

**CDK4 and MDM2 Gene Alterations Mainly Occur in Highly Proliferative and Aggressive Mantle Cell Lymphomas with Wild-type INK4a/ARF Locus**

Luis Hernández, Silvia Beá, Magda Pinyol, German Ott, Tiemo Katzenberger, Andreas Rosenwald, Francesc Bosch, Armando López-Guillermo, Jan Delabie, Dolors Colomer, Emili Montserrat, and Elias Campo

Abstract

Amplification of 12q13 locus occurs in some mantle cell lymphomas (MCL), potentially involving CDK4 and MDM2 genes. To determine the role of these genes in MCL, we have examined their gene status and expression and their relationship to INK4a/ARF and p53 gene aberrations in 69 tumors. Increased CDK4 gene copy number was detected in 4 of 19 (21%) highly proliferative blastoid variants and was associated with mRNA and protein overexpression. Three additional cases showed mRNA overexpression with no structural alterations of the gene. MDM2 gene overexpression was detected in three blastoid tumors (16%) with no relationship to gene copy gains. INK4a/ARF and p53 aberrations were observed in 13 and 12 tumors, respectively. Four of the seven lymphomas with CDK4 aberrations had concurrent inactivation of p53 gene, whereas only one case had a concomitant homozygous deletion of INK4a/ARF. No other gene aberrations were found in the three cases with MDM2 overexpression. Patients with INK4a/ARF deletions or simultaneous aberrations of p53 and CDK4 had a significantly shorter median survival (17 months) than patients with isolated alterations of p53, MDM2, or CDK4 (32 months) and patients with no alterations in any of these genes (77 months). The prognostic impact of the concomitant oncogenic alterations of the p14ARF/p53 and p16INK4a/CDK4 pathways was independent of the proliferation of the tumors. These findings indicate that CDK4 and MDM2 gene alterations mainly occur in MCL with a wild-type INK4a/ARF locus and may contribute to the higher proliferation and more aggressive behavior of the tumors.

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Introduction

Mantle cell lymphoma (MCL) is an aggressive B-cell lymphoma genetically characterized by the t(11;14)(q13;q32) translocation leading to the overexpression of the cyclin D1 gene (1). In addition to the primary oncogenic activation of cyclin D1, alterations in other G1 cell cycle regulators have also been found, particularly in blastoid variants with a higher proliferation and more aggressive clinical behavior (2–4). A recent microarray analysis of a large series of MCL has shown that the proliferation gene signature is the most powerful prognostic indicator in these tumors (5). Previous studies have indicated that the high proliferative activity of MCL is associated with different oncogenic aberrations involving p53, INK4a/ARF locus, BMI-1, and structural abnormalities in the 3′ untranslated region of the cyclin D1 gene (2–7). However, these oncogenic alterations only account for a subset of highly proliferative and aggressive variants of MCL, suggesting that other oncogenic events targeting cell cycle regulation may occur in these tumors.

CDK4 and CDK6 are the catalytic subunits of the CDK-cyclin D complexes in G1 cell cycle phase (8, 9). The kinase activity of these complexes is involved in the transit of the cell through the G1 restriction point mainly by the phosphorylation of pRb (10). Several experiments have shown that the oncogenic potential of CDK4 gene may be also necessary for the transforming and immortalizing effect of cyclin D1 (11, 12). CDK4 gene maps to 12q13, a region frequently amplified in several human tumors, including malignant lymphomas (13–16). In addition to CDK4, the 12q13 locus also contains MDM2, a negative regulator of p53 that mediates its proteasome-dependent degradation (17). Similarly to CDK4, MDM2 gene amplification and overexpression have been described in different types of solid tumors (18). MDM2 overexpression, but rarely gene amplification, has been observed in malignant lymphomas, including occasional MCL (19, 20).

