[CANCER RESEARCH 60, 2113–2115, April 15, 2000]

*Advances in Brief*

# Alterations of the *INK4a/ARF* Locus in Human Intracranial Germ Cell Tumors[1]

**Masayuki Iwato, Osamu Tachibana, Yasuo Tohma, Yasuaki Arakawa, Hisashi Nitta, Mitsuhiro Hasegawa, Junkoh Yamashita, and Yutaka Hayashi[2]**

*Department of Neurosurgery, Kanazawa University School of Medicine, 920-8641 Kanazawa [M. I., O. T., Y. T., Y. A., M. H., J. Y., Y. H.], and Department of Neurosurgery, Fukui Prefectural Hospital, 910-8526 Fukui [H. N.], Japan*

## Abstract

**Little is known about the molecular mechanisms responsible for the development of intracranial germ cell tumors (ICGTs). Recently, we demonstrated that the balance of the p53-mdm2 interactions is disrupted in ICGTs. The *p14$^{ARF}$* product, a tumor suppresser gene located on the *INK4a/ARF* locus, acts as one of the major factors affecting p53-mdm2 interactions via its binding to mdm2 and the stimulation of mdm2 degradation. To evaluate whether genetic alterations of the INK4a/ARF locus occur in the genesis of ICGTs, we analyzed the *INK4a/ARF* genes in 21 ICGTs—10 pure germinomas and 11 nongerminomatous germ cell tumors. Fifteen (71%) of the 21 ICGTs displayed genetic alterations, including 14 homozygous deletions and 1 frameshift mutation. Furthermore, the frequency of the alterations was higher in pure germinomas [9 (90%) of the 10] than in nongerminomatous germ cell tumors [6 (55%) of the 11; $P = 0.09$]. These data suggested that *INK4a/ARF* gene abnormalities could play an important role in the genesis of ICGTs, especially in pure germinoma.**

## Introduction

ICGTs[3] are rare neoplasms. The reported frequency of the tumor is 0.3–3.4% of primary intracranial tumors in Western countries and 2.1–12.7% in Japan (1–3), and their etiologies remain largely unknown. Studies on the histological nature of ICGTs revealed that ICGTs comprise five interrelated neoplasms: germinoma, teratoma, embryonal carcinoma, yolk sac tumor, and choriocarcinoma (2, 4). These five types can be divided into two groups, *i.e.,* pure germinoma and nongerminomatous germ cell tumors (teratoma, embryonal carcinoma, yolk sac tumor, and choriocarcinoma). Prognosis varies between these groups and is highly dependent on the response to chemotherapy or radiotherapy (2, 5, 6). In general, pure germinoma has a better prognosis than nongerminomatous germ cell tumors.

We recently reported that a disruption in the balance of the p53-mdm2 interactions could play an important role in the tumorigenesis of these neoplasms. *MDM2* gene amplification is found at a moderate frequency in ICGTs, whereas *TP53* gene mutations are very rare. Furthermore, mdm2 protein is overexpressed at a very high frequency in ICGTs (7).

Increasing knowledge on the molecular genetic mechanisms underlying many types of tumors has demonstrated a high frequency of genetic alterations in a single locus, *INK4a/ARF* (8–10). The *INK4a/ARF* locus has two promoters and encodes two completely different proteins, p16$^{INK4a}$ and p14$^{ARF}$ (the mouse homologue is called p19$^{ARF}$; Refs. 10–12). By acting as an inhibitor of cyclin-dependent kinases, the p16$^{INK4a}$ protein decreases the phosphorylation of retinoblastoma protein and results in cell cycle arrest at $G_1$ (13–15). The other encoded protein, p14$^{ARF}$, interacts with mdm2 and stimulates the degradation of mdm2 protein (16–19). The tumor-suppression function of p14$^{ARF}$ is dependent upon the presence of wild-type p53 (20), and ARF-deficient mice tend to develop carcinoma and tumors of the nervous systems (21).

Molecular genetic findings of the ICGTs, namely, the disruption of the balance of p53-mdm2 interactions, led us to speculate that alteration in the *INK4a/ARF* locus could be a major factor affecting p53-mdm2 interaction, and the tumorigenesis of ICGTs.

To evaluate whether *INK4a/ARF* gene alterations play a role during ICGTs development, we examined a series of 21 ICGTs to identify mutations of both the *p16$^{INK4a}$* and *p14$^{ARF}$* genes.

