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Deletions of the Cyclin-Dependent Kinase Inhibitor Genes $p16^{INK4A}$ and $p15^{INK4B}$ in Non-Hodgkin's Lymphomas

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The tumor suppressor genes $p16^{INK4A}$ and $p15^{INK4B}$ map to the 9p21 chromosomal locus and are either homozygously deleted or mutated in a wide range of human cancer cell lines and tumors. Although chromosome 9 abnormalities have been described in non-Hodgkin's lymphomas (NHLs), to date, the mutational status of these genes has not been determined for these malignancies. A total of five cell lines and 75 NHLs were examined for homozygous deletions or point mutations in the coding regions of both the p15 and p16 genes using Southern blot and/or polymerase chain reaction–single-strand conformation polymorphism analyses. Homozygous deletions of either the p16 gene or both the p15 and p16 genes were observed in one diffuse large B-cell lymphoma cell line and two uncultured lymphomas consisting of one large B-cell and one mixed T-cell lymphoma. In contrast, point mutations were not detected in either the cell lines or lymphomas. These results indicate that the rate of alterations in the p15 and p16 genes is low for lymphomas, but loss of p16 and/or p15 may be involved in the development of some lymphomas.

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CONNECTIONS BETWEEN cell cycle regulation and cancer have multiplied rapidly. The cell cycle regulators implicated in cancer are primarily involved in the transition from the G1 to S phases.[1] These include the D-type cyclins (1, 2, and 3) and their respective cyclin-dependent kinases (CDKs) 4 and 6. The cyclins bind to and activate the CDKs, which leads to phosphorylation of the tumor suppressor protein, Rb. Rb is underphosphorylated throughout the G1 phase and is phosphorylated just before the S phase.[2-4] Release of E2F transcription factors from phosphorylated Rb presumably allows transcription of genes critical for driving the cell cycle through START.[5] Therefore, the activation and subsequent inactivation of a series of cyclins and CDKs is required for normal progression of the cell cycle.[2-4] A newly identified family of functionally related proteins termed cyclin-dependent kinase inhibitors (CDKIs) plays a critical role in the inactivation of CDK activity, particularly in the G1 phase.[1,6] These include $p21^{CIP1/WAF1/CAP20}$, $p27^{KIP1}$, $p28^{ICK}$, $p16^{INK4A}$, $p15^{INK4B}$, and p18.[7-17] CDKIs bind to their respective CDKs or CDK-cyclin complexes and inhibit the activity of the kinase.[7,8,13-17]

Perturbations in the functions and/or expression of the above cell cycle regulatory proteins, as well as loss of Rb, would predictably lead to deregulation of the cell cycle and contribute to tumorigenesis. To overcome regulation of the cell cycle by Rb, either Rb function can be lost or CDK 4 or 6 activity increased. Increased CDK activity could result from either overexpression of the D-type cyclins or CDKs, or loss of CDKIs like p15 or p16. Such alterations may lead to uncontrolled cellular proliferation. Examples of each have been observed in different types of cancers. The cyclin D1 protein, which is encoded by the CCND1/PRAD1/BCL1, protooncogene, is involved in various chromosome abnormalities or gene amplifications that lead to overexpression in certain cancers.[18-20] In addition, overexpression of the cyclin D1 protein leads to transformation of primary rodent cells, as well as the development of mammary hyperplasia in transgenic mice.[21,22] Moreover, amplification of CDK4 expression has been observed in cancers such as sarcomas and gliomas.[23-25] These studies indicate that cyclins and CDKs are possible oncogenes, and their activation contributes to tumorigenesis.

On the other hand, biochemical, cell culture, and molecular studies indicate that the CDKIs may function as tumor suppressors. Overexpression of the CDKIs in cultured cells inhibits cell cycle progression and, in some cases, tumorigenesis.[7-9,11,12,17,26] In addition, the $p16^{INK4A}$ and $p15^{INK4B}$ (inhibitor of CDK4) genes, also known as CDK4I/MTS-1 (CDK4 inhibitor/multiple tumor suppressor-1) and MTS2, respectively, were mapped to chromosome 9p21 during a detailed analysis of the most frequently deleted region at this locus in a wide variety of cell lines derived from human malignancies, including non–small-cell lung cancers, gliomas, melanomas, and leukemias.[27-36] Recent studies also showed that p16 is deleted or mutated in uncultured cells from the same malignancies, but at a lower rate than in the cell lines.[25,37-49] Point mutations have not been observed in the p15 gene from either cultured or uncultured cancer cells.[27,46] However, p15 is homozygously deleted along with the p16 gene in primary brain tumors and fresh leukemia samples.[27,45] This suggests that loss of both genes may be involved in the development of both solid tumors and hematopoetic malignancies.