CDK4 and MDM2 are downstream elements negatively regulated by p16INK4a and p14ARF proteins, respectively, and are encoded by the INK4a/ARF locus. Inactivation of INK4a/ARF is a common oncogenic event in aggressive MCL associated with high proliferation of the tumors and poor outcome of the patients (21). The previous identification of 12q13 gains and amplifications in MCL (15) suggest that CDK4 and MDM2 may be targets of this genetic alteration and may participate in the pathogenesis of MCL as alternative mechanisms to CDK4/ARF inactivation. The role of these genes in two different pathways, ARF/MDM2/p53 and p16INK4a/CDK4/pRB, regulating cell proliferation, senescence, and apoptosis (10, 22) would suggest that oncogenic aberrations in these elements may play an important role in determining the tumor behavior and clinical outcome of the patients. In this study, we have investigated the implications of CDK4 and MDM2 gene alterations in the pathogenesis of MCL and the possible relationship of these alterations with the inactivation of p53 and INK4a/ARF locus and their impact on the clinical and biological behavior of the tumors.

Requests for reprints: Elias Campo, Department of Pathology, Hospital Clinic, Institut d’Investigacions Biomediques August Pi i Sunyer, University of Barcelona, Villarroel 170, 08036 Barcelona, Spain. Phone: 34-93-227-5450; Fax: 34-93-227-5717; E-mail: ecampos@clinic.ub.es.

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Materials and Methods

Case Selection. Tumor specimens from 69 MCLs were obtained from the Department of Pathology, Hospital Clinic, University of Barcelona (Barcelona, Spain; 37 cases); University of Würzburg (Würzburg, Germany; 25 cases); and Norwegian Radium Hospital (Oslo, Norway; 7 cases). These tumors consisted of 50 typical and 19 blastoid variant of MCL according to the WHO classification (23). A series of nonneoplastic lymphoid samples were also included as normal references for the real-time quantitative PCR (qPCR) assays, including 4 tonsils, 3 reactive lymphoid nodes, and 5 samples of normal peripheral blood lymphocytes (PBL) obtained from different healthy blood donors.

**CDK4 and MDM2 Gene Amplification Analysis.** Genomic DNA was obtained from frozen material of the 69 MCL cases using proteinase K/RNase treatment and phenol/chloroform extraction. At least three replicates of 40 ng genomic DNA were subjected to qPCR analysis to study the presence of CDK4 and MDM2 gene dosage. Sequences of the CDK4 detection probe and primers were designed using the Primer Express software as follows: CDK4 forward 5'-CATGTAGGACCAGGACTAAGAGA-3' and CDK4 reverse 5'-GATGTTTGGCAGTGGCAA-3'. The CDK4 probe 5'-CTGGCAAAGGCACCCCCACCA-3' was labeled with 6-carboxyfluorescein as the reporter dye and 6-carboxytetramethylrhodamine as the quencher fluorescent and exposed as described previously (1).

**Gene Amplification Analysis.** Genomic DNA was digested with EcoRl, BamHl, and Hindll restriction enzymes (Bethesda Research Laboratories, Gaithersburg, MD), separated on 0.8% agarose gels, and transferred to Hybond-N membranes (Amersham, Buckinghamshire, United Kingdom). The membranes were prehybridized and hybridized with the full cDNA CDK4 probe (26) previously radiolabeled using a random primer labeling kit (Promega Corp., Madison, WI) with [α-32P]dCTP (Amersham). The membrane was washed and exposed as described previously (1).

**P53 Gene Analysis.** A screening for mutations of the p53 gene was done on exons 4 to 8 using primers and PCR conditions described previously by single-strand conformational polymorphism and direct sequencing of both DNA strands (21). Deletions of p53 gene had been analyzed previously in 27 MCL cases by Southern blot using the same conditions above described for CDK4 gene analysis. p53 probe was a 2.0-kb EcoRI-BamHl fragment of the pLAd5 (pArgPS3) cDNA clone containing the entire coding region of the p53 gene, which was kindly provided by Dr. L.V. Crawford (Imperial Cancer Research Foundation, Cambridge, UK; ref. 30). The β-actin probe was also used as a loading control.

**INK4a Locus Deletion Analysis.** Deletions of the INK4a/ARF locus were analyzed in the whole series by qPCR using primers, and probe conditions published elsewhere (33). p16INK4a/ARF and β-actin were used as control genes in this assay. Five control DNA samples obtained from PBLs of different healthy blood donors were used to establish the cutoff ratio for the INK4a/ARF exon 2 deletions. This cutoff ratio was set as the mean ratio minus 3 SD units (5). The cutoffs for INK4a/ARF and INK4a/β-actin were 0.66 and 0.5, respectively. A sample that yielded an amplification ratio below the cutoff ratio was considered to have a genomic deletion of INK4a/ARF locus.

