[CANCER RESEARCH 55, 1206–1210, March 15, 1995]

Advances in Brief

Assignment of the Human *p27*^{Kip1} Gene to 12p13 and Its Analysis in Leukemias[1]

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Abstract

The *p27*^{Kip1} (*p27*) gene encodes an inducible inhibitor of cyclin-dependent kinase activity. Using a murine *p27* cDNA as probe, we obtained a human cDNA clone and subsequently used it to isolate a genomic clone of this gene. The coding region of the human *p27* gene was contained in two exons. Both the amino acid sequence and intron-exon organization of *p27* were similar to those previously found for the related cyclin-dependent kinase inhibitor p21^{Waf1} (p21). The *p27* gene was localized to chromosome band 12p13 by a combination of somatic cell hybrid and fluorescence *in situ* hybridization analyses. The *p27* gene product is thought to control the leukocyte cell cycle and the 12p13 chromosomal band is known to be deleted in leukemias, suggesting that the *p27* gene may act as a tumor suppressor gene in leukemias. Although *p27* was found to reside in the minimal region of chromosomal loss in hematological malignancies, no mutations of *p27* were observed in leukemia samples. Haploinsufficiency of *p27* may confer a growth advantage to leukemia cells.

Introduction

Cell cycle progression in mammalian cells is controlled by the ordered activation and inactivation of a series of cyclin-CDK[3] complexes (1). Four genes encoding inhibitors of these complexes have been reported. In mammalian cells, the *p15* and *p16* genes encode related proteins that primarily inhibit cyclin D-CDK4 and cyclin D-CDK6 complexes (2, 3). The *p21* and *p27* genes encode homologous proteins which are potent inhibitors of all the cyclin-CDK complexes implicated in G_1 and S (4–12).

Connections are emerging between cyclin-CDK inhibitors and cancer. Alterations of the *p16*^{CDKN2} gene, on chromosome 9p21, have been reported in several types of human tumors (13–18), and germline mutations of this gene have been identified in a subset of patients with familial melanoma (4, 19, 20). The identification of *p15* as another CDK inhibitor located at 9p21 (3, 13) and the finding that both *p15* and *p16* genes are homozygously deleted in brain tumors (21) raise the possibility that inactivation of both genes is important for some tumors. The expression of *p21* has been shown to be induced by DNA damage, with the induction-dependent on wild-type *p53* expression (22). However, there is no evidence that *p21* mutations are directly involved in human tumors (23). Both *p15* and *p27* have been implicated in the cell cycle arrest that occurs following transforming growth factor β treatment of cells (3, 9, 10). Resistance to transforming growth factor β growth inhibition is a hallmark of many cancer cell lines, further linking CDK inhibitors to neoplasia (24).

On the basis of the above, we attempted to determine whether mutations of *p27* might be involved in human tumorigenesis. We obtained cDNA and genomic clones of this gene and determined its sequence, intron-exon structure, and chromosomal location. We then analyzed its potential involvement in leukemias with cytogenetic alterations affecting its chromosomal locus.

Materials and Methods

***p27* Cloning and Characterization.** A human fetal brain cDNA library in the Lambda ZAP II vector (Stratagene) was screened with a ^{32}P-labeled mouse *p27* cDNA probe (10). Positive plaques were identified and purified, and the inserts were rescued from the phage as pBS SK(=) phagemids. The sequences of the inserts were determined using external primers and additional primers from the sequences generated.

A human genomic P1 library (Genome Systems, Inc.) was screened by PCR using primers designed from the cDNA sequence of human *p27*. Two P1 clones, 2096 and 2097, were identified using primers that yielded a 48-base pair genomic product (nucleotides 509 to 556 of the *p27* sequence in Table 1). Confirmation of the cDNA sequence and definition of the intron-exon borders of *p27* were determined by cycle sequencing of the P1 genomic clones using ^{32}P end-labeled primers chosen from the cDNA sequence (25).

Mutational Analysis. Bone marrow samples were obtained from nine patients with leukemia or myelodysplastic syndrome at the University of Chicago. Genomic DNA was prepared from the samples as described (26). For mutational analysis, the genomic DNA fragment containing the entire *p27* coding region was amplified with the following primers: 5′-TAATTTGCCAGCAACCACTAA-3′ and 5′-TCGTGCAGACCCGGGAG-3′. All PCR reactions were carried out in a 50-μl volume using 50 ng genomic DNA. The conditions for amplification were 95°C for 30 s, 55°C for 90 s, and 70°C for 90 s (30 cycles). The 1200-base pair products were purified and directly cycle sequenced as described (25) using ^{32}P-labeled primers designed from the cDNA.