Results from our own laboratory, as well as others, revealed a striking rate of p15 and p16 deletions in acute lymphoblastic leukemias (ALLs).[45,48,49,49a] However, the rate of deletions in chronic and acute myeloid leukemias (CML and AML, respectively), myelodysplastic syndrome (MDS), chronic lymphocytic leukemia (CLL), and multiple myeloma is very low.[48,49,49b] The mutational status of the p15 and p16 genes has not been well characterized for cultured or

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Table 1. Characteristics of NHLs

	N	Southern Blot Analysis* (p16/p15/total)
B-cell phenotype	52	1/1/37
Immunoblastic (7 HIV+)	19†	0/0/13
Lymphoblastic	2	0/0/2
Mixed	15	0/0/11
Large cell	10‡	1/1/6
Monocytoid	1	0/0/1
Mantle cell lymphoma	3	0/0/2
Burkitt-like, small non-cleaved (HIV+)	2	0/0/2
T-cell phenotype	23	1/0/16
Large cell (1 HIV+, 1 HTLV+)	7§	0/0/4
Mixed	4‖	1/0/4
Small cell peripheral	2	0/0/1
Lymphoblastic	3	0/0/3
Angioimmunoblastic	5	0/0/4
Mycosis fungoides	2	0/0/0
Total	75	

Abbreviations: HIV, human immunodeficiency virus; HTLV, human T-cell leukemia virus.

* Number of homozygous deletions in p16/p15/total number analyzed by Southern blot.

† Hemizygous deletion of p15 exon 2 in one sample.

‡ One sample contains hemizygous deletions in p16 exon 2 and p15 exon 2.

§ One sample contains a hemizygous deletion in p15 exon 2; a second contains hemizygous deletions in exon 2 of p15 and p16.

‖ Possible rearrangement of p15 exon 2 in sample with homozygous deletion of p16.

uncultured non-Hodgkin's lymphomas (NHLs). By cytogenetic and molecular analyses, various chromosome 9p and 9q aberrations have been described in NHLs, including deletions of the 9p21 locus.[50,51] This, together with the role of p16 alterations in some leukemias, suggests that p15 and p16 alterations may be involved in lymphomagenesis. To determine if loss of p16 and/or p15 function plays a role in lymphomagenesis, we evaluated B-cell and T-cell lymphomas and B-cell lymphoma cell lines for p16 and p15 gene alterations using both Southern blot and polymerase chain reaction (PCR)–single-strand conformational polymorphism (SSCP) analyses.

MATERIALS AND METHODS

Human lymphoma samples and cell lines. Fresh tissue from patients with malignant lymphoid proliferations were obtained at the time of biopsy from the Hematopathology Division of Cedars-Sinai Medical Center (Los Angeles, CA). The characteristics of the 75 NHL cases are described in Table 1. All cases were characterized by routine immunophenotyping as previously described.[52] As a normal control, bone marrow was obtained from a healthy individual after proper consent.

The human Burkitt's B-cell lymphoma cell lines Namalwa, Daudi, Raji, and Ramos were obtained from American Type Culture Collection (ATCC; Rockville, MD). The cell line OZ was established from a diffuse large B-cell lymphoma containing a t(14,18) chromosomal translocation and was provided by Masami Nagai (Kagawa Medical School, Kagawa, Japan). All cell lines were maintained in RPMI 1640 medium plus 10% to 20% fetal calf serum without antibiotics. Genomic DNA was isolated from frozen tumors and cell lines using standard methods.[53]

Southern blot analysis. Genomic DNA for Southern blot analysis was digested with *Eco*RI (GIBCO/BRL, Gaithersburg, MD) and resolved on either 0.8% or 1% agarose gels. The DNA was transferred to positively charged nylon membranes under alkaline conditions according to the supplier's protocol (Amersham, Arlington Heights, IL). The probe DNA for exon 1 of the $p16^{INK4A}$ gene (p16X1) was a PCR product (amplification described below) containing the entire coding portion of exon 1. The probe DNA specific for exon 2 of p16 (p16X2) was a *Kpn* I restriction enzyme fragment isolated from a p16 cDNA clone, provided by David Beach (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), representing the 3′-coding portion of exon 2.[15] A second probe for p16 exon 2 was a PCR product (amplification described below) containing the entire coding portion of exon 2. It shares homology with exon 2 of both p15 and p16. As a control, blots were hybridized with a portion of the myeloperoxidase (MPO) gene.[54] Probe DNAs were labeled with [α-^{32}P]-deoxycytidine triphosphate (dCTP) (3,000 mCi/mmol; NEN/Dupont, Boston, MA) using a random primer system as described by the supplier (Gibco/BRL). Hybridization was performed using standard conditions with a final wash of either 0.1× or 0.5× saline sodium citrate (SSC) and 0.1% sodium dodecyl sulfate (SDS) at 68°C for 30 minutes.[53]

Visual inspection of the autoradiographs was used to determine the presence of homozygous or hemizygous deletions. Suspected deletions were more objectively determined by measuring the intensity of the signals by densitometry using the Imagestore 5000 Gel Documentation System (Ultra Violet Products Ltd, Cambridge, UK). Of the 53 lymphomas examined by Southern blot hybridization, 36 were analyzed by densitometry.

