] This translocation activates the cyclin D1 (PRAD-1/BCL-1/CCND1) gene located 110 to 130 kb downstream from the major breakpoint of this rearrangement.[6–8] Recent studies have shown that cyclin D1 is overexpressed at both mRNA and protein levels in virtually all MCLs independently of the detection of t(11;14) translocation or *bcl*-1 rearrangements, indicating that deregulation of this gene is an important mechanism in the pathogenesis of this lymphoma.[8–12]

Cyclin D1 has been recognized as a G1 cyclin that participates in the control of the cell cycle progression at the G1 to S phase transition. Cyclin-D1-overexpressing cells have abnormal proliferative characteristics with shortened G1 phase and less dependence on growth factors.[13,14] Conversely, microinjection of anti-cyclin-D1 antibodies or antisense vectors arrest the cells in G1.[14–17] Cyclin D1 may also function as an oncogene cooperating with other oncogenes in cellular transformation.[18–20] However, the tumorigenic and transforming properties of cyclin D1 seem to be less effective than the conventional oncogenes. The possible additional oncogenic factors cooperating with cyclin D1 in the development of MCLs are unknown.

Recent studies have shown that cyclin D1 participates in the regulation of G1 phase by interacting with the retinoblastoma gene product (pRb), suggesting that both proteins may be involved in the same cell cycle control pathway. Cyclin D1 physically binds to pRb and, coupled with cyclin-dependent kinases, is capable of its phosphorylation, contributing to the inactivation of the pRb growth-suppressive effect.[21–23] Cyclin D1 overexpression may also overcome the growth-suppressing effect of pRb independently of pRb phosphorylation.[24] In addition, several observations suggest the existence of a regulatory loop between pRb and cyclin D1 in which pRb controls the expression of cyclin D1 at a transcriptional level[25–27]

MCLs may present with a variable mitotic activity, which is considered to be of prognostic significance in these tumors. Particularly, a blastic variant has been recognized with a high proliferative fraction and more aggressive behavior.[28,29] Inactivation of the Rb tumor suppressor gene has been implicated in the development of different types of human solid tumors in addition to retinoblastoma. Gene alterations and reduced pRb expression have been also observed in some non-Hodgkin's lymphomas, particularly in tumors with a high proliferative activity and high grade morphology.[30–32] The interactions between cyclin D1 and pRb have been studied in some solid tumors,[16,33,34] but the possible implication of the Rb gene in MCLs in which cyclin D1 is constantly up-regulated has not been well characterized.

To determine the role of the retinoblastoma gene in the development and progression of MCLs and its possible interaction with cyclin D1, we have examined the structure and expression of the Rb gene in a series of MCLs including typical and blastic variants. The results were compared with the cyclin D1 mRNA levels and the proliferative activity of the tumors.

Materials and Methods

Tissues

Tumor specimens from 23 MCLs were obtained from the files of the Pathology Departments of the Hospital Clinic, University of Barcelona, and Hospital Virgen de la Salud. The specimens included 16 lymph nodes, 3 spleens, 2 gastrointestinal specimens, 1 tonsil, and 1 skin biopsy. The cases were classified as nodular or diffuse and typical or blastic variants according to histological criteria previously described.[28,29,35] All of the cases were immunophenotyped using immunohistochemistry on frozen or fixed, paraffin-embedded tissue sections and/or cell suspensions by flow cytometry. *bcl*-1 rearrangement was studied by Southern blot analysis using a 2.3-kb *Sac*I fragment for the MTC probe and/or by polymerase chain reaction according to a previously described technique.[36]

Immunohistochemistry

Retinoblastoma protein expression was immunohistochemically assessed on formalin-fixed, paraffin-embedded sections of 22 cases. The immunohistochemical analysis of 1 blastic case was not evaluated because of a fixation artifact. The immunostaining was performed using the anti-Rb monoclonal antibody PMG3–245 (PharMingen, San Diego, CA), which recognizes an epitope between amino acids 300 and 380 of both phosphorylated and unphosphorylated human Rb protein. The growth rate and immunoreactivity preservation were similarly examined on formalin-fixed, paraffin-embedded sections using the anti-Ki-67 monoclonal antibody MIB-1 (Immunotech, Marseille, France), which recognizes a fixation-resistant epitope of this molecule.[37] Before the application of the primary antibodies, an antigen retrieval technique was performed. The deparaffinized and rehydrated slides were placed in 10 mmol/L citrate buffer, pH 6, and heated in a micro-