FISH. To map the location of the *p27* gene, DNA isolated from two P1 clones (2096, 2097) was biotinylated by nick translation and hybridized to metaphase spreads of a healthy male. Probe labeling, slide preparation, hybridization, washing, and fluorescent tagging of hybridization sites were performed as described (27) with four modifications: (*a*) cells were synchronized before harvest by a methotrexate block and released into bromodeoxyuridine (28); (*b*) approximately 100 ng labeled probe were applied to the slides in a 10-μl hybridization volume; (*c*) antibodies and avidin were applied to slides in 4× SSC/5% nonfat dry milk; [1× SSC, 0.15 M NaCl + 0.015 M sodium citrate (ph 7.0)]; and (*d*) slides were washed between detection layers in 2× SSC containing 0.005% 3-[(3-cholamidopropyl)dimethylamino]-1-propanesulfate detergent, as recommended by L-C. Yu (University of California at San Francisco). After these steps, the metaphase chromosomes were R-banded by Hoechst staining, UV irradiation, and propidium iodide staining (28). The positions of probe hybridization sites and bands were viewed simultaneously through a FITC/Texas red dual-band pass filter (Chromatechnology). Ten metaphase cells were evaluated for each P1 clone.

For FISH analysis of the patient samples, the DNA of P1 clone 2096 was sequence- independently amplified and PCR labeled with BIO-11-dUTP as described previously (29) and hybridized to metaphase cells as described (30). The probe was detected with FITC-conjugated avidin and the chromosomes

Received; 12/1/94; accepted 2/10/95.

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[1] This research was supported in part by NIH Grants CA 43460 (B. V.) and CA42577 (J. D. R.), Department of Energy Grants DE-FGO2-86ER60408 (J. D. R.) and DE-FG06-93ER61553 (B. T.), and the Pruess Foundation. B. V. is an American Cancer Society Research Professor.

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[3] The abbreviations used are: CDK, cyclin-dependent kinase; FISH, fluorescence *in situ* hybridization.

Table 1 *Intron-exon borders*

Exon	Exon size (base pairs)	Codon	Sequence[a]
1[b]	>474	1–158	5′-GGCGGTCGTGCAGACCCGGGAGAAAGATGTCAAACGTGCGAGTGTCT^{nt21} AACGGGAGCCCTAGCCTGGAGCGGATGGACGCCAGGCAGGCGGATCAC^{nt69} CCCAAGCCCTCGGCCTGCAGGAACCTCTTCGGCCCGGTGGACCACGAA^{nt117} GAGTTAACCCGGGACTTGGAGAAGCACTGCAGAGACATGGAAGAGGCG^{nt165} AGCCAGCGCAAGTGGAATTTCGATTTTCAGAATCACAAACCCCTAGAG^{nt213} GGCAAGTACGAGTGGCAAGAGGTGGAGAAGGGCAGCTTGCCCGAGTTC^{nt261} TACTACAGACCCCCGCGGCCCCCCAAAGGTGCCTGCAAGGTGCCGGCG^{nt309} CAGGAGAGCCAGGATGGCAGCGGGAGCCGCCCGGCGGCGCCTTTAATT^{nt357} GGGGCTCCGGCTAACTCTGAGGACACGCATTTGGTGGACCCAAAGACT^{nt405} GATCCGTCGGACAGCCAGACGGGGTTAGCGGAGCAATGCGCAGGAATA^{nt453} AGGAAGCGACCTGCAACCGACGgtaatgccctttcccaaccatagaat^{nt475} gtgtttggggccttcagacctcacgatacctgatcttactggttgatg gcaaattaaaagcttatgggg-3′
2[c]	120	159–198	5′-gggtttttctacccctgactatggggccaacttctgccagccattgt ttttctaataaagattgtgtgttctttttaaaaatttcccctgcgct tagATTCTTCTACTCAAAACAAAAGAGCCAACAGAACAGAAGAAAATG^{nt520} TTTCAGACGGTTCCCCAAATGCCGGTTCTGTGGAGCAGACGCCCAAGA^{nt568} AGCCTGGCCTCAGAAGACGTCAAACGTAAACAGCTCGgtgggttgatc^{nt594} actaaaggagcacgcactggaacccggggccttcagacctcacgatac ctgatcttactggttgctggcaaattaaaagcttatgggg-3′

[a] Upper case letters correspond to exons, lower case to introns. nt, numbering of nucleotides begins with the initiating AUG, including only the coding nucleotides.
[b] Includes 26 base pairs of untranslated sequence upstream of the initiating ATG (*underlined*).
[c] Includes 8 base pairs of untranslated sequences downstream of the stop codon (*underlined*).

were identified by counter staining with 4,6-diamidino-2-phenylendole. Between 2 and 16 (mean, 11) metaphase cells were scored for the presence or absence of a hybridization signal on the normal and deleted chromosome 12 by 2 observers. The cytogenetic findings on six of the patients reported here have been described previously; patients 1–6 in Table 2 correspond to patients 4, 11, 17, 18, 20, and 21, respectively, by Kobayashi *et al.* (31).