PCR-SSCP analysis. For PCR-SSCP analysis, p15 and p16 exons 1 and 2 were amplified from approximately 100 ng of tumor or cell line DNA by PCR in the presence of radiolabeled nucleotides.[55] The PCR products were denatured and electrophoresed through a nondenaturing 5% polyacrylamide Mutation Detection Enhancement (MDE; J.T. Baker Inc, Phillipsburg, NJ) gel at 6 W for 24 hours, dried, and exposed to Kodak X-OMAT AR film (Eastman Kodak, Rochester, NY) at −80°C with an intensifying screen.

The PCR primers used for amplification of exons 1 and 2 of p15 and p16 are described in Table 2. The PCR conditions for each exon were as follows: p16 exon 1: 35 cycles of 95°C for 45 seconds, 52.5°C for 30 seconds, and 72°C for 60 seconds; p16 exon 2: 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 120 seconds; p15 exon 1: 35 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 40 seconds; p15 exon 2: 30 cycles of

Table 2. Primers Used to Amplify Exons 1 and 2 of the p16 and p15 Genes

Primers	Oligonucleotide Sequence	Expected PCR Product Size (bp)
p16 exon 1		
p16X1S4	5′-GGAGAGGGGGAGAACAGACAACGG-3′	≈270
p16X1A1	5′-GCGCTACCTGATTCCAATTC-3′	
p16 exon 2		
p16X2S2	5′-ACCCTGGCTCTGACCATTCTGTTCT-3′	371
p16X2A2	5′-GTACAAATTCAGATCATCAGTCC-3′	
p15 exon 1		
p154BS1	5′-CTGCGCGTCTGGGGGCTGC-3′	164
p154BA1	5′-CCTCCCGAAACGGTTGACTCC-3′	
p15 exon 2		
p154BS2	5′-CCCGGCCGGCATCTCCCATA-3′	351
p154BA2	5′-CGTTGTGGGCGGCTGGGGAACCT-3′	

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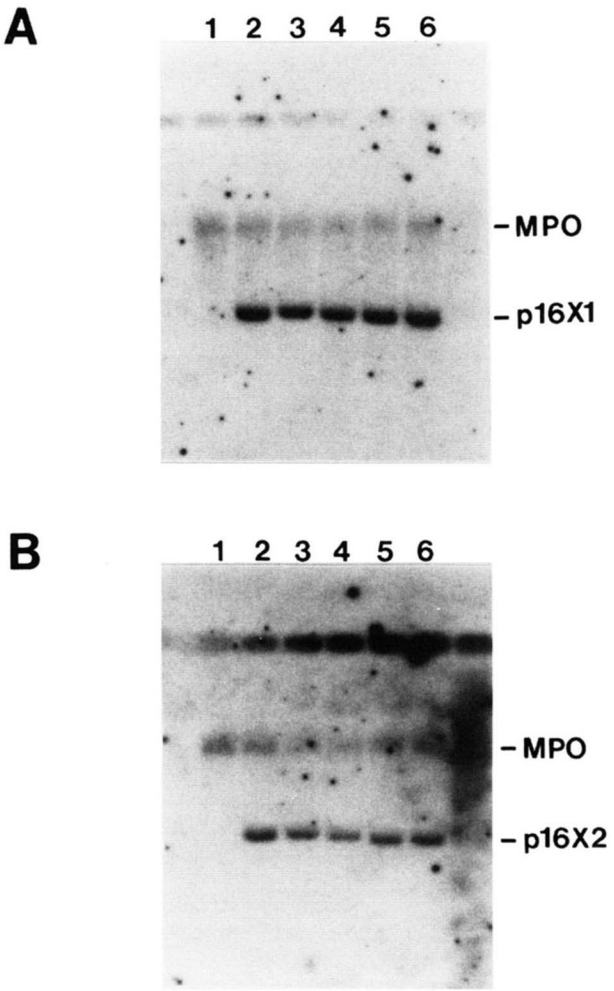

Fig 1. Southern blot analysis of lymphoma cell line DNAs for p16 deletions. Cell line genomic DNAs (5 μg per lane) digested with *Eco*RI were electrophoresed on 1% agarose gels, transferred to nylon membranes, and hybridized simultaneously with a probe for the MPO gene and probes for p16 exon 1 (p16X1; A) and exon 2 (p16X2; B). Lane numbers are indicated across the top of each panel: lane 1, Oz; lane 2, Ramos; lane 3, Namalwa; lane 4, Raji; lane 5, Daudi; and lane 6, normal human bone marrow.

94°C for 60 seconds, 57°C for 60 seconds, and 72°C for 60 seconds, followed by 72°C for 10 minutes. The PCR products were labeled by the inclusion of 1 to 2 μCi of [α-^{32}P]dCTP per 20 μL reaction.

RESULTS

Examination of lymphoma cell lines and lymphomas for homozygous deletion of the p15 and p16 genes. Using Southern blot analysis, we examined five lymphoma cell lines and 53 uncultured NHLs for homozygous deletion of the p15 and p16 genes (Table 1). Hybridization with probes specific for p16 exons 1 and 2 and a control gene, MPO, showed that the diffuse large B-cell lymphoma cell line OZ contained homozygous deletions of both exons (Fig 1A and B, lane 1). Signals for exons 1 and 2 were detected in the control DNA from normal human bone marrow and all of the remaining cell lines (Fig 1A and B, lanes 2 through 6). The signal appearing at the top of the blot was due to nonspecific hybridization of the probe to the wells. Subsequent hybridization of the same blot with a probe homologous for exon 2 of both p15 and p16 showed that p15 was also homozygously deleted in cell line Oz (Fig 2, lane f), but not in the other cell lines (Fig 2, lanes a through e).