wave oven for 15 minutes at 700 W.[37] The anti-Rb and MIB monoclonal antibodies were incubated at a concentration of 10 μg/ml and 2μg/ml, respectively, overnight at 4°C. The antibodies were detected by means of the streptavidin-biotin-alkaline phosphatase (Biogenex, San Ramon, CA) technique using Fast-Red as chromogen. Levamisole was used to inhibit endogenous alkaline phosphatase. The slides were counterstained with hematoxylin. Sections from hyperplastic tonsils were used as positive control.

Quantitative immunohistochemical analysis was performed using a computerized digital analyzer (Microm Image Processing IMCO, Kontron Electronik, Munich, Germany) to score the nuclei of the tumor cells for the presence of Rb or Ki-67 proteins. The value measured was the percentage of the immunohistochemically stained area against that of the total nuclear area. Nuclear boundary optical density and antibody threshold were adjusted for each case examined. Five or more fields of each tumor were analyzed, until a minimum of 1000 cells had been examined. The fields were selected in each slide from the areas with the greatest number of stained cells.

Western Blot

Protein extraction was obtained from the frozen blocks of four blastic and five typical MCLs. In addition, protein samples were extracted from a reactive tonsil and the BT-20 breast carcinoma cell line. In each case, 10 frozen sections of 30 μm were incubated in 300 μl of ice-cold lysis buffer (50 mmol/L Tris-HCl, pH 8, 150 mmol/L NaCl, 0.02% sodium azide, 0.1% sodium dodecyl sulfate, 1% Nonidet P-40, and 0.5% sodium deoxycholate) containing 150 μg/ml phenylmethylsulfonyl fluoride and 50 μg/ml aprotinin for 30 minutes at 4°C. The cell debris was sedimented by centrifugation at 14,000 rpm at 4°C for 20 minutes. The clarified supernatants were collected, the protein content of the lysate was determined by the Bradford protein assay (Bio-Rad, Richmond, CA), and 40 μg of total cellular protein was run per lane on an 8% sodium dodecyl sulfate polyacrylamide gel and electroblotted to a nitrocellulose membrane (Amersham, Arlington Heights, IL). The membrane was blocked by overnight incubation in 5% dry milk and 0.1% Tween-20 at 4°C. The blocked membrane was then incubated with the same anti-Rb monoclonal antibody used for the immunohistochemical study at a final dilution of 1:100 for 1.5 hours. The membrane was then washed three times with phosphate-buffered saline, 0.1% Tween-20 for 15 minutes each time and exposed to sheep anti-mouse conjugated to horseradish peroxidase (Amersham) at a 1:1000 dilution for 1.5 hours. After washing, antibody binding was detected with chemiluminescence detection procedures according to the manufacturer's recommendations (Amersham).

Cytogenetic Analysis

Cytogenetic analysis was performed in five patients with leukemic expression. Four of these patients had a typical MCL and one was a blastic variant. Tumor cells isolated from peripheral blood were analyzed using G banding after cultures were stimulated with phytohemagglutinin and lipopolysaccharide from *Escherichia coli* for 72 hours. The blastic MCL had a complex karyotype and was also analyzed by a chromosome *in situ* hybridization technique using biotinylated probes of the whole chromosomes 1, 9, 11, 13, 14, 17, and 18 (Cambio, Cambridge, UK). In these cases, a specific study of the locus 13q14 was also performed using the probe P5116 (Oncor, Gaithersburg, MD). The karyotypes were established according to the International System for Human Cytogenetic Nomenclature.[38]

Northern and Southern Blot Analyses

Total RNA was isolated from frozen tissues in 20 cases by guanidine isothiocyanate extraction and cesium chloride gradient centrifugation.[39] Eight micrograms of total RNA were electrophoresed on a denaturing 1.2% agarose formaldehyde gel and transferred to Hybond-N membranes (Amersham, Buckinghamshire, UK). The membranes were prehybridized, hybridized, and washed as previously described.[11,40] The blots were hybridized with the PRAD-1/cyclin D1 probe, and the signals were quantified using a UVP-GDS5000 video densitometer (UVP, San Gabriel, CA) as described.[11,40] The PRAD-1/cyclin D1 gene expression of 14 cases were included in a previous report.[11]