Results

Characterization and Localization of the Human *p27* Gene. A full-length mouse *p27* cDNA was used to screen a human fetal brain cDNA library. Positively reacting clones were isolated, and after the hybridizing fragments were sequenced, a sequence homologous to mouse *p27* was identified (Table 1). The cDNA had an open reading frame of 594 nucleotides coding for a protein of 198 amino acids. The sequence is the same as previously reported (10) with the exception of codon 109. We found GGC (glycine) at this codon instead of the previously identified GTC (valine).

A human genomic P1 library was screened by PCR using primers designed from the cDNA sequence. Two P1 clones were identified that contained the entire *p27* coding region. Analysis of the P1 clones revealed that the gene contained two introns, one located within the coding region and one located after the stop codon (Table 1). The position of intron 1 (Table 1) was identical to that found in the related human *p21* gene.

Utilizing somatic cell hybrid panels, *p27* was localized to chromosome arm 12p (data not shown). FISH was used to define the chromosomal location of p27 as 12p13.1–13.2 (Fig. 1). The two P1 genomic clones were localized relative to fluorescent R bands. In independent experiments, the two P1 clones gave hybridization signals in the same location. A total of 77 hybridization signals were observed at 12p13.1–p13.2 in 20 metaphase cells (10 for each clone). The efficiency of hybridization was therefore 96% (77 of 80). No double signals were seen on other chromosomes. The *p27* gene has been independently localized to the 12p12–12p13 boundary by other investigators (32, 33).

Mutational Analysis in Leukemia Patients. Translocations and deletions of the short arm of chromosome 12 occur in a wide spectrum of hematological malignant diseases. In a recent study of 20 patients with lymphoid and myeloid malignancy, Kobayashi *et al.* (31) showed that the majority of 12p13 translocation breakpoints were clustered between 2 cosmid markers, *D12S133* (telomeric) and *D12S142* (centromeric). Additionally, in 11 patients with 12p deletions, the common segment of deletion spanned 12p12.2 to 12p13.1. This common segment is flanked by markers *D12S133* distally and *D12S140* proximally and includes *D12S142*.

The *p27* gene thus became a candidate for the "target" of 12p13 deletions in leukemias. The "target" refers to the gene within the deletion, the inactivation of which is responsible for a growth advan-

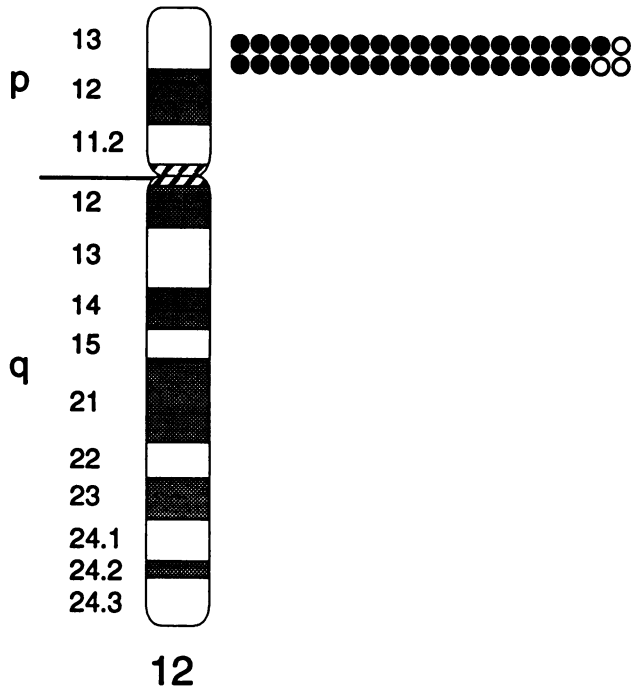

Fig. 1. FISH analysis of *p27* in normal cells. The chromosomal location of fluorescent signals observed in FISH with *p27* probes is diagrammed. Fluorescence-tagged probe sites were mapped relative to red fluorescent R bands in metaphase chromosomes of a normal male. A total of 20 metaphase cells were evaluated, 10 for each of the 2 P1 clones used. Because the results of the two clones were similar, observations were pooled. ●, presence of signal on both chromatids at the indicated site; ○, presence of signal on only one chromatid at the indicated site.