Blots containing DNA from uncultured NHLs were hybridized in a manner similar to that described for the cell lines. Representative results are presented in Fig 3. Densitometry analysis was performed to assist in determining the presence of homzygous and possible hemizygous deletions. Of the 53 NHLs, visual inspection and densitometry analysis of the autoradiograph indicated that in one large B-cell lymphoma, exon 1 of p16 and exon 2 of p15 were homozygously deleted; however, p16 exon 2 was hemizygously deleted (Fig 3, lane d). In addition, in a mixed T-cell lymphoma, exons 1 and 2 of p16 were homozygously deleted (Fig 3, lane p). Exon 2 of p15 was not deleted, but appeared to be rearranged due to the homozygous deletion of both exons 1 and 2 of p16 (data not shown). The faint signals observed for the deleted samples were most likely due to contamination by normal cells. Hemizygous deletions of p15 exon 2 occurred in four samples, with two of the four containing hemizygous deletions of p16 exon 2 (Table 1). Hemizygous deletions in p16 exon 1 were not detected. These results indicate that the rate of homozygous deletions in the p15 and p16 genes for lymphomas is low.

Examination of lymphoma cell lines and lymphomas for small deletions and point mutations in the p16 gene. To detect the presence of small deletions or point mutations, we performed PCR-SSCP analysis for exons 1 and 2 of both p15 and p16 on the lymphoma cell lines and uncultured NHL samples. The OZ cell line, which contains a homozygous deletion of the p16 gene, did not amplify for either exon 1 or 2 of p16 or exon 2 of p15 as expected (data not shown). For the remaining cell lines, the mobility patterns observed for both exons of p16 were identical to that of the control DNA from normal human bone marrow (data not shown). Of the NHLs examined for p16 (exon 1, 35 of 75; exon 2, 67 of 75), no point mutations or polymorphisms were detected. For p15 exon 2, 64 of 75 NHLs were examined, and a pattern, previously observed in 6 of 36 matched, tumor-normal non–small-cell lung cancer samples,[56] was observed, indicating the presence of a polymorphism. This polymorphism, identified in 12 lymphomas (data not shown), was due to a silent base substitution in codon 38.[57] The rate of heterozygousity for this polymorphism in the lymphomas was 13%. For p15 exon 1, 69 of 75 NHLs were examined, and no point mutations or polymorphisms were found. It should be noted that the samples with hemizygous deletions did not display altered mobility patterns after SSCP analysis. This indicates that point mutations in the remaining allele, which might lead to loss of protein function, were not present. The above PCR-SSCP results indicate that point mutations are extremely rare or nonexistent in both lymphoma cell lines and lymphomas.

DISCUSSION

Two interesting observations were made in this study. First, the overall rate of p15 and p16 alterations is low for

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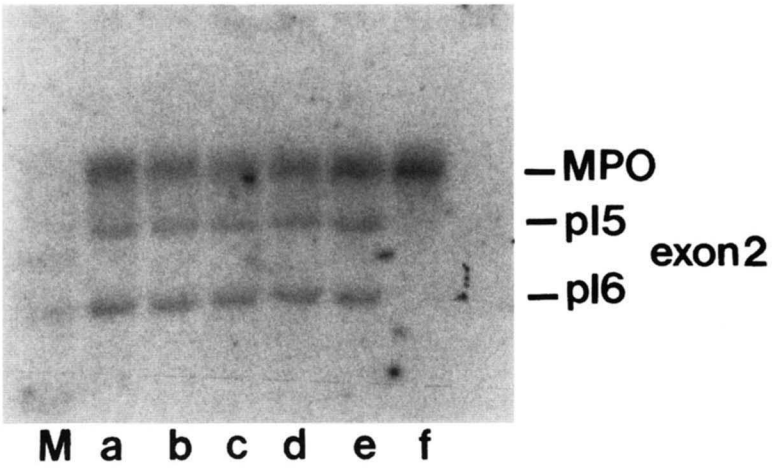

Fig 2. Southern blot analysis of lymphoma cell line DNAs for p15 deletions. The blot described in Fig 1 was hybridized with a probe containing the entire coding region of p16 exon 2. This probe shares homology with exon 2 of both p15 and p16. A second probe for the control gene MPO was included. Lane M, DNA markers; lane a, normal human bone marrow; lane b, Daudi; lane c, Raji; lane d, Namalwa; lane e, Ramos; and lane f, Oz.