Genomic DNA was extracted from additional frozen material available in 13 cases using proteinase K/RNAse treatment and phenol chloroform extraction. DNA from each case (10 μg) was digested with *Eco*RI, *Hin*dIII, and *Bam*HI, separated on 0.8% agarose gels, and transferred to Hybond-N membranes (Amersham). The membranes were hybridized with 3.8- and 0.9-kb retinoblastoma probes as previously described.[11,40]

Probes

Probes were radiolabeled using a random primer DNA labeling kit (Promega Corp., Madison, WI) with

[^{32}P]dCTP. The retinoblastoma probes used were Rb0.9 and Rb3.8 representing the 5′ and 3′ portions of Rb cDNA. These probes were a kind gift from Dr. R. A. Weinberg, Whitehead Institute, Cambridge, MA.[41] The PRAD-1 probe used was the 1.4-kb *Eco*RI fragment (λP1–4) of the pPL-8 partial cDNA clone of the PRAD-1 gene (kindly provided by Dr. A. Arnold, Massachussets General Hospital, Boston, MA).[7]

Flow Cytometry

DNA ploidy and S phase fraction were studied by flow cytometry in 14 cases (11 typical and 3 blastic variants). The analysis was performed on 50-μm-thick sections from formalin-fixed, paraffin-embedded tissues using a technique previously described.[42] The nuclear samples were stained with propidium iodide and analyzed with an Epics Profile II flow cytometer (Coulter Co., Hialeah, FL). Non-neoplastic cells in the section under study were used as the internal standard of the diploid channel as recommended by the DNA Cytometry Consensus Conference.[43] Discrimination of doublets and aggregates was performed on the basis of the pulse peak *versus* pulse area analysis with the Power Pack system (Coulter). Single-parameter histograms analyzed with the Multicycle software (Phoenix Flow Systems, San Diego, CA) were classified according to the guidelines on nomenclature of the Society for Analytical Cytology.[44]

Statistical Analysis

Differences among the histological subgroups in terms of proliferative activity, pRb expression, and cyclin D1 mRNA levels were compared by Mann-Whitney test. To determine the possible correlation between Rb expression, the proliferative activity of the tumors, and cyclin D1 levels, a linear regression analysis was performed by means of the Pearson product moment correlation. Data were analyzed with the BMDP statistical software package (BMDP Statistical Software, Los Angeles, CA).

Results

pRb Expression

Retinoblastoma protein was immunohistochemically detected in all MCLs. The staining pattern was always nuclear, although in mitotic cells the immunoreaction was distributed throughout the whole cytoplasm. Immunoreactive cells were homogeneously distributed within the tumor. The levels of pRb expression varied from case to case ranging from 1.3 to 42% of the cells (Table 1). The blastic variants of MCL showed significantly higher numbers of positive cells (29 ± 10%, mean ± SD) than typical cases (10 ± 6%; Table 2 and Figure 1). pRb expression in one nodular MCL included in this study showed very low pRb expression (4%). Residual germinal centers present in some tumors showed an intense pRb immunostaining, usually stronger than the surrounding lymphoma.

Immunoblot analysis confirmed the immunohistochemical observations. The four blastic MCLs examined had higher levels of pRb expression than the typical MCLs (Figure 2). The blastic MCL in which the immunohistochemical staining was not assessed showed a strong pRb expression by Western blot. In addition, the four blastic cases showed increased levels of phosphorylated Rb protein that was not detectable or imperceptible in the typical MCLs.

Proliferative Activity of MCLs

The proliferative activity of the tumors was variable. The values obtained with the three different parameters used in this study (mitotic index, Ki-67 growth fraction, and S phase determined by flow cytometry) were concordant. A significant correlation was found between the mitotic index and the Ki-67 growth fraction (r = 0.83; $P < 0.001$), mitotic index and S phase (r = 0.88; $P < 0.001$), and Ki-67 growth fraction and S phase (r = 0.82; $P < 0.001$). The blastic variants showed a higher proliferative activity than typical cases (Figure 1 and Table 2). The only nodular case included in the study showed low levels of cell proliferation.