Table 2 *Clinical, cytogenetic, and p27 polymorphism data for 9 patients with 12p abnormalities*

Patient[a]	Diagnosis[b]	% of abnormal cells[c]	Karyotype	Location of P1 p27 signals[d]	Cells examined (FISH)	p27 codon 109 sequence
1	AML-MO	84	45,add(X)(p11),Y,−3,der(3)t(3;15)(q12;q15),t(4;7)(q21;q22),−5**t(9;12;14)(q34;p13;q22),del(12)(p11p13)**,der(15)del(15)(q11q15)t(3;15)(q12;q15),der(18)t(3;18)(q11;q1?2),+mar[26]/related clones [5]/46,XY[1]	12p	15	gtc
2	ALL-L3	72	47,XX,**del(12)(p11p13),+del(12)(p11p13),t(12;22)(p13;q13)**[17]/46,XX[7]	12p	8	gtc
3	RAEB	58	46,XY,inv(15)(q13q24)[6]/46,idem,**del(12)(p11.2p13)**[14]/46,XY[4]	12p	16	gtc
4	MDS	72	47,XX,del(5)(q13q33),+8[6]/47,idem,**del(12)(p11p12)**[14]	12p	7	gtc
5	AML-M6	71	46,XX,del(5)(q15q33 or q21q34)[4]/45,idem,−7+8,**del(12)(p12p13)**,15,del(16)(q13q24)[2]/47,idem,+10[4]/46,XX,del(5),−7+11,**del(12)**, del(16)[8]/46,XX[2]	12p	8	gtc
6	T-AML	100	44,XY,der(3;6)(q10;p10),del(5)(q11q34),−7,**del(12)(p11,p13)**[24]/45,idem,+r[18]/46,idem,+r,+mar[2]	12p	15	gtc
7	ALL-L2	93	45,XY,−1,del(3)(p23p26),der(10)t(1;10)(q21;p15),**der(12)t(1;12)(p32.2;p13.2 or p31;p11)**[15]/46,idem,+mar[11]/46,XY[2]	12p	12	gtc
8	AML-M1	91	46,X,−Y,−2,add(4)(p11),add(5(q15),del(5)(p11p15),+6,inv(11)(q13q25),**der(12)t(2;12)(q13;p13)**,−17,+2mar1[25]/47,idem,t(9;10)(q34;q22)+mar2[4]/46,XY[3]	12p	2	ggc
9	T-AML	89	45,XX,der(5)t(5;7)(q12;q11),−7,**del(12)(p11p13)**[2]/45,idem,del(4)(q25q31),der(11)ins(11;?)(q14;?)t(2;11)(q13;q14)[15]	12p	15	gtc

[a] Patients 1 through 6 are patients 4, 11, 17, 18, 20, and 21 in Ref. 30.

[b] MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; T-AML, therapy-related acute myeloid leukemia; BM, bone marrow; RAEB, refractory anemia with excess blasts.

[c] Analyses were on pretreatment bone marrow samples.

[d] Using the p27 P1 clone as a probe in two-color FISH analysis, p27 was localized between *D12S133* (telomeric) and *D12S142* (centromeric) in all cases.

tage. According to the hypothesis enunciated by Knudson (34), one expects the target gene to be doubly mutated in the tumor cells; one allele is usually deleted through a gross chromosomal event, while the other is inactivated through an intragenic mutation. Evidence that *p27* is a target of genetic alteration in these leukemias would thereby follow from demonstration of intragenic *p27* mutations in tumor cells that have lost one allele.

Bone marrow samples from 45 leukemia patients with cytogenetic abnormalities involving 12p13, excluding those with balanced translocations, were analyzed by FISH using a p27 P1 probe. Thirty-four patients were found to have hemizygous deletions which included *p27* (35). Nine of these patients were analyzed for *p27* mutations. All nine had a deletion of the p27 FISH probe on the del(12p) chromosome, whereas one FISH signal was visible on the other chromosome 12 (Table 2; Fig. 2). There was a high proportion of leukemic cells in each of the bone marrow samples, as judged by cytogenetic analysis in each of the bone marrow samples evaluated (Table 2). A DNA fragment containing the entire *p27* coding region was amplified from bone marrow DNA. A PCR fragment of the expected size was obtained in every case and the entire coding sequence and intron-exon borders were examined by cycle sequencing both strands of the fragment. The only variant noted was at codon 109, where valine was found in eight patients and glycine in one (Table 2). This variant confirmed that nucleotide substitutions could be detected when pre-