lymphomas (2 of 75, 2.5%). This rate is much lower than that found for ALLs, but higher than the rate of deletion in other hematopoetic malignancies such as CML, AML, MDS, and CLL.[45,48,49] However, when the samples are analyzed according to their clinicopathogenic classifications, it is tempting to speculate that p15 and p16 deletions may occur more frequently in particular lymphoma classes. The collection that we analyzed contained 10 uncultured and one cultured large B-cell lymphoma (the Oz cell line), and four mixed T-cell lymphomas. One of six (16%) uncultured large B-cell lymphomas, the Oz cell line, and one of four (25%) mixed T-cell lymphomas that were analyzed by Southern hybridization contained homozygous deletions in the p16 gene. In addition, homozygous deletions of the p15 gene were noted in the large B-cell lymphoma and the cell line. The p15 gene of the mixed T-cell lymphoma was not deleted but appeared to be rearranged. The effect of this on the expression of p15 is unclear. However, the rearrangement appears to be due to the loss of both p16 exons, and the breakpoints would be downstream of the p15 gene and its promoter, suggesting that expression may not be affected. Our results differ from those of Chaganti et al.[51] They examined 13 cases of diffuse large-cell lymphomas in which greater than 25% of the samples with loss of heterozygosity (LOH) in 9p contained heterozygous or homozygous deletions in various 9p loci. However, none of the lymphomas exhibited loss or alterations when *Hin*dIII-digested DNAs were hybridized with a p16 probe.[51] This may be due to differences in the collections. We did not find deletions of p15 or p16 in Burkitt's lymphoma cell lines, which is similar to previously reported results.[51] It would be of interest to examine a larger collection of large B-cell and mixed T-cell lymphomas to determine if the rates observed in this study are maintained.

The second observation was that homozygous deletions of p15 occurred only when p16 was homozygously deleted, but did not occur in all cases where p16 was deleted. A similar finding was observed for 22 ALL samples that contained homozygous deletions of p16, but only 17 had lost the p15 gene.[45] The predominance of codeletion suggests

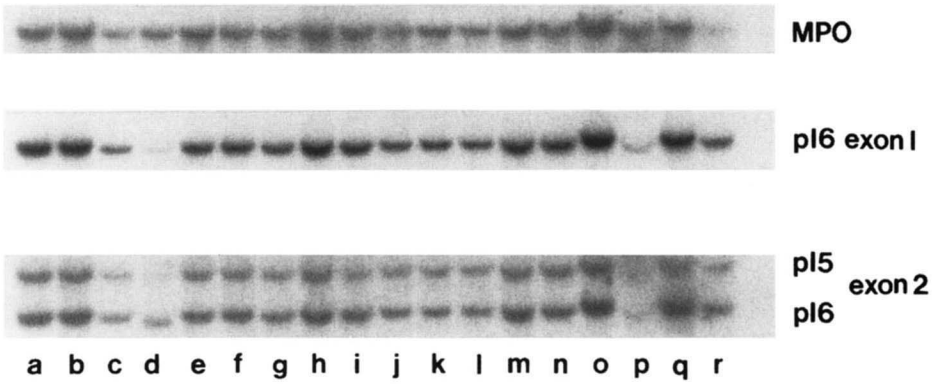

Fig 3. Southern blot analysis of representative lymphoma DNAs. Genomic DNAs (10 μg per lane) digested with *Eco*RI were electrophoresed on a 0.8% agarose gel, transferred to nylon membranes, and hybridized subsequently with probes for the control gene MPO (top panel), p16 exon 1 (middle panel), and a probe homologous to p15 and p16 exon 2 (lower panel). Lane a, B-cell immunoblastic (BIBS); lane b, T-cell lymphoblastic lymphoma (TLBL); lane c, BIBS; lane d, large B-cell lymphoma (BLL); lane e, mixed B-cell lymphoma (BM); lane f, hyperplastic lymph node; lane g, BIBS; lane h, BLL; lane i, BIBS; lane j, large T-cell lymphoma (TLL); lane k, BLL; lane l, BLL; lane m, BLL; lane n, monocytoid; lane o, TLL; lane p, mixed T-cell lymphoma; lane q, angioimmunoblastic; lane r, BM.

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that loss of both genes is often required for tumorigenesis.[45,47] However, the absence of p15 alterations in some cancers suggests that p16 is the main target of the deletion. As the p15 and p16 genes are localized to 9p21, share extensive predicted amino acid sequence similarity, and bind to CDKs 4 and 6, it is possible that p15 can compensate for p16 loss.[16,17,27,28] Loss of p16 may be required for tumorigensis, but the additional loss of p15 may make the process more efficient. This could explain the predominance of homozygous deletions, which would be the most efficient way to inactivate both genes. The homozygous deletions of both p15 and p16 in the cell line Oz will allow us to test whether or not both genes need to be lost during lymphomagenesis. It is possible to express these genes in Oz and determine if one or the other, or both are required to inhibit proliferation or tumorigenicity of this cell line.