Southern blot analysis was done previously in 27 of these MCL cases using the same conditions above described for CDK4 gene analysis, and...
p16INK4a probe was a fragment of exon 2 obtained by PCR with the use of primers described previously (3). The β-actin probe was also used as a loading control.

Bicolor fluorescence in situ hybridization (FISH) could be done in 18 additional MCL cases. To obtain well-preserved and separately located mononuclear cells for bicolor FISH, methanol/acetic acid (3:1)-fixed cell suspension from cytogenetic preparations were dropped onto glass slides. The analyses of the p16INK4a deletion status were carried out using LSI p16(9p21)/CEP 9 dual-color probe according to the manufacturer’s advice (Abbott GmbH & Co. KG, Wiesbaden, Germany). At least 100 intact nuclei per case were evaluated, and an aberrant clone was defined according to the cutoff level evaluated in control studies with five reactive lymph node cases. To obtain well-preserved and separately located specimens by a Zeiss Axioskop2 fluorescence microscope (Zeiss, Jena, Germany).

The mean ± SD, 1.21 RU). Reactive lymphoid tissues showed significantly higher levels of mRNA expression than all other groups of tumors (mean ± SD, 65.74 ± 41.6 RU; P < 0.01; Fig. 2). In addition to the four cases with gene amplification, three cases, one typical and two blastoid MCL tumors, with no apparent structural alterations of the gene, also showed very high levels of CDK4 mRNA expression ranging from 23.45 to 35.31 RU, similar to some cases with amplification of the gene. A Northern blot study could be done in 27 tumors, including 2 cases with gene amplification and mRNA overexpression by qPCR analysis. These two tumors had concordant high levels of CDK4 mRNA detected by Northern blot (Fig. 1). All the remaining cases with no overexpression by reverse transcription-PCR showed relatively similar lower CDK4 levels than those observed in reactive lymphoid tissues.

CDK4 protein expression was also examined by Western blot in 18 tumors, including 3 cases with increased CDK4 gene copy number (2 amplifications and 1 allelic gain) and mRNA overexpression. These 3 cases had the highest CDK4 protein levels in the whole series (mean ± SD, 3.3 ± 0.4 RU), whereas the remaining 15 tumors showed lower protein expression (mean ± SD, 1.1 ± 0.5 RU), with this difference being significant (P = 0.008; Fig. 1). These results indicate that CDK4 protein expression is up-regulated concordantly with the increased mRNA levels and the amplification of the gene.

### Results

**CDK4 Gene Amplification and Overexpression.** Increased CDK4 gene copy number was detected in 4 of the 69 (6%) MCL, including 3 amplifications and 1 allelic gain (Table 1; Fig. 1). The four cases with CDK4 gene alterations were blastoid MCL variants (21%). A Southern blot analysis of the CDK4 gene could be done in 37 of these cases, including 2 of the 4 cases with increased CDK4 gene dosage detected by qPCR. This study showed a CDK4 increased gene copy number only in the 2 cases detected by qPCR. The values obtained by Southern blot were concordant although slightly higher than the qPCR (Southern blot versus qPCR: 2 versus 1.6 and 8 versus 5 gene copy number, respectively).