A significant linear correlation was found between the pRb expression levels and the proliferative activity of the tumors. This correlation was found with all of the parameters assessed including the mitotic index (r = 0.85; $P < 0.001$), Ki-67 growth fraction (r = 0.71; $P < 0.001$), and the S phase (r = 0.73; $P < 0.003$; Figure 3).

Cyclin D1 was overexpressed in all of the cases examined. The levels of mRNA expression determined by densitometric analysis were heterogeneous, ranging from 0.5 to 2.4 arbitrary units (Table 1). No correlation was found between the mRNA levels and the proliferative activity of the tumors with any of the parameters examined. Concordantly, the pRB expression was independent of the cyclin D1 mRNA levels (r = 0.01).

The analysis of DNA ploidy by flow cytometry identified only three cases as aneuploid. These three cases were typical variants, and no particular alter-

Table 1. *Principal Characteristics of the 23 MCLs*

Biopsy	Age/sex	Stage	Histology	*bcl*-1 rearrangement	pRB (%)	Mitotic index*	Ki-67 (%)	S phase (%)	DNA ploidy	Cyclin D1 gene expression (AU)
3858	62/M	III	Blastic	GL	42	9.4	48.2	29.0	D	2.4
4958	59/M	IV(PB)	Blastic	R	18	3.2	36.8	8.0	D	1.9
3747	63/M	IV(PB)	Blastic	t(11;14)	33.3	7.5	34.4	11.0	D	1.3
441	63/M	IV	Blastic	R	31.1	5.1	36.4	ND	ND	1
1104	73/F	IV	Blastic	GL	21.1	5.0	35.4	ND	ND	1.4
15566	60/F	IV	Blastic-skin	ND	NE†	4.5	NE	ND	ND	ND‡
346	54/M	IV(PB)	Diffuse	R	11.2	1.3	14.1	5.3	D	1.6
5752	51/F	III	Diffuse	R	6	0.8	17.6	3.2	D	0.9
5377	71/M	IV(PB)	Diffuse	GL	8.5	1.5	12.6	5.8	A	1.9
3153	63/M	IV(PB)	Diffuse	GL	1.3	0.1	5.5	0.9	D	1.6
445	64/M	IV	Diffuse	ND	6	0.7	17.8	5.2	D	ND
2166	58/M	IV(PB)	Diffuse	GL	19.1	1.2	12.8	1.4	A	1
8482	60/M	IV(PB)	Diffuse	R;t(11;14)	8	0.4	20.7	1.5	D	ND
6449	76/M	IV	Diffuse	GL	16	2.9	0.5	0.6	A	1.2
9180	61/F	IV(PB)	Diffuse	R;t(11;14)	24.6	0.3	2.6	2.5	D	0.5
T-3537	68/M	IV	Diffuse	ND	9.9	0.4	8.6	ND	ND	1.3
T-3049	70/M	IV	Diffuse-GI	GL	11.8	0.2	8.1	ND	ND	0.8
T-3419	77/M	IV	Nodular-GI	GL	4	0.4	2.8	ND	ND	1
T-3230	63/M	IV	Diffuse	GL	12.2	0.9	10.2	ND	ND	0.9
T-2308	87/F	IV	Diffuse	R	3.8	0.5	8.3	ND	ND	2.2
T-692	60/F	IV	Diffuse	R	5.1	0.7	8.3	ND	ND	2
14192	72/M	III	Diffuse	GL	18.5	1.1	14.1	1.8	D	0.7
15855	68/M	III	Diffuse	GL	6.2	1.9	12.7	5.1	D	3

AU, arbitrary units of mRNA cyclin D1 overexpression; PB, peripheral blood involvement; GI, gastrointestinal localization; pRB, retinoblastoma protein expression; R, rearrangement; GL, germline; D, diploid; A, aneuploid; ND, not determined; NE, not evaluated due to a fixative artifact.

*Mitoses per high power field.

†This case showed strong pRb expression by Western blot (see Figure 2).

‡Cyclin D1 overexpression was detected in this case by Western blot.

ations in the pRb expression, proliferative activity, or cyclin D1 expression were detected (Table 1).

Southern Blot and Cytogenetic Studies

The DNA structure of the Rb gene was examined by Southern blot analysis in 13 cases including 3 blastic variants using the 3.8- and 0.9-kb Rb cDNA probes specific for the 3′ and 5′ regions of the Rb locus. No abnormalities in the intensity or distribution of the expected bands were observed with any of the restriction enzymes (Figure 4).