In summary, this study further elucidates the connections between cell cycle and lymphomagenesis. NHLs display a wide clinicopathologic heterogeneity, which is reflected at the molecular level by various lesions in both tumor suppressor genes and oncogenes.[58] Therefore, it is quite possible that alterations in different genes involved in regulating cell cycle may occur in particular classes of lymphomas. The cyclin D1 protein is frequently overexpressed in mantle cell lymphomas and presumably disrupts the cell cycle in G1 by increasing CDK activity, thereby increasing Rb phosphorylation and cellular proliferation.[18-20,21,22,59] Our results suggest that a similar disruption of G1-S phase regulation occurs in some NHLs via deletion of the tumor suppressor genes p15 and p16, which negatively regulate CDK activity.

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REFERENCES

1. Hunter T, Pines J: Cyclins and cancer II: Cyclin D and CDK inhibitors come of age. Cell 79:573, 1994

2. Sherr CJ: Mammalian G1 cyclins. Cell 73:1059, 1993

3. Pines J: Cyclins and cyclin-dependent kinases; take your partners. Trends Biochem Sci 18:195, 1993

4. Reed SI: The role of p34 kinases in the G1 to S-phase transition. Ann Rev Cell Biol 8:529, 1992

5. Hinds PW, Weinberg RA: Tumor suppressor genes. Curr Opin Genet Dev 4:135, 1994

6. Hunter T: Braking the cycle. Cell 75:839, 1993

7. Xiong Y, Hannon GJ, Zhang H, Casso D, Kobayashi R, Beach D: p21 is a universal inhibitor of cyclin kinases. Nature 366:701, 1993

8. Harper JW, Adami GR, Wei N, Keyomarsi K, Elledge SJ: The p21 cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. Cell 75:805, 1993

9. El-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, Lin D, Mercer WE, Kinzler KW, Vogelstein B: WAF1, a potential mediator of p53 tumor suppression. Cell 75:817, 1993

10. Gu Y, Turck CW, Morgan DO: Inhibition of CDK2 activity in vivo by an associated 20K regulatory subunit. Nature 366:707, 1993

11. Polyak K, Kato J, Solomon MJ, Sherr CJ, Massague J, Roberts JM, Koff A: $p27^{Kip1}$, a cyclin-Cdk inhibitor, links transforming growth factor-beta and contact inhibition to cell cycle arrest. Genes Dev 8:9, 1994

12. Polyak K, Lee MH, Erdjument-Bromage H, Koff A, Roberts JM, Tempst P, Massague J: Cloning of $p27^{Kip1}$, a cyclin-dependent kinase inhibitor and a potential mediator of extracellular antimitogenic signals. Cell 78:59, 1994

13. Toyoshima H, Hunter T: p27, a novel inhibitor of G1 cyclin-Cdk protein kinase activity, is related to p21. Cell 78:67, 1994

14. Hengst L, Dulic V, Slingerland JM, Lees E, Reed SI: A cell cycle-regulated inhibitor of cyclin-dependent kinases. Proc Natl Acad Sci USA 91:5291, 1994

15. Serrano M, Hannon GJ, Beach D: A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. Nature 366:704, 1993

16. Hannon GJ, Beach D: $p15^{INK4B}$ is a potential effector of TGF-β–induced cell cycle arrest. Nature 371:257, 1994

17. Guan KL, Jenkins CW, Li Y, Nichols MA, Wu X, O'Keefe CL, Matera AG, Xiong Y: Growth suppression by p18, a $p16^{INK4/MTS1}$- and $p14^{INK4B/MTS2}$-related CDK6 inhibitor, correlates with wild-type pRb function. Genes Dev 8:2939, 1994

18. Motokura T, Bloom T, Kim HG, Juppner H, Ruderman JV, Kronenberg HM, Arnold A: A novel cyclin encoded by a bcl-1 linked candidate oncogene. Nature 350:512, 1991

19. Rosenberg CL, Wong E, Petty EM, Bale AE, Tsujimoto Y, Harris NL, Arnold A: PRAD1, a candidate BCL-1 oncogene: Mapping and expression in centrocytic lymphoma. Proc Natl Acad Sci USA 88:9638, 1991

20. Withers DA, Harvey RC, Faust JB, Melnyk O, Carey K, Meeker TC: Characterization of a candidate bcl-1 gene. Mol Cell Biol 11:4846, 1991

21. Hinds PW, Dowdy SF, Eaton EN, Arnold A, Weinberg RA: Function of a human cyclin gene as an oncogene. Proc Natl Acad Sci USA 91:709, 1994

22. Wang TC, Cardiff RD, Zukerberg L, Lees E, Arnold A, Schmidt EV: Mammary hyperplasia and carcinoma in MMTV-cyclin D1 transgenic mice. Nature 369:669, 1994

23. Khatib ZA, Matsushime MV, Valentine M, Shapiro DN, Sherr CJ, Look AT: Coamplification of the CDK4 gene with MDM2 and GLI in human sarcomas. Cancer Res 53:5535, 1993

24. Reifenberger G, Reifenberger J, Ichimura K, Meltzer PS, Collins VP: Amplification of multiple genes from chromosomal region 12q13-14 in human malignant gliomas: Preliminary mapping of the amplicon shows preferential involvement of CDK4, SAS, and MDM2. Cancer Res 54:4299, 1994