**CDK4 mRNA expression was examined by real-time reverse transcription-qPCR in 62 MCL, 46 typical, and 16 blastoid variants, including the 4 cases with increased gene dosage, 7 reactive lymphoid tissues (4 tonsils and 3 lymph nodes), and 5 samples of PBLs. CDK4 mRNA expression was very low in quiescent PBLs (mean ± SD, 1.21 ± 0.71 RU).** Reactive lymphoid tissues showed significantly higher mRNA levels (mean ± SD, 8.34 ± 1.4 RU) than PBLs (P = 0.004) and were relatively similar to those observed in typical MCL (mean ± SD, 8.56 ± 4.93 RU). Blastoid MCL without CDK4 gene amplification showed a moderate 1.6-fold increased expression (mean ± SD, 13.78 ± 8.95 RU) in comparison with typical variants. However, the four MCLs with CDK4 increased gene dosage showed significantly higher levels of mRNA expression than all other groups of tumors (mean ± SD, 65.74 ± 41.6 RU; P < 0.01; Fig. 2). In addition to the four cases with gene amplification, three cases, one typical and two blastoid MCL tumors, with no apparent structural alterations of the gene, also showed very high levels of CDK4 mRNA expression ranging from 23.45 to 35.31 RU, similar to some cases with amplification of the gene. A Northern blot study could be done in 27 tumors, including 2 cases with gene amplification and mRNA overexpression by qPCR analysis. These two tumors had concordant high levels of CDK4 mRNA detected by Northern blot (Fig. 1). All the remaining cases with no overexpression by reverse transcription-PCR showed relatively similar lower CDK4 levels than those observed in reactive lymphoid tissues.

CDK4 protein expression was also examined by Western blot in 18 tumors, including 3 cases with increased CDK4 gene copy number (2 amplifications and 1 allelic gain) and mRNA overexpression. These 3 cases had the highest CDK4 protein levels in the whole series (mean ± SD, 3.3 ± 0.4 RU), whereas the remaining 15 tumors showed lower protein expression (mean ± SD, 1.1 ± 0.5 RU), with this difference being significant (P = 0.008; Fig. 1). These results indicate that CDK4 protein expression is up-regulated concordantly with the increased mRNA levels and the amplification of the gene.

**MDM2 Gene Amplification and Expression.** MDM2 allelic gains were observed in eight MCL cases (two typical and six blastoid variants), but no gene amplification was found. Three of these cases were associated with CDK4 gene amplifications. MDM2 mRNA expression was low in quiescent PBLs (mean ± SD, 1.5 ± 0.4 RU). Reactive lymphoid tissues showed higher mRNA levels (mean ± SD, 2.5 ± 0.9 RU) that were relatively similar to those observed in typical MCL (mean ± SD, 2.2 ± 1.6 RU) and blastoid MCL (mean ± SD, 3.59 ± 2.9 RU). Only three blastoid MCL cases had extremely high levels of mRNA, but no clear relationship to the gene copy number was observed. Although two of these cases showed an allelic gain, the expression levels in the remaining six tumors with allelic gains were similar to other tumors with a normal gene dosage (Fig. 2, bottom). Interestingly, the mRNA overexpression of these three cases was concordant with high protein expression detected by immunohistochemistry.
because these cases had >40% of positive cells, whereas all the remaining cases with low levels of mRNA showed <5% of positive cells.

**Correlation among CDK4, MDM2, INK4a, and p53 Alterations.**

Aggressive MCL is characterized by frequent inactivation of p53 and INK4a/ARF genes (2, 3, 7). To determine whether the alterations in CDK4 and MDM2 genes could be concomitant or alternative molecular phenomena to aberrations in other elements of these related oncogenic pathways, we examined the status of the p53 gene and the INK4a/ARF locus in the same series of tumors.

p53 gene alterations were observed in 12 (17%) cases, including 3 (6%) typical and 9 (47%) blastoid variants (Table 1). These alterations were two homozygous deletions, an insertion of 5 bp resulting in a truncated protein (FS506STOP), and nine missense mutations (T155N, V172F, G245S, I255F, R273C, P278L, E286K, and two cases with the identical R273H mutation). The structural and functional information of these p53 variants was searched in several available databases (35, 36). Thus, the p53 mutations were in the functional domains L2 loop (T155N, G245S, and V172F), L3 loop (G245S), and LSH motif (P278L, E286K, R273C, and R273H). I255F is in the conserved domain IV and it has been recorded 29 times in different neoplasias in the IARC database (version R9, July 2004 update; ref. 36). No differences in the survival of the patients were observed according to the specific p53 mutated functional domains.