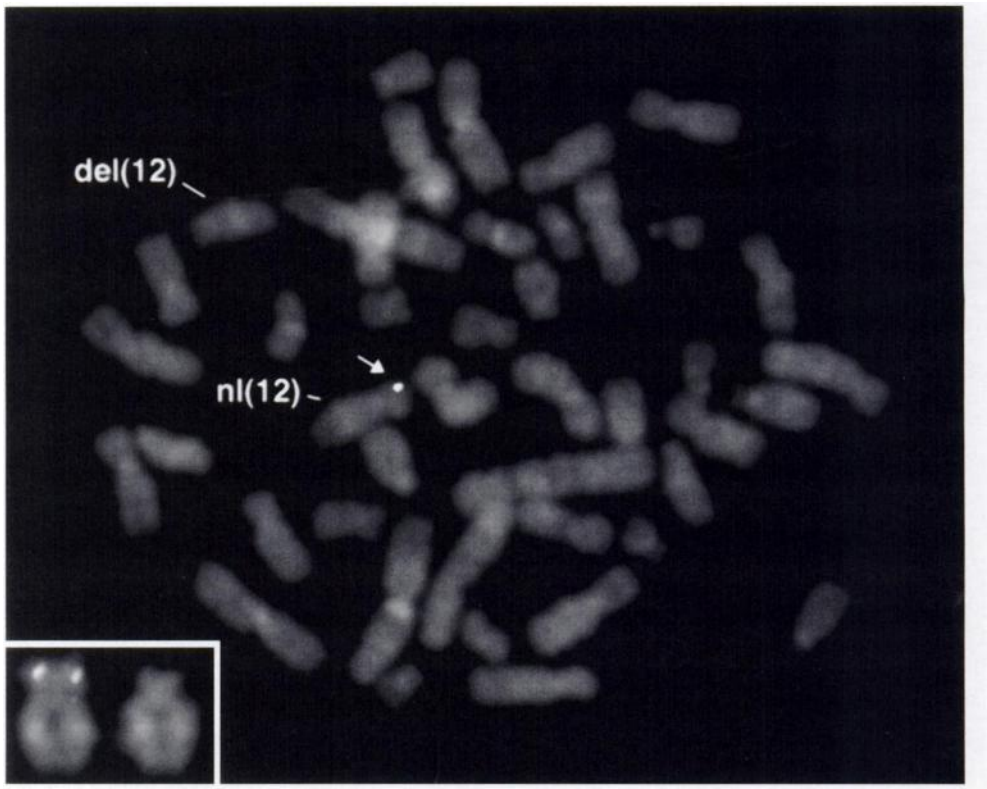

Fig. 2. FISH analysis of leukemia patients. Metaphase cells from leukemia patient 9 showing the *in situ* hybridization of *p27* P1 clone 2096. The deleted chromosome 12 [*del(12)*] shows no hybridization signal, whereas a clear signal can be seen on one chromatid of the normal 12 [*nl(12)*]. *Inset*, chromosomes 12 from patient 6 hybridized with the same probe. The normal chromosome shows signals on both chromatids at 12p13, whereas no signal is present on the deleted chromosome 12. Separate gray-scale images were taken with charge-coupled device camera for the FITC and 4,6-diamidino-2-phenylindole images. The images were adjusted to different gray levels and digitally superimposed using the NIH image program.

sent. We assume that this change represented a polymorphism rather than a functional change, because either a valine or glycine were encoded at codon 109 of *p27* in eight normal individuals (data not shown).

Discussion

Tumor suppressor genes encode proteins that antagonize the transformed state of cells. Their inactivation through chromosome loss and/or mutation is thought to be required for progression through the various stages of malignancy. Based on the inhibitory effect of known tumor suppressor genes on cell division and on other characteristics of the transformed state, it is reasonable to assume that certain types of gene products might act as tumor suppressors. In light of the role of cyclin-CDK complexes in the control of the cell cycle, inhibitors of this activity are attractive candidates in this regard.