25. He J, Allen JR, Collins VP, Allalunis-Turner MJ, Godbout R, Day RS III, James CD: CDK4 amplification is an alternative mechanism to p16 gene homozygous deletion in glioma cell lines. Cancer Res 54:5804, 1994

26. Serrano M, Gomez-Lahoz E, DePinho RA, Beach D, Bar-Sagi D: Inhibition of ras-induced proliferation and cellular transformation by $p16^{INK4}$. Science 267:249, 1995

27. Kamb A, Gruis NA, Weaver-Feldhaus J, Liu Q, Harshman K, Tavtigian SV, Stockert E, Day RS, Johnson BE, Skolnick MH: A cell cycle regulator potentially involved in genesis of many tumor types. Science 264:436, 1994

28. Nobori T, Miura K, Wu DJ, Lois A, Takabayashi K, Carson DA: Deletions of the cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. Nature 368:753, 1994

29. Olopade OI, Jenkins RB, Ransom DT, Malik K, Pomykala H, Nobori T, Cowan JM, Rowley JD, Diaz MO: Molecular analysis of deletions of the short arm of chromosome 9 in human gliomas. Cancer Res 52:2523, 1992

30. Lukeis R, Irving L, Garson M, Hasthorpe S: Cytogenetics

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of non-small cell lung cancer: Analysis of consistent non-random abnormalities. Genes Chromosom Cancer 2:116, 1990

31. Diaz MO, Ziemin S, Le Beau MM, Pitha P, Smith SD, Chilcote RR, Rowley JD: Homozygous deletion of the alpha- and beta1-interferon genes in human leukemia and derived cell lines. Proc Natl Acad Sci USA 85:5259, 1988

32. Fountain JW, Karayiorgou M, Ernsthoff MS, Kirkwood JM, Vlock DR, Titus-Ernsthoff L, Bouchard B, Vijayasaradhi S, Houghton AN, Lahti J, Kidd VJ, Housman DE, Dracopoli NC: Homozygous deletions of human chromosome 9p21 in melanoma. Proc Natl Acad Sci USA 89:10557, 1992

33. Olopade OI, Bohlander SK, Pomykala H, Maltepe E, Van Melle E, Le Beau MM, Diaz MO: Mapping of the shortest region of overlap of deletions of the short arm of chromosome 9 associated with human neoplasia. Genomics 14:437, 1993

34. Olopade OI, Buchhagen DL, Malik K, Sherman J, Nobori T, Bader S, Nau MM, Gazdar AF, Minna JD, Diaz MO: Homozygous loss of the interferon genes defines the critical region on 9p that is deleted in lung cancer. Cancer Res 53:2410, 1993

35. James CD, He J, Carlbom E, Nordenskjold M, Cavenee WK, Collins VP: Chromosome 9 deletion mapping reveals interferon alpha and beta-1 gene deletions in human glial tumors. Cancer Res 51:1684, 1991

36. Diaz MO, Rubin CM, Harden A, Ziemin S, Larson RA, Le Beau MM, Rowley JD: Deletions of interferon genes in acute lymphoblastic leukemia. N Engl J Med 322:77, 1990

37. Spruck CH, Ganzalez-Zulueta M, Shibata A, Simoneau AR, Lin MF, Gonzales F, Tsai YC, Jones PA: p16 gene in uncultured tumors. Nature 370:183, 1994

38. Cairns P, Mao L, Merlo A, Lee DJ, Schwab D, Eby Y, Tokino K, van der Reit P, Blaugrund JE, Sidransky D: Rates of p16 (MTS1) mutations in primary tumors with 9p loss. Science 265:415, 1994

39. Caldas C, Hahn SA, da Costa LT, Redston MS, Schutte M, Seymour AB, Weinstein CL, Hruban RH, Yeo CJ, Kern SE: Frequent somatic mutations and homozygous deletions of the p16 (MTS1) gene in pancreatic adenocarcinoma. Nat Genet 8:27, 1994

40. Mori T, Miura K, Aoki T, Nishihira T, Mori S, Nakamura Y: Frequent somatic mutation of the MTS1/CDK4I (multiple tumor suppressor/cyclin-dependent kinase 5 inhibitor) gene in esophageal squamous cell carcinoma. Cancer Res 54:3396, 1994

41. Kamb A, Shattuck-Eidens D, Eeles R, Liu Q, Gruis NA, Ding W, Hussey C, Tran T, Miki Y, Weaver-Feldhaus J, McClure M, Aitken JF, Anderson DE, Bergman W, Frants R, Goldgar DE, Green A, MacLennan R, Martin NG, Meyer LJ, Youl P, Zone JJ, Skolnick MH, Cannon-Albright LA: Analysis of the p16 gene (CDKN2) as a candidate for the chromosome 9p melanoma susceptibility locus. Nat Genet 8:22, 1994

42. Hussussian CJ, Struewing JP, Goldstein AM, Higgins PAT, Ally DS, Sheahan MD, Clark WH, Tucker MA, Dracopoli NC: Germline p16 mutations in familial melanoma. Nat Genet 8:15, 1994