Table 2. *Comparison of pRb Expression, Proliferative Activity, and cyclin D1 mRNA Levels between Morphological Variants of MCL*

	MCL		
	Typical (n = 17)	Blastic (n = 6)	*P*
pRb (%)	10.1 ± 6.3	29.1 ± 9.7	0.001
Mitotic index*	0.9 ± 0.7	5.8 ± 2.2	0.003
Ki-67 (%)	10.4 ± 5.7	38.2 ± 5.6	0.001
S phase (%)	3.1 ± 1.9	16 ± 11.4	0.011
Cyclin D1 (AU)	1.3 ± 0.5	1.6 ± 0.6	>0.05

pRb, retinoblastoma protein expression; AU, Arbitrary units.

Cytogenetic studies were performed in five cases including one blastic variant. No abnormalities of chromosome 13 were observed in any of the four typical MCLs. A t(11;14)(q13;q32) was detected in two typical cases and in the blastic variant. The blastic MCL showed a complex karyotype: 44, XY, del(1), del(9)(p12)t(11;14)(q13;q32), -13, dup(13) der(17) t(13;17)(q11;p11). The presence of the derivative der(17) implies that a copy of part of chromosome 13 was translocated to the short arm of chromosome 17. The analysis with a specific probe for the locus 13q14 showed the presence of two hybridization signals, one localized in each chromatid. No DNA was available in this case for Southern blot analysis. Despite chromosome 13 abnormalities, pRb expression in this case was one of the highest in the series (33%).

Discussion

In this study we have examined the structure and expression of the Rb gene in a series of MCLs. Our