Deletions of the INK4a/ARF exon 2 by qPCR were found in 13 (19%) MCL, 8 of 50 (16%) typical and 5 of 19 (26%) blastoid variants.
(Table 1). To confirm the results obtained by PCR, the INK4a/ARF locus deletions were also examined by Southern blot and FISH in 27 and 18 cases, respectively. INK4a/ARF homozygous deletions were detected by Southern blot in 3 MCL cases that were also detected by qPCR, whereas the remaining 24 cases showed a germ line INK4a/ARF by both techniques. FISH analysis showed a deletion at INK4a/ARF locus in 5 cases that were also detected by PCR, whereas no deletions were observed in 12 cases by FISH and PCR. Only one case was found to be deleted by PCR but not by FISH. These findings indicate a good correlation among these three techniques in the detection of INK4a/ARF locus deletions.

CDK4 gene amplification and/or overexpression were detected as an isolated molecular alteration in two cases and concurrent with p53 aberrations in four additional tumors. However, CDK4 gene alterations were associated with INK4a/ARF deletions only in one case. Similarly, only one case harbored a simultaneous inactivation of both p53 gene and INK4a/ARF locus. The three tumors with MDM2 overexpression did not show alterations in any of the other genes (Table 2; Fig. 3).

**CDK4, MDM2, p53, and INK4a/ARF Alterations and Cell Proliferation.** CDK4, MDM2, p53, and INK4a/ARF genes are involved in cell cycle regulatory pathways. To determine the relationship among these gene alterations and the proliferation of the tumors, we analyzed the proliferation index of these tumors by immunostaining for the Ki-67 proliferation related antigen. The median proliferative index in this series was 30%. Alterations of these four genes were more frequently found in tumors with a high proliferative rate (Ki-67 >30%). Thus, aberrations in one or two of these genes was observed in 21 of 30 (70%) tumors with high proliferation but only in 5 of 32 (16%) cases with a low proliferative index (P < 0.01; Fig. 3). However, no significant differences in the proliferation rate were observed among tumors with only CDK4 (mean Ki-67+ 31%), MDM2 (mean Ki-67+ 52%), or p53 (mean Ki-67+ 45%) alterations and cases with concurrent aberrations of the p53 and CDK4 (mean Ki-67+ 55%) and tumors with inactivation of the INK4a/ARF locus (mean Ki-67+ 42%).

**CDK4, p53, and INK4a/ARF Alterations and Survival.** Inactivation of p53 and INK4a/ARF locus has been associated with poor survival in MCL (2, 21). The INK4a/ARF locus encodes for p14ARF and p16INK4a genes, which act as upstream negative regulators of MDM2/p53 and CDK4/pRb, respectively. Deletions of this locus inactivate simultaneously both p53 and CDK4/pRb pathways. Similarly, the concomitant occurrence of p53 and CDK4 aberrations may also inactivate both pathways simultaneously. To investigate the prognostic significance of CDK4 and MDM2 alterations and its potential effect in comparison with the aberrations of p53 and INK4a/ARF in MCL, 44 patients with appropriate clinical information and follow-up were included in the analysis. Patients were grouped in three categories: group A, 23 patients with no alterations in any of these genes; group B, 8 patients with alterations only in p53 (4 patients), MDM2 (2 patients), or CDK4 (2 patients); and group C, 13 patients with concomitant alterations in both regulatory pathways including 4 cases with p53 mutations and CDK4 amplification/over-expression and 9 cases with deletions of the INK4a/ARF locus. The group of patients with inactivation of both pathways (group C) had a significantly worse prognosis (median survival, 17 months) than patients with alterations only in p53 or CDK4 (group B; median survival, 32 months) or no alterations in any of these genes (group A; median survival, 77 months; P < 0.001; Fig. 4).

Patients with high proliferative tumors (Ki-67 >30%) also showed a significantly worse outcome (P < 0.01) than patients with low proliferation (median survival, 25 versus 95 months, respectively; Fig. 4).