43. Hayashi N, Sugimoto Y, Tsuchiya E, Ogawa M, Nakamura Y: Somatic mutations of the MTS (multiple tumor suppressor) 1/CDK4I (cyclin-dependent kinase-4 inhibitor) gene in human primary non-small cell lung carcinomas. Biochem Biophys Res Commun 202:1426, 1994

44. Okamoto A, Demetrick DJ, Spillare EA, Hagiwara K, Hussain SP, Bennet WP, Forrester K, Gerwin B, Serrano M, Beach DH, Harris CC: Mutations and altered expression of $p16^{INK4}$ in human cancer. Proc Natl Acad Sci USA 91:11045, 1994

45. Hebert J, Cayuela JM, Berkeley J, Sigaux F: Candidate tumor-suppressor genes MTS1 ($p16^{INK4A}$) and MTS2 ($p15^{INK4B}$) display frequent homozygous deletions in primary cells from T- but not from B-cell lineage acute lymphoblastic leukemias. Blood 84:4038, 1994

46. Schmidt EE, Ichimura K, Reifenberger G, Collins VP: CDKN2 (p16/MTS1) gene deletion or CDK4 amplification occurs in the majority of glioblastomas. Cancer Res 54:6321, 1994

47. Jen J, Harper JW, Bigner SH, Bigner DD, Papadopoulos N, Markowitz S, Willson JKV, Kinzler KW, Vogelstein B: Deletion of p16 and p15 genes in brain tumors. Cancer Res 54:6353, 1994

48. Ogawa S, Hirano N, Sato N, Takahashi T, Hangaishi A, Tanaka K, Kurokawa M, Tanaka T, Mitani K, Yazaki Y, Hirai H: Homozygous loss of the cyclin-dependent kinase 4-inhibitor (p16) gene in human leukemias. Blood 84:2431, 1994

49. Quesnel B, Preudhomme C, Philippe N, Vanrumbeke M, Dervite I, Lai JL, Bauters F, Wattel E, Fenaux P: p16 gene homozygous deletions in acute lymphoblastic leukemia. Blood 85:657, 1995

49a. Takeuchi S, Bartram CR, Seriu T, Miller CW, Tobler A, Janssen JWG, Reiter A, Ludwig W-D, Zimmermann M, Schwaller J, Lee E, Miyoshi I, Koeffler HP: Analysis of a family of cyclin-dependent kinase inhibitors: p15/MTS2/INK4B, p16/MTS1/INK4A and p18 genes in acute lymphoblastic leukemia (ALL) of childhood. Blood (in press)

49b. Nakimaki T, Kawamata N, Schwaller J, Tobler A, Fey M, Pakkala S, Lee YY, Kim BK, Fukuchi K, Tsuroka N, Kahan J, Miller CW, Koeffler HP: Structural integrity of the cyclin-dependent kinase inhibitor genes, p15, p16 and p18 in myeloid leukemias. Br J Haematol (in press)

50. Offit K, Parsa NZ, Jhanwar SC, Filippa D, Wachtel M, Chaganti RSK: Clusters of chromosome 9 aberrations are associated with clinico-pathologic subsets of non-Hodgkin's lymphoma. Genes Chromosome Cancer 7:1, 1993

51. Chaganti SR, Gaidano G, Louie DC, Dalla-Favera R, Chagnti RSK: Diffuse large cell lymphomas exhibit frequent deletions in 9p21-22 and 9q31-34 regions. Genes Chromosom Cancer 12:32, 1995

52. Pinkus GS, Said JW: Characterization of non-Hodgkin's lymphomas using multiple cell markers. Immunologic, morphologic, and cytochemical studies of 72 cases. Am J Pathol 94:349, 1979

53. Ausebel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K: Current Protocols in Molecular Biology, vol 1. New York, NY, Greene Publishing Associates, and Wiley, 1993

54. Johnson KR, Nousseef WM, Care A, Wheelock MJ, Hudson S, Koeffler HP, Selsted M, Miller C, Rovera G: Characterization of cDNA clones for human MPO: Predicted amino acid sequence and evidence for multiple mRNA species. Nucleic Acids Res 15:2012, 1987

55. Orita M, Suzuki Y, Sekiya T, Hayashi K: Rapid and sensitive detection of point mutations and genetic polymorphisms using polymerase chain reaction. Genomics 5:874, 1989

56. Kawamata N, Miller CW, Koeffler HP: The molecular analysis of the cyclin-dependent kinase inhibitors p15 and p18 in non-small cell lung cancers. Mol Carcinogen (manuscript submitted)

57. Park DP, Pham E, Koeffler HP: (manuscript in preparation)

58. Gaidano G, Dalla-Favera R: Biologic and molecular characterization of non-Hodgkin's lymphoma. Curr Opin Oncol 5:776, 1993

59. Seto M, Yamamoto K, Iida S, Akao Y, Utsumi KR, Kubonishi I, Miyoshi I, Ohtsuki T, Yawata Y, Namba M, Motokura T, Arnold A, Takahasi T, Ueda R: Gene rearrangement and overexpression of PRAD-1 in lymphoid malignancy with t(11;14)(q13;q32) translocation. Oncogene 7:1401, 1992

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