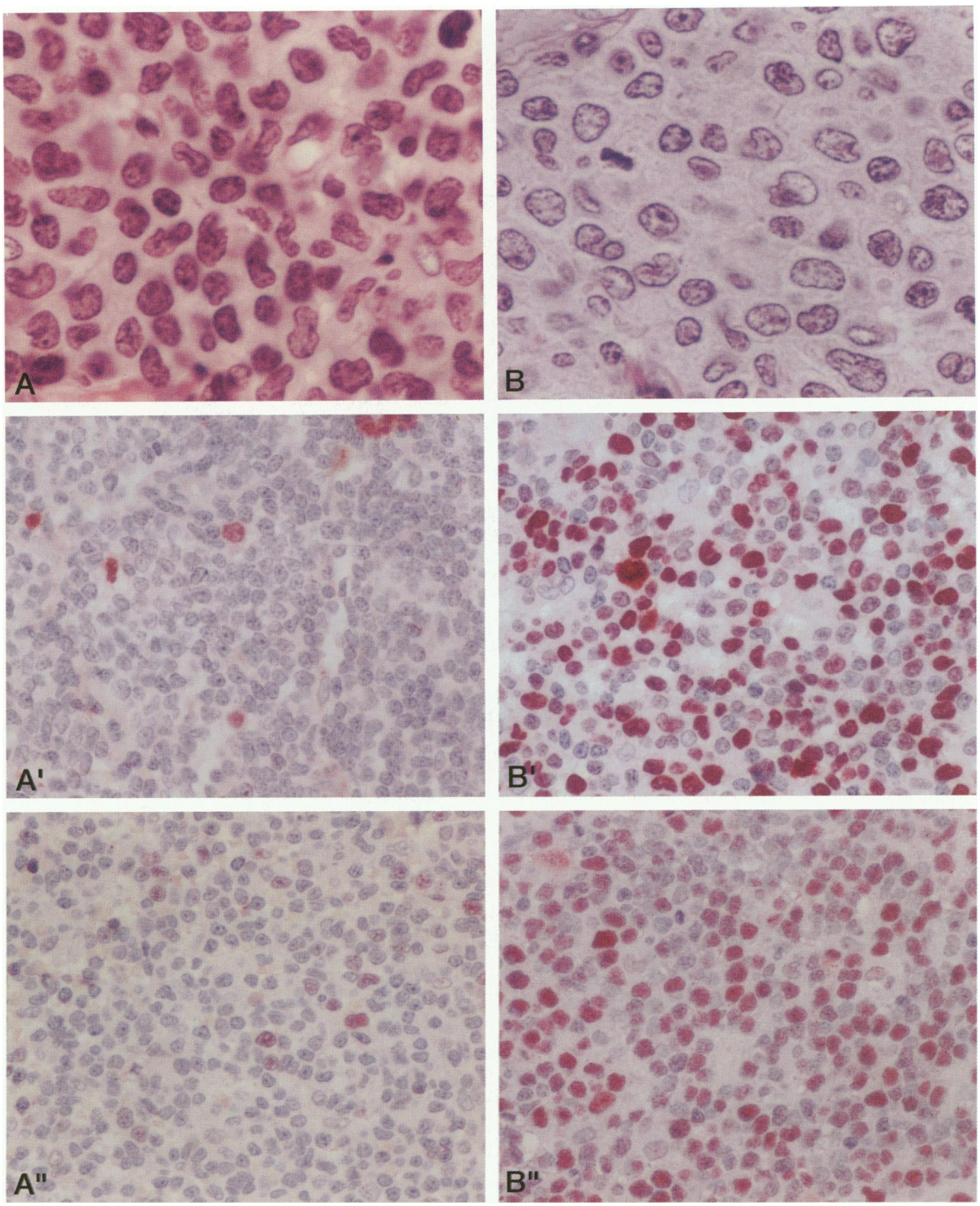

Figure 1. *pRb expression and Ki-67 growth fraction in two representative typical* (**A**) *and blastic* (**B**) *MCLs.* **A**: *Typical MCL (H&E; magnification, ×1000) with the respective Ki-67 immunostaining (alkaline phosphatase, MIB antibody; ×650;* **A'**), *and the pRb expression level (alkaline phosphatase, anti-pRb antibody; ×650;* **A"**). **B**: *Blastic MCL (H&E; ×1000) with the respective Ki-67 immunostaining (alkaline phosphatase, MIB antibody; ×650;* **B'**) *and the pRb expression level (alkaline phosphatase, anti-pRb antibody; ×650;* **B"**).

results indicate that pRb is expressed in all of these tumors, but its levels varied from case to case with a significant correlation between the levels of expression and the proliferative activity of the tumors measured by the mitotic index, Ki-67 growth fraction, and flow cytometry. In this sense, the blastic variants

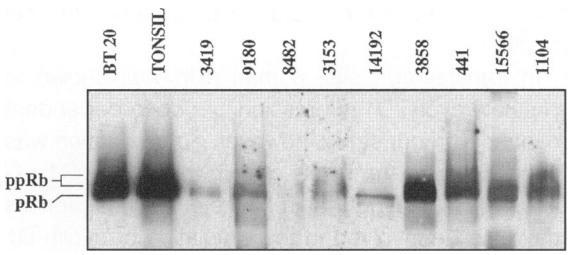

Figure 2. *Western blot analysis of retinoblastoma protein in MCLs. The blastic variants of MCLs (cases 3858, 441, 15566, and 1104) show higher expression of pRb than the typical variants (cases 3419, 9180, 8482, 3153, and 14192). In addition, the blastic cases also have an increased expression of phosphorylated protein.*

included in our series showed the highest levels of pRB expression. In addition, blastic MCLs showed increased levels of phosphorylated Rb protein.

Several studies have demonstrated that pRb expression in normal and neoplastic cells oscillates during the cell cycle with very low levels in resting G0/G1 cells and a gradual increase when the cell progresses to S and G2/M phases.[31,45] Similarly, proliferative lymphocytes in thymic cortex and reactive germinal centers are strongly positive for pRb.[31] Martinez et al[31] have also found a significant correlation between pRb expression in non-Hodgkin's lymphomas and Ki-67 growth fraction. Our findings suggest that pRb expression in MCLs may be normally regulated with low levels in tumors with low growth fraction and a progressive increase in expression and phosphorylation in tumors with a higher proliferation. The increased expression observed in highly proliferative tumors has been suggested to represent a growth-inhibitory response to control cell proliferation.[45]

Alteration in the regulatory control of pRb may be important in the pathogenesis of a subset of high grade non-Hodgkin's lymphomas in which Rb expression is not detectable despite a high proliferative activity.[31,32] In a previous study, Ginsberg et al[30] described a deletion in the Rb locus associated with undetectable levels of mRNA in 1 of 15 intermediately differentiated lymphomas, now considered to be the same entity as MCL.[35] This particular case was a blastic variant with a very aggressive evolution, suggesting that Rb alterations could play a role in the progression of some MCLs. In our study, however, no gross alterations in the structure of the gene were observed by Southern blot analysis in any of the MCLs including the 3 blastic variants examined. This finding was concordant with the apparently nor-