The hypothesis that *p27* was a tumor suppressor was based on three observations: (*a*) it functions biochemically as a cell cycle inhibitor (9–12); (*b*) its expression in leukocytes may negatively control the transition from quiescence to proliferation (12, 36); (*c*) it is localized to a chromosomal region commonly deleted in leukemias (31). As shown previously, chromosomal deletions or unbalanced translocations involving 12p13 are frequent events in lymphoid and myeloid malignancies (31). Several leukemias were identified in this study that had hemizygous deletions involving the *p27* locus. However, the search for intragenic *p27* mutations in leukemic cells with chromosomal deletion of one *p27* allele revealed no evidence of an abnormal sequence in the remaining allele.

At least three explanations of these data can be offered: (*a*) the possibility exists that the relevant mutations of *p27* reside outside the coding region and intron-exon borders examined in this study. However, it seems unlikely that nine independent mutations would occur in such unevaluated regions; (*b*) another gene may be the altered target that resides within the several-megabase region of 12p that defines the minimal region consistently lost in leukemia (31); (*c*) haploinsufficiency of p27 may provide a selective growth advantage. Reduced levels of p27 in cells due to hemizygosity at 12p13 could alter the stoichiometric relationship of p27 with other components of the cyclin-CDK-regulatory complexes (10, 11) and result in a loss or reduction of CDK-inhibitory activity. Similarly, it has been proposed that the precise levels of p21 protein may be critical in defining the probability of cell cycle progression (4–7). As with *p27*, *p21* mutations have not been found, even in tumors with 6p21 loss (23)[4]. Ponce-Castañeda *et al.* (32) have independently noted the absence of *p27* mutations in a variety of solid tumors. Low levels of *p21* and *p27* may be necessary for normal cell cycle progression, whereas a higher level may be required for growth-inhibitory pathways. A reduction in the protein level following loss of one allele would therefore provide a cell with a selective growth advantage while inactivation of both alleles would be a lethal event. This hypothesis can be tested through the development of appropriate "knock-out" cells or mice.

Acknowledgments

We thank Dr. Michelle M. Le Beau for help with the karyotypes and Rafael Espinosa III and Elizabeth Davis for their expert assistance in the FISH analyses of the patient samples.