To assess whether the prognosis significance of the concomitant inactivation of the two ARF/MDM2/p53 and p16/CDK4 pathways was independent of the proliferative activity of the tumors, we did a multivariate Cox analysis, including these two variables. In the model, proliferation considered either as a continuous variable (P = 0.0001; relative risk, 1.04; 95% confidence interval, 1.02-1.06) or as a categorical variable (Ki-67 cutoff >30%; P = 0.006; relative risk, 5.7; 95% confidence interval, 1.65-19.77) kept its prognostic significance along with the inactivation of the two pathways (P = 0.0001; relative risk, 3.1; 95% confidence interval, 1.82-5.29 and P = 0.006; relative risk, 2.2; 95% confidence interval, 1.25-3.82, respectively). A further evidence of the independence of these two factors can be observed in Fig. 4 (bottom). Patients with high proliferation could be subdivided in two groups with a significantly different survival according to the inactivation of the two pathways (median survival, 32 versus 17 months, respectively; P < 0.01) in spite that the two groups of tumors had a similar proliferative activity (means, 51.8% versus 51.5%, respectively).

**Discussion**

MCL is genetically characterized by the oncogenic activation of the G1-phase regulatory element cyclin D1 (1). In addition to this primary event, inactivation of other cell cycle regulators, such as p53 and INK4a/ARF genes, confer a more aggressive behavior to the

| Table 2. Relationship between gene alterations and histologic subtype of MCL |
|-------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                   | n               | One pathway impaired | Two pathways impaired |
|                                   | CDK4 only       | MDM2 only         | p53 only         | INK4a/ARF only  | CDK4 + INK4a   | CDK4 + p53     | p53 + INK4a     |
| Typical (%)                       | 46              | 1 (2)             | 3 (6)            | 8 (17)          | 0              | 1 (2)          | 0              |
| Blastoid (%)                      | 16*             | 2 (3)             | 3 (5)            | 10* (16)        | 1 (2)          | 4 (6)          | 1 (2)          |
| Total (%)                         | 62*             | 3 (19)            | 5* (8)           |                 |                |                |                |

*Two blastoid MCL cases with p53 mutation and one with an INK4a/ARF deletion were excluded from this comparison, because RNA was not available to perform CDK4 and MDM2 expression analysis.
that the genesis of MCL is not known. In the present study, we have shown however, the potential role of these elements in the pathogen-

Figure 3. Relationship among INK4a deletion, p53, MDM2, and CDK4 alterations, and proliferation activity measured by Ki-67 immunostaining. MCL cases are ordered by their Ki-67 degree of immunostaining (0-100% from left to right). Red squares, altered cases. CDK4 alterations are frequently associated with p53 but not with INK4a alterations. The majority of the altered cases corresponded to tumors with high proliferative activity, >30% of Ki-67 immunostaining (70% versus 16%; P < 0.01).

tumor usually associated with higher proliferation (2–4). CDK4 and MDM2 are two oncogenic elements in chromosome 12q13 frequently gained or amplified in MCL (15). CDK4 and MDM2 regulate pRb and p53, respectively, and are in turn negatively modulated by the upstream elements p14ARF and p16INK4a (10), often deleted in MCL. Several studies have shown the oncogenic alterations of CDK4 and MDM2 in human tumors (18, 37). However, the potential role of these elements in the pathogen-

Figure 4. Overall survival of 44 MCL cases according to the gene alterations at diagnosis and its relationship with the tumor proliferation. Top, group of patients with inactivation of both pathways (four cases with p53 mutations and CDK4 amplification/overexpression and nine cases with deletions of the INK4a/ARF locus) had a significantly worse prognosis than patients with alterations only in one pathway (eight cases with p53, MDM2, or CDK4 altered) or no alterations in any of these genes (P < 0.001). Bottom, overall survival in 40 MCL cases with proliferation information by Ki-67 immunohistochemistry. The cutoff of 30% Ki-67 immunostaining defined a high proliferative group with a poor prognosis (P < 0.001). Further stratification of these cases according to the presence of INK4a/ ARF or p53/CDK4 alterations showed that patients with alterations in the two pathways had a significantly worse prognosis (P < 0.05) than patients without concomitant aberrations. The proliferation index in these two groups was similar (means, 51.8% versus 51.5%, respectively).

