[CANCER RESEARCH 55, 1941–1945, May 1, 1995]

# Loss of P16$^{INK4}$ Expression Is Frequent in High Grade Gliomas[1]

**Ryo Nishikawa, Frank B. Furnari,[2] Hong Lin, Wadih Arap, Mitchel S. Berger, Webster K. Cavenee, and H-J. Su Huang[3]**

*Ludwig Institute for Cancer Research [R. N., F. B. F., H. L., W. A., W. K. C., H-J. S. H.], Department of Medicine [W. K. C., H-J. S. H.], and Center for Molecular Genetics [W. K. C.], University of California-San Diego, La Jolla, California 92093; Cancer Biology Program, Stanford University, Stanford, California 94305 [W. A.]; and Department of Neurological Surgery, University of Washington School of Medicine, Seattle, Washington 89195 [M. S. B.]*

## ABSTRACT

**P16$^{INK4}$ is a cell cycle regulator that specifically binds to and inactivates cyclin-dependent kinase 4 (CDK4). Its encoding gene (*p16/CDKN2*) maps to chromosome 9p21, a region that undergoes frequent loss of heterozygosity in a variety of human tumors. We have analyzed the *p16/CDKN2* gene and its expression in a series of primary glioma samples. Although homozygous deletion or mutation of the *p16/CDKN2* gene was uncommon in this series and P16$^{INK4}$ protein was detectable in all grade II tumors, it was present in only 50% of grade III and grade IV samples. Conversely, in some grade IV tumors the level of P16$^{INK4}$ protein was elevated; in these cases, its target, CDK4, was amplified and overexpressed. These results suggest: (*a*) the involvement of P16$^{INK4}$ in glioma progression; (*b*) that mechanisms other than mutation or deletion can down-regulate expression of the *p16/CDKN2* gene; and (*c*) that the balance between CDK4 and its cognate inhibitor, P16$^{INK4}$, may confer a cell growth advantage and facilitate tumor progression.**

## INTRODUCTION

Mammalian cell cycle progression is regulated by the sequential activation and subsequent inactivation of various cyclin-dependent kinases at different stages of the cell cycle (1). CDK4[4] associates with and is activated by D-type cyclins in $G_1$ (2), and it can be negatively modulated by the cell cycle inhibitor, P16$^{INK4}$ (3). The mechanism for this inhibition is not well characterized, but *in vitro* evidence suggests that P16$^{INK4}$ may compete with cyclin D in binding to CDK4 and thereby prevent the kinase from being activated (3). Thus, mutation or deletion of *p16/CDKN2* may affect the balance between P16$^{INK4}$ and D cyclins resulting in abnormal cell growth. In agreement with this hypothesis, homozygous mutation or deletion of the *p16/CDKN2* gene has been found to be very common in tumor cell lines of different origins (4, 5). However, subsequent studies have demonstrated a much lower frequency of mutations in corresponding primary tumors (4–10), with only a few exceptions (8, 11–13), raising questions about the role of *p16/CDKN2* in tumor development or progression.

The *p16/CDKN2* gene is located in chromosome 9p21 (14), a region with frequent loss of heterozygosity in malignant (grade III and grade IV) gliomas (15, 16). Recently, several authors have studied large numbers of glioma samples and shown that 27 to 34% of the clinical samples of malignant cases (17–19) and 68% of xenografts of glioblastomas (20) have homozygous deletion of *p16/CDKN2* locus. However, thus far, only a single case of missense mutation has been reported in clinical samples of gliomas that had lost the second allele (21). To date, it has been unclear whether the *p16/CDKN2* gene is the target of the 9p21 deletions seen in this tumor type or whether the actual target is another gene located nearby (20, 21). Here we have analyzed the *p16/CDKN2* gene and its expression in a series of primary low and high grade gliomas. Despite mutation or deletion of the *p16/CDKN2* gene being uncommon in this series, its protein was undetectable in one-half of the high grade tumors. Moreover, some of the remaining cases in which the protein could be detected had amplified or overexpressed *CDK4* genes. These results support the role of P16$^{INK4}$ in glioma progression and suggest that its inactivation can occur by mechanisms apart from mutation or deletion.

## MATERIALS AND METHODS

**Tumor Samples.** Glioma samples were obtained from 21 brain tumor patients who had operations at the University of Washington, Seattle, WA. All tumors were classified on the basis of the WHO criteria for tumors of the central nervous system (22) and quick frozen at the time of resection until analysis. The series consisted of 6 cases of low grade astrocytoma (WHO grade II), and 15 cases of high grade disease [6 cases of anaplastic astrocytoma (WHO grade III) and 9 cases of glioblastoma multiforme (WHO grade IV)].