References

1. Sherr, C. J. Mammalian G1 cyclins. Cell, *73:* 1059–1065, 1993.
2. Serrano, M., Hannon, G. L., and Beach, D. A new regulatory motif in cell-cycle control causing specific inhibition of cyclinD/CDK4. Nature (Lond.), *366:* 704–707, 1993.
3. Hannon, G. J., and Beach, D. $p15^{INK4B}$ is a potential effector of TGF-β-induced cell cycle arrest. Nature (Lond.), *371:* 257–261, 1994.
4. Harper, J. W., Adami, G. R., Keyomarsi, K., and Elledge, S. J. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. Cell, *75:* 805–816, 1993.
5. El-Deiry, W. S., Tokino, T., Velculescu, V. E., Levy, D. B., Parsons, R., Trent, J. M., Lin, D., Mercer, E., Kinzler, K. W., and Vogelstein, B. WAF1, a potential mediator of *p53* tumor suppression. Cell, *75:* 817–825, 1993.
6. Xiong, Y., Hannon, G. J., Zhang, H., Casso, D., Kobayashi, H., and Beach, D. p21 is a universal inhibitor of cyclin kinases. Nature (Lond.), *366:* 701–704, 1993.
7. Gu, Y., Turck, C. W., and Morgan, D. O. Inhibition of CDK2 activity *in vivo* by an associated 20K regulatory subunit. Nature (Lond.), *366:* 707–710, 1993.
8. Noda, A., Ning, Y., Venable, S. F., Periera-Smith, O. M., and Smith, J. R. Cloning of senescent cell-derived inhibitors of DNA synthesis using an expression screen. Exp. Cell Res., *211:* 90–98, 1994.
9. Polyak, K., Kato, J. Y., Solomon, M. J., Sherr, C. J., Massague, J., Roberts, J. M., and Koff, A. $p27^{Kip1}$, a cyclin-Cdk inhibitor, links transforming growth factor-B and contact inhibition to cell cycle arrest. Genes Dev., *8:* 9–22, 1994.
10. Polyak, K., Lee, M-H., Erdjument-Bromage, H., Koff, A., Roberts, J. M., Tempst, P., and Massaque, J. Cloning of $p27^{Kip1}$, a cyclin-dependent kinase inhibitor and a potential mediator of extracellular antimitogenic signals. Cell, *78:* 59–66, 1994.
11. Toyoshima, H., and Hunter, T. p27, a novel inhibitor of G1 cyclin-cdk protein kinase activity, is related to p21. Cell, *78:* 67–74, 1994.
12. Firpo, E. J., Koff, A., Solomon, M. J., and Roberts, J. Inactivation of a Cdk2 inhibitor during interleukin 2-induced proliferation of human T lymphocytes. Mol. Cell. Biol., *14:* 4889–4901, 1994.
13. Kamb, A., Gruis, N. A., Weaver-Feldhaus, J., Liu, Q., Harshman, K., Tavitigian, S. V., Stochert, E., Day, R. S., III, Johnson, B. E., and Skolnick, M. H. A cell cycle regulator potentially involved in genesis of many tumor types. Science (Washington DC), *264:* 436–440, 1994.
14. Nobori, T., Miura, K., Wu, D. J., Lois, A., Takabayashi, K., and Carson, D. A. Deletions of the cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. Nature (Lond.), *368:* 753–756, 1994.
15. Okamoto, A., Demetrick, D. J., Spillare, E. A., Hagiwara, K., Hussain, S. P., Bennett, W. P., Forrester, K., Gerwin, B., Serrano, M., Beach, D. H., and Harris, C. C. Mutations and altered expression of $p16^{INK4}$ in human cancer. Proc. Natl. Acad. Sci. USA, *91:* 11045–11049, 1994.
16. Mori, T., Miura, K., Aoki, T., Nishihira, T., Mori, S., and Nakamura, Y. Frequent somatic mutation of the *MTS1/CDK4I* (multiple tumor suppressor/cyclin-dependent kinase 4 inhibitor) gene in esophageal squamous cell carcinoma. Cancer Res., *54:* 3396–3397, 1994.
17. Caldas, C., Hahn, S. A., da Costa, L. T., Redston, M. S., Schutte, M., Seymour, A. B., Weinstein, C. L., Hruban, R. H., Yeo, C. J., and Kern, S. E. Frequent somatic mutations and homozygous deletions in the *p16 (MTS1)* gene in pancreatic adenocarcinoma. Nat. Genet., *8:* 27–32, 1994.
18. He, J., Allen, J. R., Collins, V. P., Allalunis-Turner, M. J., Godbout, R., Day, R. S., III, and James, C. D. CDK4 amplification is an alternative mechanism to *p16* gene homozygous deletion in glioma cell lines. Cancer Res., *54:* 5804–5807, 1994.
19. Hussussian, C. J., Struewing, J. P., Goldstein, A. M., Higgins, P. A. T., Ally, D. S., Sheahan, M. D., Clark, W. H., Tucker, M. A., and Dracopoli, N. C. Germline *p16* mutations in familial melanoma. Nat. Genet., *8:* 15–21, 1994.
20. Kamb, A., Shattuuck-Eidens, D., Eeles, R., Liu, Q., Gruis, N. A., Ding, W., Hussey, C., Tran, T., Miki, Y., Weaver-Feldhaus, J., McClure, M., Aitken, J. F., Anderson, D. E., Bergman, W., Frants, R., Goldgar, D. E., Green, A., MacLennan, R., Martin, N. G., Meyer, L. J., Youl, P., Zone, J. J., Skolnick, M. H., and Cannon-Albright, L. A. Analysis of the *p16* gene (*CDKN2*) as a candidate for the chromosome 9p melanoma susceptibility locus. Nat. Genet., *8:* 22–26, 1994.
21. Jen, J., Harper, J. W., Bigner, S. H., Bigner, D. D., Papadopoulos, N., Markowitz, S., Willson, J. K. V., Kinzler, K. W., and Vogelstein, B. Deletion of *p16* and *p15* genes in brain tumors. Cancer Res., *54:* 6353–6358, 1994.
22. El-Deiry, W. S., Harper, J. W., O'Connor, P. M., Velculescu, V. E., Canman, C. E., Jackman, J., Pietenpol, J. A., Burrell, M., Hill, D. E., Wang, Y., Wiman, K. G., Mercer, W. E., Kastan, M. B., Kohn, K. W., Elledge, S. J., Kinzler, K. W., and Vogelstein, B. WAF/CIP1 is induced in p53-mediated G_1 arrest and apoptosis. Cancer Res., *54:* 1169–1174, 1994.
23. Shiohara, M., El-Deiry, W. S., Wada, M., Nakamaki, T., Takeuchi, S., Yang, R., Chen, D-L., Vogelstein, B., and Koeffler, H. P. Absence of *WAF1* mutations in a variety of human malignancies. Blood, *84:* 3781–3784, 1994.
24. Moses, H. L., Tucker, R. F., Leof, E. B., Coffey, R. J., Jr., Halper, J., and Shipley, G. D. Type β transforming growth factor is a growth stimulator and a growth inhibitor. Cancer Cells (Cold Spring Harbor), *3:* 65–71, 1985.
25. Papadopoulos, N., Nicolaides, N. C., Wei, Y-F., Rube, S. M., Carter, K. C., Rosen, C. A., Haseltine, W. A., Fleischmann, R. D., Fraser, C. M., Adams, M. D., Venter, J. C., Hamilton, S. R., Petersen, G. M., Watson, P., Lynch, H. T., Peltomaki, P., Mecklin, J-P., de la Chapelle, A., Kinzler, K. W., and Vogelstein, B. Mutation of a *mutL* homolog in hereditary colon cancer. Science (Washington DC), *263:* 1625–1629, 1994.
26. Goelz, S. E., Hamilton, S. R., and Vogelstein, B. Purification of DNA from formaldehyde fixed and paraffin embedded human tissue. Biochem. Biophys. Res. Commun., *130:* 118–126, 1985.
27. Trask, B. J. DNA sequence localization in metaphase and interphase cells by fluorescence *in situ* hybridization. Methods Cell Biol., *35:* 3–35, 1991.