CDK4 mRNA expression. Concordantly with these observations, two previous studies examining CDK4 mRNA expression in a few MCL had observed a moderate, although significant, increased expression from resting B cells to typical and blastoid MCL variants (39, 40). However, these studies failed to detected the extremely high mRNA levels observed in our tumors with amplification of the gene probably due to the small number of cases examined. These findings suggest that the moderate progression in CDK4 mRNA levels observed among reactive lymphoid tissue, typical, and blastoid MCL variants with no gene alterations may reflect the increasing proliferative activity of the cells. However, the extremely high levels of mRNA and protein expression present in a subset of tumors with amplification of the gene would indicate that CDK4 aberrations may be an oncogenic mechanism involved in the pathogenesis of the tumor.
The oncogenic potential of CDK4 activation has been related to the deregulation of the G1 phase by increasing the hyperphosphorylation of pRb (10). In addition, CDK4-cyclin D complexes may also enhance tumornogenesis by sequestering CDK inhibitors, such as p16INK4a and p21Cip1, and facilitate the activation of downstream cyclin-CDK complexes (11, 41). In addition, CDK4 overexpression may also contribute to the progression of the tumors by variant mechanisms, such as providing an alternative way to bypass telomere-dependent replicative senescence (22), interfering with the growth arrest signals induced by DNA damage signals (22), and interacting with STAT proteins (42).

INK4a/ARF locus alterations are a relatively frequent event in many cancers, including hematologic malignancies (3, 37). Deletions of this locus have been detected in 15% to 21% of MCL lymphomas, particularly highly proliferative and aggressive variants, and involve both p16INK4a and p14ARF genes (3, 7, 21, 43). Point mutations or promoter hypermethylation of this locus have been observed in some types of malignant lymphomas. However, they are extremely unusual in MCL, indicating that in these tumors homozygous deletions are the main mechanism inactivating this locus (3, 21, 44). In this study, we have confirmed the relative high frequency of INK4a/ARF deletions in highly proliferative and aggressive MCL. Interestingly, the CDK4 gene aberrations observed in these cases were mainly observed in tumors with no deletions in the INK4a/ARF locus. The alternative occurrence of CDK4 and p16INK4a or pRb gene aberrations has been also described in gliomas and other tumors (45, 46) but not in hematologic malignances. These findings parallel previous observations, indicating that INK4a/ARF and p53 alterations may also be alternative mechanisms in MCL and other non-Hodgkin’s lymphomas (21, 44). INK4a/ARF locus encodes for both p16INK4a and p14ARF proteins, upstream regulators of CDK4 and p53, respectively; consequently, inactivation of one of these critical loci may diminish the selective pressure for accumulation of other alterations in genes of the same regulatory pathway.

Interestingly, four of the seven MCL with CDK4 aberrations had also a concomitant p53 mutation, suggesting that simultaneous oncogenic alterations of these genes may have a synergistic effect on the progression of the tumors by inactivating both CDK4/pRb and p53 pathways. MDM2 overexpression was not associated with p53 alterations in any case. These findings in human MCL are concordant with recent in vitro and transgenic animal models, indicating that the concomitant alterations in elements of the same regulatory pathways, such as CDK4 overexpression and pRb loss, did not produce a cooperating oncogenic effect (47) as observed for alterations in elements of synergizing growth regulatory pathways, such as p53 and CDK4 or pRb (48–50). Interestingly, patients with simultaneous disruption of the two p14ARF/MDM2/p53 and p16INK4a/CDK4 pathways in MCL had a significantly worse prognosis than those with only isolated CDK4, p53, or MDM2 aberrations or no alterations in these genes. Similarly to previous studies in MCL (5, 51), the proliferation of the tumors was a strong predictor of the outcome of the patients, and alterations in all these genes were more common in highly proliferative tumors. However, the impact on survival of the inactivation of the two p14ARF/MDM2/p53 and p16INK4a/CDK4 pathways was independent of the proliferation, suggesting that the combination of these gene alterations may reflect the disruption of different biological functions with important impact on the behavior of the tumors.

In conclusion, our results indicate that CDK4 and MDM2 gene alterations occur in a subset of highly proliferative and clinically aggressive MCLs with wild-type INK4a/ARF locus. The shorter survival of patients with INK4a/ARF deletions or concomitant aberrations of CDK4 and p53, independently of the proliferative activity of the tumors, suggests that the use of a model combining the proliferative index and the presence of molecular alterations in these two pathways may improve our ability to predict survival in MCL patients.

References


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