**Nucleic Acid Isolation and Analysis.** High molecular weight genomic DNA was prepared from tumor tissues by proteinase K digestion and phenol/chloroform extraction. Approximately 10 μg of DNA were digested with *Eco*RI, separated by electrophoresis through 0.9% agarose gels and transferred to nylon membranes. A *p16/CDKN2* cDNA probe covering nucleotides 25 to 960 (3) and a full length human CDK4 cDNA probe (23) were $^{32}$P-labeled by random priming (24). The amount and integrity of the loaded DNA were determined by reprobing the membranes with the *ABL* gene located on the chromosome 9q arm. Quantitative densitometric analysis of Southern blot hybridizations was performed using a laser densitometer (UltroScan XL; LKB, Bromma, Sweden). Densities of each signal corresponding to *p16/CDKN2* and *CDK4* were normalized to that of the *ABL* gene. A relative increase in gene dosage of more than 5 times that of DNA extracted from peripheral blood lymphocytes of a healthy volunteer was considered amplification. RNA was isolated using the guanidinium thiocyanate method (25) Approximately 20 μg of total cellular RNA were electrophoresed through a 1% denaturing agarose-formaldehyde gel and blotted onto nylon membranes. Northern blot hybridization and washing was performed using Quickhyb solution (Stratagene, La Jolla, CA). A 5′ region *p16/CDKN2*-specific $^{32}$P-labeled probe containing 172 base pairs [nucleotides −40 to 132 (5′ sequences were acquired from GeneBank)] was generated by PCR using primers 5′-CGGAGAGGGGGAGAACAGACAACG-3′ (sense) and 5′-GCCTCCGACCGTAACTATTCGG-3′ (antisense). RNA quality and blotting efficiency was assessed by reprobing the membranes with the glyceraldehyde-3-phosphate dehydrogenase cDNA (26).

**p16/CDKN2 Genomic DNA Sequencing.** For exons 1 and 3, direct *p16/CDKN2* DNA $^{35}$S sequencing in both directions was accomplished using a Cyclist kit (Stratagene) with tumor genomic DNA as template. Each exon sequence was obtained independently with the primer sets (sense/antisense) 5′-TGGCTGGTCACCAGAGGGTGGG-3′/5′-TGCAAACTTCGTCCTCCAGAGTCGCC-3′ for exon 1 and 5′-TTGCGAGAACTTTATCCATA-3′/5′-ATGAAAACTACGAAAGCGGG-3′ for exon 3. PCR reactions were performed under standard conditions, except for the presence of 10% dimethyl sulfoxide (American Type Culture Collection, Bethesda, MD) in exon 1 amplifications. Annealing temperatures used were 65°C for exon 1 and 60°C for exon 3. For exon 2, genomic PCR using primers 5′-TCTGACCATTCTGTTCTCTC-3′ (sense) and 5′-CTCAGCTTTGGAAGCTCTCA-3′ (antisense) was performed at an annealing temperature of 60°C. PCR products from each tumor sample were subcloned into TA vectors (Invitrogen, San Diego, CA) and at least 10 clones/tumor sample were pooled. Sequencing of each pool was accomplished using a

Received 12/30/94; accepted 3/1/95.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work is supported in part by the CNPq/Brazilian National Research Council (W. A.).

[2] Fellow of the Robert Steel Foundation for Pediatric Cancer Research.

[3] To whom requests for reprints should be addressed, at Ludwig Institute for Cancer Research, 9500 Gilman Drive, La Jolla, CA 92093-0660.

[4] The abbreviation used is: CDK4, cyclin-dependent kinase 4.



Downloaded from cancerres.aacrjournals.org on October 25, 2021. © 1995 American Association for Cancer Research.