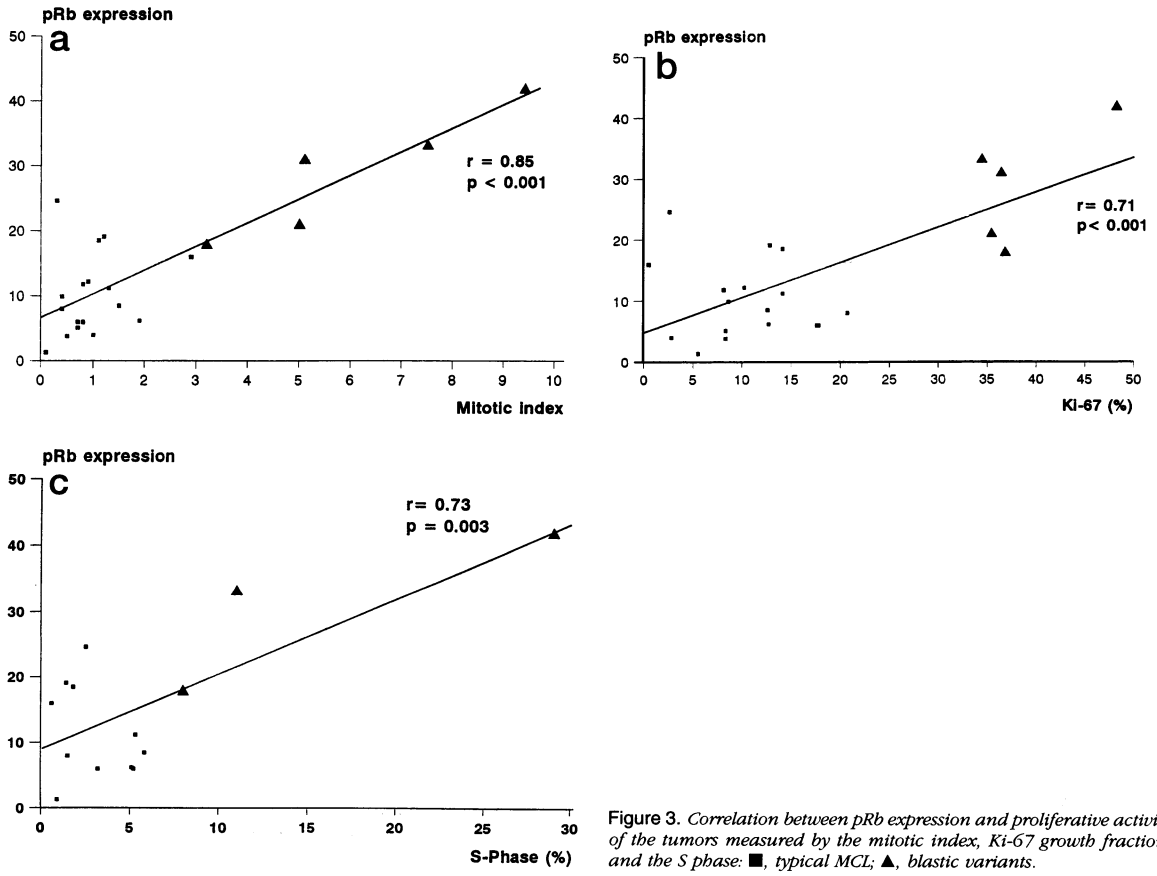

Figure 3. *Correlation between pRb expression and proliferative activity of the tumors measured by the mitotic index, Ki-67 growth fraction, and the S phase: ■, typical MCL; ▲, blastic variants.*