[4] Unpublished data.

28. Cherif, D., Julier, C., Delattre, O., Derre, J., Lathrop, G. M., and Berger, R. Simultaneous localization of cosmids and chromosome R-banding by fluorescence microscopy: application to regional mapping of human chromosome 11. Proc. Natl. Acad. Sci. USA, *87:* 6639–6643, 1991.
29. Bohlander, S. K., Espinosa, R., Fernald, A. A., Rowley, J. D., Le Beau, M. M., and Diaz, M. O. Sequence-independent amplification and labeling of yeast artificial chromosomes for fluorescence *in situ* hybridization. Cytogenet. Cell Genet., *65:* 108–110, 1994.
30. Rowley, J. D., Diaz, M. O., Espinosa, R., Patel, Y. D., van Melle, E., Ziemin, S., Taillon-Miller, P., Lichter, P., Evans, G. A., Kersey, J. H., Ward, D. C., Domer, P. H., and Le Beau, M. M. Mapping chromosome band 11q23 in human acute leukemia with biotinylated probes: identification of 11q23 translocation breakpoints with a yeast artificial chromosome. Proc. Natl. Acad. Sci. USA, *87:* 9358–9362, 1990.
31. Kobayashi, H., Montgomery, K. T., Bohlander, S. K., Adra, C. N., Lim, B. L., Kucherlapati, R. S., Donis-Keller, H., Holt, M. S., Le Beau, M. M., and Rowley, J. D. Fluorescence *in situ* hybridization mapping of translocations and deletion involving the short arm of human chromosome 12 in malignant hematologic diseases. Blood, *84:* 3473, 1994.
32. Ponce-Castañeda, M. V., Lee, M-H., Latres, E., Polyak, K., Lacombe, L., Montgomery, K., Mathew, S., Krauter, K., Schinfeld, J., Massague, J., and Cordon-Cardo, C. $p27^{Kip1}$: chromosomal mapping to 12p12 and absence of mutations in human tumors. Cancer Res., *55:* 1211–1214, 1995.
33. Bullrich, F., MacLachlan, T. K., Sang, N., Druck, T., Veronese, M. L., Allen, S. L., Chlorazzi, N., Koff, A., Heubner, K., Croce, C. M., and Giordano, A. Chromosomal mapping of members of the cdc2 family of protein kinases cdk3, cdk6, PISSLRE, and PITALRE, and a cdk inhibitor, $p27^{Kip1}$, to regions involved in cancer. Cancer Res., *55:* 1199–1205, 1995.
34. Knudson, A. G. Antioncogenes and human cancer. Proc. Natl. Acad. Sci. USA, *90:* 10914–10921, 1993.
35. Sato, Y., Suto, Y., Pietenpol, J. A., Golub, T. R., Gilliland, D. G., Roberts, J. M., Trask, B. J., Le Beau, M. M., Vogelstein, B., Rowley, J. D., and Bohlander, S. K. *TEL* and the gene for $p27^{Kip1}$ are frequently deleted in patients with 12p13 rearrangements and hematopoietic malignancies. Blood, *84:* 292a, 1994.
36. Nourse, J., Firpo, E., Flanagan, W. M., Coats, S., Polyak, K., Lee, M-H., Massaque, J., Crabtree, G., and Roberts, J. Rapamycin prevents interleukin-2-mediated elimination of the cyclin-dependent-kinase inhibitor, $p27^{Kip1}$. Nature (Lond.), in press, 1995.