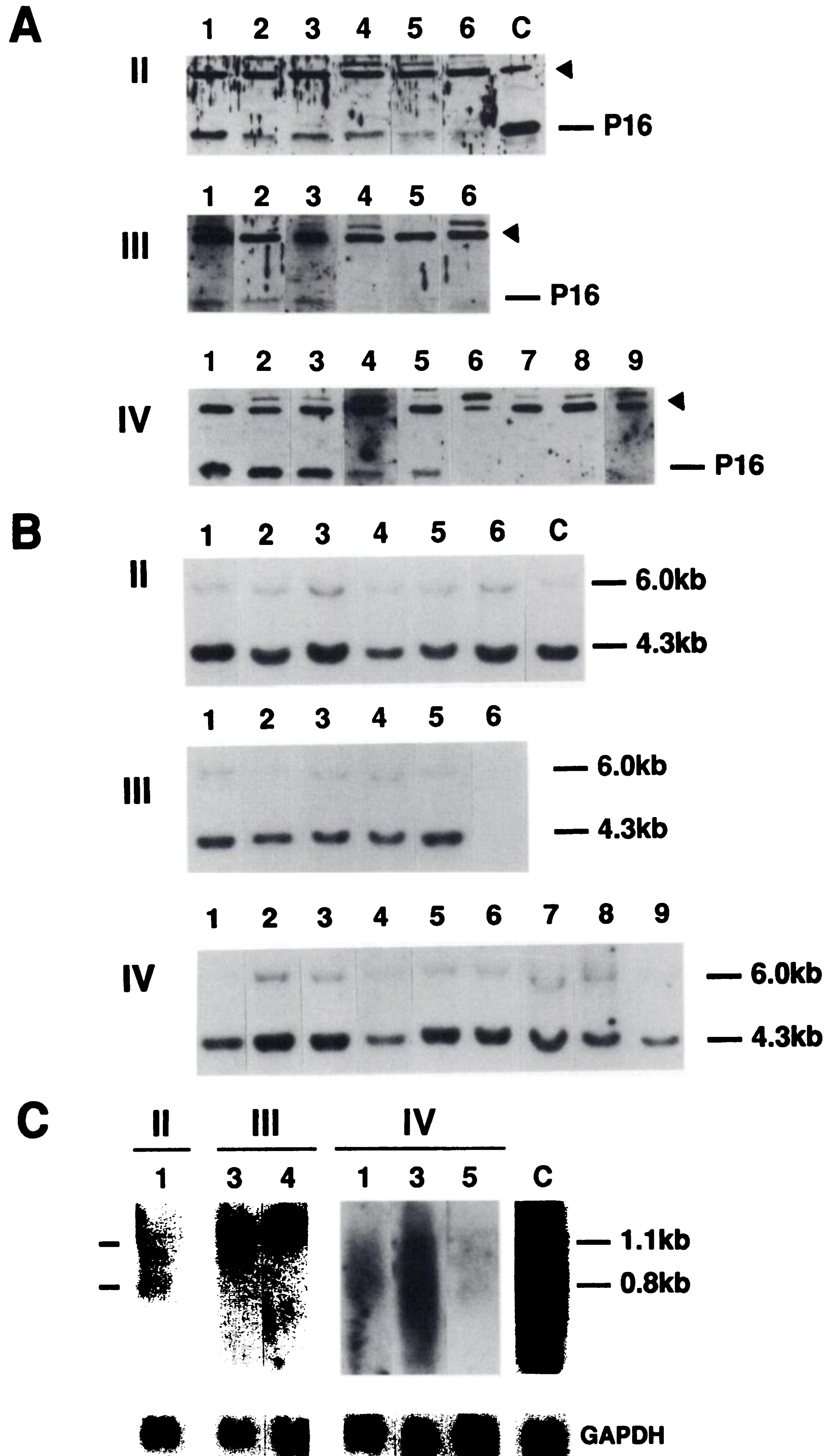


Fig. 1. Analysis of *p16/CDKN2* in human gliomas. *II*, astrocytoma (WHO grade II); *III*, anaplastic astrocytoma (WHO grade III); *IV*, glioblastoma multiforme (WHO grade IV). The case numbers are marked on the top of each panel. *Lane C*, positive control sample (a P16$^{INK4}$ expressing human glioma cell line, LN-Z308). *A*, Western blot analysis. *P16*, P16$^{INK4}$ protein; *Arrowheads*, nonspecific protein cross-reaction with the anti-P16$^{INK4}$ polyclonal antibody serves as an internal loading control. *B*, Southern blot analysis. *Right*, size of the DNA fragments hybridizing to a *p16/CDKN2* probe. The 4.3-kilobase (*kb*) band represents the *p16/CDKN2* gene. The 6.0-kilobase band may represent cross-hybridization with *p15$^{INK4B}$*. *C*, Northern blot analysis. The two expected *p16/CDKN2* transcripts of 0.8 and 1.1 kilobases which hybridize to a *p16/CDKN2*-specific probe are indicated on the *right*. The quantity and integrity of RNA are demonstrated by rehybridizing the blot with a human glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) probe.



Downloaded from cancerres.aacrjournals.org on October 25, 2021. © 1995 American Association for Cancer Research.

Sequenase kit (USB, Cleveland, OH), with the same primer set described above and additional internal primers, 5'-CCCCGCCACTCTCACCCG-3' (sense) and 5'-CAGCACCACCAGCGTGT-3' (antisense). For cases with questionable analyses of exon 2 using this approach, direct sequencing using the Cyclist kit described above was performed for confirmation.