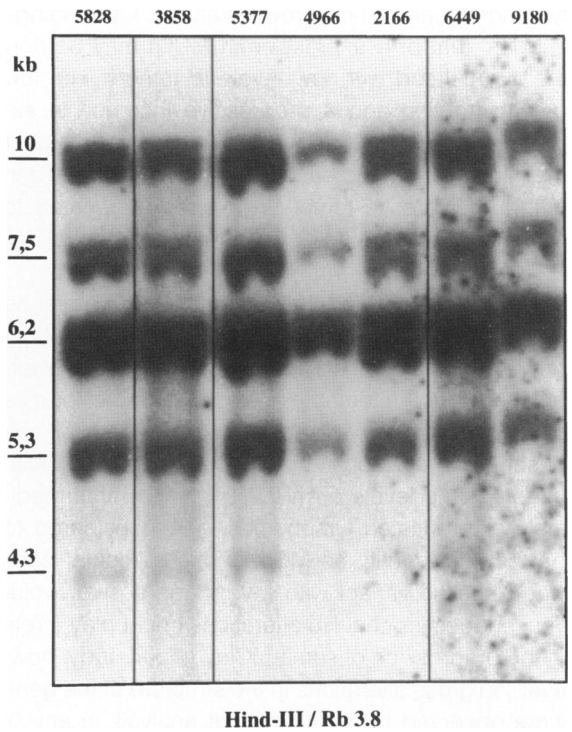

Figure 4. *Southern blot analysis of genomic DNA restricted with* Hin*dIII and hybridized with the 3.8-kb cDNA probe. No alterations are seen in the distribution or relative intensity of the expected bands.*

mal pattern of pRb expression in all of the cases. Interestingly, a blastic MCL included in our study showed a complex karyotype with abnormalities of both chromosomes 13. One chromosome 13 was translocated to chromosome 17, and the other showed a duplication of part of the long arm. In this case, however, the pattern of pRb expression followed the general model with high immunostaining in 33% of the cells and a high proliferative activity.

Several studies have indicated that pRb and cyclin D1 are closely interdependent in the regulation of cell cycle progression. One of the interactions between these two proteins seems to be the stimulation of cyclin D1 transcription by a functional pRb.[27] On the other hand, cyclin D1 coupled with cyclin-dependent kinases, particularly cyclin-dependent kinase-4, may phosphorylate pRb leading to its inactivation.[21–23] Concordantly with this postulated regulatory loop between pRb and cyclin D1, cell lines and human tumors with inactivated pRb show very low or undetectable levels of cyclin D1, whereas in neoplasms with cyclin D1 overexpression, pRb seems to be normal.[26,33,34] These findings suggest that the regulatory growth control of pRb in some tumors may be overcome either by Rb inactivation or cyclin D1 overexpression. The apparently normal expression of pRb in MCLs in which cyclin D1 is constantly up-regulated would be consistent with this model.

In transfected cells, normal pRb was shown to stimulate cyclin D1 expression in a dose-dependent manner.[27] In our study, however, no correlation was found between the levels of pRb and cyclin D1 expression, indicating that in MCLs other mechanisms may also be involved in the regulation of cyclin D1 levels. In some MCLs, cyclin D1 transcripts may have a shorter 3′ untranslated region with a loss of regulatory sequences that lead to a longer half-life of the messages.[46,47]

Normal lymphoid cells and lymphomas usually express cyclin D2 and D3 instead of cyclin D1. However, in malignant lymphoid neoplasms bearing the t(11;14) translocation, overexpressed cyclin D1 is functional and becomes essential for G1 progression, suggesting a role of this protein in the maintenance of an autonomous growth in these neoplasms.[48] In our study we have observed a lack of correlation between the cyclin D1 mRNA levels and the proliferative activity of MCLs.[11] Similarly, in mouse skin tumors, cyclin D1 expression did not correlate with the proliferation index and it was suggested to have a role in the acquisition of autonomous growth.[49] Although we cannot rule out that protein levels may be better related to the growth fraction than mRNA, these findings suggest that other factors may cooperate with cyclin D1 in the pathogenesis of the blastic variants of MCLs. In this sense, we have shown recently that p53 gene mutations may be implicated in the pathogenesis of a subset of aggressive variants of MCLs. p53 gene mutations and protein ovexpression were found in 50% of aggressive MCLs but in none of 37 typical cases.[50]

In conclusion, our findings suggest that pRb expression in MCLs is normally regulated in relation to the proliferative growth fraction of the tumors. The constant cyclin D1 overexpression may play a role in the maintenance of cell proliferation by overcoming the suppressive growth control of pRb. Additional studies are needed to understand the additional oncogenic factors that may cooperate with cyclin D1 in the progression of MCLs.

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