**Western Blot Analysis.** Frozen tumor samples were processed in a tissue grinder and lysed in a solution containing 100 mM Tris-HCl (pH 6.8), 2% SDS, 3.2 M urea, 4% 2-mercaptoethanol, 5 mg/ml leupeptin, 5 mg/ml pepstatin A, 5 mg/ml antipain, 1 mM phenylmethylsulfonyl fluoride, and 0.25% bromophenol blue. Tumor lysates were boiled, size-fractionated through SDS-12.5% polyacrylamide gels, and transferred onto nitrocellulose membranes. Membranes were probed with anti-p16/CDKN2 rabbit polyclonal antibody (PharMingen, San Diego, CA) or anti-CDK4 mouse monoclonal antibody (Transduction Laboratories, Lexington, KY) and enhanced chemiluminescence detection was performed according to the manufacturer's protocol (Amersham Corp., Arlington Heights, IL).

## RESULTS

We first examined P16$^{INK4}$ protein expression in a series of low and high grade glioma samples by Western blot analysis. While P16$^{INK4}$ was clearly detectable in each of the low grade tumors, it was apparent in only a little over one-half of the high grade samples (6 of 6 grade II, 3 of 6 grade III, and 5 of 9 grade IV tumors) (Fig. 1*A*; Table 1). Since the *p16/CDKN2* gene maps to chromosome 9p21, a region with frequent loss of heterozygosity in high grade gliomas, we reasoned that the lack of detectable P16$^{INK4}$ protein might be due to mutation or deletion of the gene. Southern blot analysis revealed that homozygous deletion of the *p16/CDKN2* gene was uncommon in this series of gliomas (Fig. 1*B*); among the high grade tumors, only one (grade III, case 6) had clearly lost both alleles. While this low frequency is concordant with one study (21), it is lower than others (17–19). It is unlikely that this was due to contaminating normal tissue inasmuch as the histopathology indicated greater than 95% tumor cells for each specimen. Moreover, wherever possible (in informative females) X-chromosome clonality assays (27, 28) were conducted and were consistent with the cytology. The 4.3-kilobase band on the blot which represents the *p16/CDKN2* gene was present in all samples except this case. A larger band of 6.0 kilobases possibly representing cross-hybridization to the recently cloned *p15$^{INK4B}$* gene (29) was also found in those tumors containing the *p16/CDKN2* gene. To further investigate whether intragenic alterations (such as point mutations or small deletions/insertions) that would be undetectable by Southern blot analysis might result in gene inactivation, we have sequenced all three exons of the *p16/CDKN2* gene from 5 of the tumors in which the protein could not be detected. The results showed not only that their coding sequences were identical to that of the wild type but also that the splice sequences were preserved (Table 1). Thus lack of P16$^{INK4}$ protein in these tumors is unlikely to be due to mutations of the gene. To confirm that those tumors expressing P16$^{INK4}$ contain a wild-type gene, we also sequenced the exons of the *p16/CDKN2* gene from 5 such samples; in no case were mutations detected (Table 1).

In order to determine if the absence of P16$^{INK4}$ protein was due to a lack of transcription of the gene or instability of the message, we examined *p16/CDKN2* mRNA by Northern blot analysis. A *p16/CDKN2*-specific probe (see "Materials and Methods") which does not cross-hybridize with p15$^{INK4B}$ mRNA revealed the two expected transcripts, 0.8 and 1.1 kilobases, in those samples expressing P16$^{INK4}$ proteins, while no message could be detected in the tumors that lacked P16$^{INK4}$ product (Fig. 1*C*). Moreover, the level of p16 message was directly correlated to the amount of p16$^{INK4}$ protein suggesting that *p16/CDKN2* expression in gliomas may be regulated at the transcriptional level or by a mechanism governing message stability.

In initial analyses of the P16$^{INK4}$ protein described above, we noted that 3 of the 5 high grade tumors were expressing the protein at unusually high levels (Fig. 1*A*, *Lanes IV-1* to *IV-3*). Since it appears that P16$^{INK4}$ exerts its effect on cell proliferation by binding to and inhibiting the function of CDK4 (3) and since amplification of the *CDK4* gene has been observed in gliomas (30), we reasoned that overexpression of CDK4 may override the P16$^{INK4}$ inhibitory activity in these cases. Western blot analysis of CDK4 showed that in 2 of 3 cases overexpressing p16$^{INK4}$, CDK4 levels were elevated (Fig. 2*A*, *Lanes IV-2* and *IV-3*). Southern blot analysis revealed that the *CDK4*

Table 1 *p16/CDKN2 and CDK4 in glioma*

| Grade | Case | p16/CDKN2 Gene: Southern blot[a] | p16/CDKN2 Gene: Sequence[b] | p16/CDKN2 mRNA (Northern blot)[c] | p16/CDKN2 Protein (Western blot)[d] | CDK4 Gene (Southern blot)[a] | CDK4 mRNA (Northern blot)[c] | CDK4 Protein (Western blot)[d] |
|---|---|---|---|---|---|---|---|---|
| II | 1 | + | ND | + | + | ND | + | ND |
| | 2 | + | ND | ND | + | + | ND | ND |
| | 3 | + | ND | ND | + | + | ND | ND |
| | 4 | + | ND | ND | + | + | ND | ND |
| | 5 | + | ND | ND | ± | + | ND | ± |
| | 6 | + | ND | ND | ± | + | ND | + |
| III | 1 | + | ND | ND | + | + | ND | ± |
| | 2 | + | WT | ND | + | ND | ND | ND |
| | 3 | + | ND | – | ± | + | + | ± |
| | 4 | + | WT | – | – | + | + | + |
| | 5 | + | WT | ND | – | ND | ND | – |
| | 6 | – | NA | ND | – | ND | ND | + |
| IV | 1 | + | WT | ++ | ++ | + | + | + |
| | 2 | + | WT | ND | ++ | ++[e] | ND | ++ |
| | 3 | + | WT | ++ | ++ | ++[e] | ++ | ++ |
| | 4 | + | ND | + | + | + | ND | ± |
| | 5 | + | WT | + | + | + | + | + |
| | 6 | + | WT | – | – | + | + | – |
| | 7 | + | WT | ND | – | + | ND | + |
| | 8 | + | ND | ND | – | + | ND | + |
| | 9 | + | WT | ND | – | + | ND | + |

[a] +, present; –, absent.
[b] WT, wild-type; ND, not done; NA, not applicable.
[c] –, not detected; +, detectable; ++, elevated expression relative to that of glyceraldehyde-3-phosphate dehydrogenase.
[d] –, not detected; ±, detectable at a minimal level; +, detectable; ++, elevated protein amount.
[e] 7.5× amplification for case IV-2 and 15× amplification for case IV-3.



Downloaded from cancerres.aacrjournals.org on October 25, 2021. © 1995 American Association for Cancer Research.

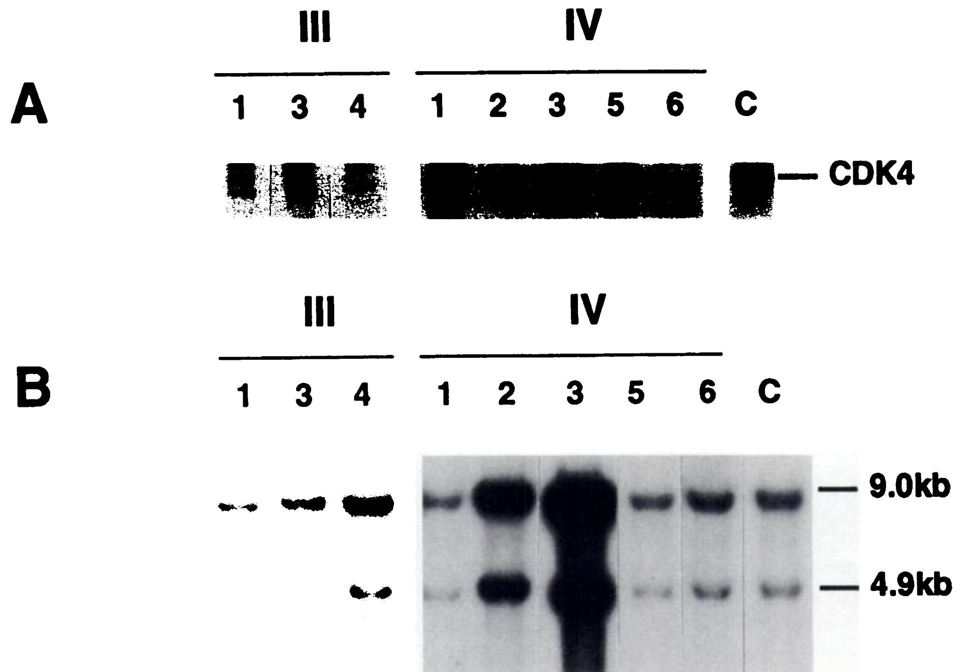


Fig. 2. Analysis of CDK4 in human gliomas. *III*, anaplastic astrocytoma (WHO grade III); *IV*, glioblastoma multiforme (WHO grade IV). The case numbers are marked on the *top* of each *panel*. *A*, Western blot analysis. *Right*, position of authentic CDK4 protein. *Lane* C, positive control sample (a CDK4 expressing the human cervical carcinoma cell line, HeLa) (35). *B*, Southern blot analysis. *Right*, size of the DNA fragments hybridized to a human CDK4 probe. *Lane* C, positive control sample (peripheral lymphocytes from a healthy volunteer).

gene in these samples was amplified (Fig. 2*B*, *Lanes IV-2* and *IV-3*) and Northern blot analysis showed a corresponding increase in their mRNA levels (data not shown). The third tumor contained relatively normal amounts of CDK4 protein (Fig. 2*A*, *Lane IV-1*) and a normal copy number of the gene (Fig. 2*B*, *Lane IV-1*).

## DISCUSSION

Homozygous deletion of the *p16/CDKN2* gene was uncommon in this series of gliomas; of 6 grade II, 6 grade III, and 9 grade IV tumors examined, only one grade III sample lost both alleles of the *p16/CDKN2* gene. This frequency is consistent with one study (21) but lower than some others (17–19). Subtle alterations, such as small deletions or insertions and point mutations, also are unlikely to be the major mechanisms for inactivating this gene, since the sequence of all three coding exons of the *p16/CDKN2* from ten high grade (grade III and IV) tumors were wild type. However, $P16^{INK4}$ protein was undetectable in about 50% of high grade tumors, while all grade II samples expressed obvious amounts of this product. This observation suggests that *p16/CDKN2* inactivation occurs in glioma progression at the transition from grade II to grade III and that *p16/CDKN2* may play an important role in glioma progression. In support of this theory, we have recently transferred a full-length *p16/CDKN2* cDNA into p16-null glioblastoma cell lines and demonstrated that expression of the exogenous *p16/CDKN2* indeed could suppress cell growth (31).

Absence of *p16/CDKN2* message in the samples lacking $P16^{INK4}$ protein suggests that *p16/CDKN2* is likely regulated at the transcriptional level or by an mRNA stability mechanism. We cannot rule out the possibility that mutation may occur in the promoter region of the *p16/CDKN2* gene with the consequence of gene inactivation. Alternate mechanisms such as aberrant methylation of the gene may also suppress *p16/CDKN2* transcription. Methylation of CpG islands around gene promoters has been documented in association with loss of gene expression in normal biological processes and pathological events (32). While mutational inactivation and allelic loss of the von Hippel-Lindau (*VHL*) gene seems to underlie the majority of spontaneous clear-cell renal carcinomas, it has also been shown that hypermethylation of a normally unmethylated CpG island in the 5′ region of the *VHL* gene is an alternative mechanism for inactivation in a significant portion of these cancers (33). Since the 5′ and coding sequences of *p16* also have CpG island characteristics, it may be that inactivation of *p16/CDKN2* expression in the high grade gliomas is commonly mediated through its aberrant methylation.

If inactivation of a cell cycle inhibitor is one of the mechanisms conferring a cell growth advantage, overexpression of cell cycle activators might be expected to exert the same effect. Consistent with this hypothesis, we have found that *CDK4* was amplified and overexpressed in two grade IV gliomas which expressed high levels of $P16^{INK4}$, but not in the low grade samples or other high grade tumors which did not express $P16^{INK4}$. *CDK4* gene amplification has been reported in about 14% of grade IV gliomas, but an analysis of CDK4 protein was not performed (30, 34). Nevertheless, these results further support the idea that perturbation of the balance of CDK4 and its cognate inhibitor may play an important role in glioma development. It is not clear whether amplification and overexpression of CDK4 takes place first thereby up-regulating the cognate inhibitor by a homeostatic mechanism, or alternatively overexpression of $P16^{INK4}$ leads to selection of a subpopulation of cells with CDK4 amplification and overexpression. Overexpression of CDK4 is also likely not the only way to overcome elevated levels of $P16^{INK4}$ in glioma as suggested by the one sample in this study containing a high amount of $P16^{INK4}$ but no CDK4 elevation. It will be interesting to determine whether other factors in the $P16^{INK4}$/CDK4 pathway, such as cyclin D or the retinoblastoma protein Rb, are deregulated or mutated, because these may have the same effect as increased levels of CDK4 (1, 2).

## ACKNOWLEDGMENTS

The authors thank Drs. Steve Hanks and David Beach for providing the plasmids used in this study and Drs. Joe Costello, Amy Larson, and Garret Hampton for helpful discussions.

## REFERENCES

1. Hunter, T., and Pines, J. Cyclins and cancer II: cyclin D and CDK inhibitors come of age. Cell, *79:* 573–582, 1994.
2. Sherr, C. $G_1$ phase progression: Cycling on cue. Cell, *79:* 551–555, 1994.



Downloaded from cancerres.aacrjournals.org on October 25, 2021. © 1995 American Association for Cancer Research.

3. Serrano, M., Hannon, G. J., and Beach, D. A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. Nature (Lond.), *366:* 704–707, 1993.
4. Kamb, A., Gruis, N. A., Weaver-Feldhaus, J., Liu, Q., Harshman, K., Tavtigian, S. V., Stockert, 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.
5. 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.
6. Cairns, P., Mao, L., Merlo, A., Lee, D. J., Schwab, D., Eby, Y., Tokino, K., van der Riet, P., Blaugrund, J. E., and Sidransky, D. Rates of *p16* (*MTS1*) mutations in primary tumors with 9p loss. Science (Washington DC), *265:* 415–417, 1994.
7. Spruck, C. H., III, Gonzalez-Zulueta, M., Shibata, A., Simoneau, A. R., Lin, M-F., Gonzales, F., Tsai, Y. C., and Jones, P. A. *p16* gene in uncultured tumours. Nature (Lond.), *370:* 183–184, 1994.
8. Zhang, S-Y., Klein-Szanto, A. J. P., Sauter, E. R., Shafarenko, M., Mitsunaga, S., Nobori, T., Carson, D. A., Ridge, J. A., and Goodrow, T. L. Higher frequency of alterations in the *p16/CDKN2* gene in squamous cell carcinoma cell lines than in primary tumors of the head and neck. Cancer Res., *54:* 5050–5053, 1994.
9. Xu, L., Sgroi, D., Sterner, C. J., Beauchamp, R. L., Pinney, D. M., Keel, S., Ueki, K., Rutter, J. L., Buckler, A. J., Louis, D. N., Gusella, J. F., and Ramesh, V. Mutational analysis of *CDKN2* (*MTS1*/p16$^{ink4}$) in human breast carcinomas. Cancer Res., *54:* 5262–5264, 1994.
10. Ohta, M., Nagai, H., Shimizu, M., Rasio, D., Berd, D., Mastrangelo, M., Singh, A. D., Shields, J. A., Shields, C. L., Croce, C. M., and Huebner, K. Rarity of somatic and germline mutations of the cyclin-dependent kinase 4 inhibitor gene, *CDK41*, in melanoma. Cancer Res., *54:* 5269–5272, 1994.
11. Mori, T., Miura, K., Aoki, T., Nishihira, T., Mori, S., and Nakamura, Y. Frequent somatic mutation of the *MTS1/CDK41* (multiple tumor suppressor/cyclin-dependent kinase 4 inhibitor) gene in esophageal squamous cell carcinoma. Cancer Res., *54:* 3396–3397, 1994.
12. Hussussian, C. J., Struewing, J. P., Goldstein, A. M., Higgins, P. A. T., Ally, D. S., Sheahan, M. D., Clark, W. H., Jr., Tucker, M. A., and Dracopoli, N. C. Germline *p16* mutations in familial melanoma. Nat. Genet., *8:* 15–21, 1994.
13. 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 of the *p16* (*MTS1*) gene in pancreatic adenocarcinoma. Nat. Genet., *8:* 27–32, 1994.
14. 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 p16INK4 in human cancer. Proc. Natl. Acad. Sci. USA, *91:* 11045–11049, 1994.
15. Olopade, O. I., Jenkins, R. B., Ransom, D. T., Malik, K., Pomykala, H., Nobori, T., Cowan, J. M., Rowley, J. D., and Diaz, M. O. Molecular analysis of deletions of the short arm of chromosome 9 in human gliomas. Cancer Res., *52:* 2523–2529, 1992.
16. James, C. D., He, J., Carlbom, E., Nordenskjold, M., Cavenee, W. K., and Collins, V. P. Chromosome 9 deletion mapping reveals interferon $\alpha$ and interferon $\beta$-1 gene deletions in human glial tumors. Cancer Res., *51:* 1684–1688, 1991.
17. Giani, C., and Finocchiaro, G. Mutation rate of the *CDKN2* gene in malignant gliomas. Cancer Res., *54:* 6338–6339, 1994.
18. Schmidt, E. E., Ichimura, K., Geifenberger, G., and Collins, V. P. *CDKN2(p16/MTS1)* gene deletion or *CDK4* amplification occurs in the majority of glioblastomas. Cancer Res., *54:* 6321–6324, 1994.
19. Walker, D. G., Duan, W., Popovic, E. A., Kaye, A. H., Tomlinson, F. H., and Lavin, M. Homozygous deletions of the multiple tumor suppressor gene 1 in the progression of human astrocytomas. Cancer Res., *55:* 20–23, 1995.
20. 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.
21. Ueki, K., Rubio, M-P., Ramesh, V., Correa, K. M., Rutter, J. L., von Deimling, A., Buckler, A. J., Gusella, J. F., and Louis, D. N. *MTS1/CDKN2* gene mutations are rare in primary human astrocytomas with allelic loss of chromosome 9p. Hum. Mol. Genet., *3:* 1841–1845, 1994.
22. Kleihues, P., Burger, P. C., and Scheithauer, B. W. Histological Typing of Tumours of the Central Nervous System. Berlin Springer-Verlag, 1993.
23. Hanks, S. K. Homology probing: identification of cDNA clones encoding members of the protein-serine kinase family. Proc. Natl. Acad. Sci. USA, *84:* 388–392, 1987.
24. Feinberg, A. P., and Vogelstein, B. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity (addendum). Anal. Biochem., *137:* 266–267, 1984.
25. Chomczynski, P., and Sacchi, N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem., *162:* 156–159, 1987.
26. Fort, P., Marty, L., Piechaczyk, M., el Sabrouty, S., Dani, C., Jeanteur, P., and Blanchard, J. M. Various rat adult tissues express only one major mRNA species from the glyceraldehyde-3-phosphate-dehydrogenase multigenic family. Nucleic Acids Res., *13:* 1431–1442, 1985.
27. Tilley, W. D., Marcelli, M., Wilson, J. D., and McPhaul, M. J. Characterization and expression of a cDNA encoding the human androgen receptor. Proc. Natl. Acad. Sci. USA, *86:* 327–331, 1989.
28. Allen, R. C., Zoghbi, H. Y., Moseley, A. B., Rosenblatt, H. M., and Belmont, J. W. Methylation of *Hpa*II and *Hha*I sites near the polymorphic CAG repeat in the human androgen-receptor gene correlates with X chromosome inactivation. Am. J. Hum. Genet., *51:* 1229–1239, 1992.
29. Hannon, G. J., and Beach, D. p15$^{INK4B}$ is a potential effector of TGF-$\beta$-induced cell cycle arrest. Nature (Lond.), *371:* 257–261, 1994.
30. Reifenberger, G., Reifenberger, J., Ichimura, K., Meltzer, P. S., and Collins, V. P. Amplification of multiple genes from chromosomal region 12q13–14 in human malignant gliomas: preliminary mapping of the amplicons shows preferential involvement of CDK4, SAS, and MDM2. Cancer Res., *54:* 4299–4303, 1994.
31. Arap, W., Nishikawa, R., Furnari, F. B., Cavenee, W. K., and Huang, H-J. S. Replacement of the *p16/CDKN2* gene suppresses human glioma cell growth. Cancer Res., *55:* 1351–1354, 1995.
32. Jones, P. A., and Buckley, J. D. The role of DNA methylation in cancer. Adv. Cancer Res., *54:* 1–23, 1990.
33. Herman, J. G., Latif, F., Weng, Y., Lerman, M. I., Zbar, B., S., L., Samid, D., Duan, D-S. R., Gnarra, J. R., M., L. W., and Baylin, S. B. Silencing of the *VHL* tumor-suppressor gene by DNA methylation in renal carcinoma. Proc. Natl. Acad. Sci. USA, *91:* 9700–9704, 1994.
34. He, J., Allen, J. R., Collins, V. P., Allalunis-Turner, 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.
35. Xiong, Y., Zhang, H., and Beach, D. Subunit rearrangement of the cyclin-dependent kinases is associated with cellular transformation. Genes Dev., *7:* 1572–1583, 1993.



Downloaded from cancerres.aacrjournals.org on October 25, 2021. © 1995 American Association for Cancer Research.

Cancer Research
The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

AACR American Association for Cancer Research

# Loss of P16$^{INK4}$ Expression Is Frequent in High Grade Gliomas

Ryo Nishikawa, Frank B. Furnari, Hong Lin, et al.

*Cancer Res* 1995;55:1941-1945.

| | |
|---|---|
| **Updated version** | Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/55/9/1941 |

| | |
|---|---|
| **E-mail alerts** | Sign up to receive free email-alerts related to this article or journal. |
| **Reprints and Subscriptions** | To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org. |
| **Permissions** | To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/55/9/1941. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site. |

Downloaded from cancerres.aacrjournals.org on October 25, 2021. © 1995 American Association for Cancer Research.