## Materials and Methods

**Tissue Samples.** Twenty-one intracranial germ cell tumor specimens (10 pure germinomas and 11 nongerminomatous germ cell tumors, including 4 mature teratomas, 3 immature teratomas, 2 choriocarcinomas, and 2 yolk sac tumors) were obtained at surgery. All of the cases were diagnosed and followed up at the Department of Neurosurgery, University Hospital, Kanazawa, Japan, between 1988 and 1998. The specimens were sent to the pathology laboratory for routine formalin fixation and paraffin embedding. All of the tumor specimens were examined microscopically, and only tumor tissue was dissected under microscope before phenolic DNA extraction.

**Homozygous Deletion Analysis of the *INK4a/ARF* Genes.** Homozygous deletions of the *INK4a/ARF* genes were analyzed by a differential PCR method (22). Because the exon2 is a common open reading frame of both of these genes and the deletion breakpoint is located between exon3 and exon 1$\beta$ (23), we coamplified a 204-bp fragment of INK4a/ARF from exon2 (primer: ex2–1) with a 134-bp fragment of the *APRT* gene. Primer sequences are listed in Table 1. One of each pair was labeled with indodicarbocyanine (Cy5) fluorescent dye (Pharmacia Biotech, Uppsala) at the 5′ end. Differential PCR was performed in a final volume of 10 $\mu$l containing 2 ng of DNA, 50 mM KCL, 1.5 mM of $MgCL_2$, 10 mM TRIS-HCL (pH 8.3), 200 $\mu$M each dNTP, 0.1% gelatin, 5 pmol of each primer set, 10% DMSO, and 0.25 units of Taq polymerase. Initial denaturation at 94°C for 3 min was followed by 28 cycles of denaturation at 94°C for 40 s, annealing at 56°C for 55 s, and extension at 72°C for 55 s on a thermal cycler (thermal cycler 480, Perkin-Elmer, CA). A final extension step of 10 min at 72°C was used.

**Mutation Analysis of the *INK4a/ARF* Genes.** For all of the cases without *INK4a/ARF* gene homozygous deletion, all of the exons of each gene were analyzed by fluorescence-based SSCP and direct sequencing (24). PCR was performed in a final volume of 10 $\mu$l containing 2ng DNA, 50 mM KCL, 1.5 mM $MgCL_2$, 10 mM TRIS-HCL (pH 8.3), 200 $\mu$M each dNTP, 0.1% gelatin, 5 pmol of each primer set, 10% DMSO, and 0.25 units Taq polymerase. Initial denaturation at 94°C for 3 min was followed by 40 cycles of denaturation at 94°C for 40 s, annealing at 52°C to 60°C for 55 s, and extension at 72°C for 55 s on a thermal cycler (thermal cycler 480, Perkin-Elmer). A final extension step of 10 min at 72°C was used. PCR products were electrophoretically separated by 8 and 10% acrylamide gels (the methylene-bis-acrylamide:acrylamide ratio was 1:99) at 40 W with water cooling to a temperature of 16°C.

Received 9/10/99; accepted 2/29/00.

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] Supported in part by a Grant-in-Aid from the Ministry of Health and Welfare on Scientific Research for pediatric brain tumors, and Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan for Scientific Research B (No. 09470292).

[2] To whom requests for reprints should be addressed, at Department of Neurosurgery, Kanazawa University School of Medicine, 13-1 Takaramachi, 920-8641 Kanazawa, Japan. Phone: 81-76-265-2384; Fax: 81-76-234-4262; E-mail: yuh@ns.m.kanazawa-u.ac.jp.

[3] The abbreviations used are: IGCT, intracranial germ cell tumor; APRT, adenine phosphoribosyltransferase; SSCP, single-strand conformational polymorphism; TGT, testicular germ cell tumor.



Downloaded from http://aacrjournals.org/cancerres/article-pdf/60/8/2113/2483904/ch080002113.pdf by guest on 07 October 2024

Once separated, the products were analyzed by an automated DNA sequencer (Pharmacia Biotech Model ALFred) running with a fragment analysis program (Pharmacia biotech AlleleLinks version 1.00).

The materials with variant SSCPs were reamplified and sequenced bidirectionally. Sequence analysis was carried out with a semiautomated sequencer (Model 373, Applied Biosystems, CA). The primers for SSCP analysis and direct sequencing are listed in Table 1.

**Statistical Analysis.** Two-tailed Fisher's exact test was carried out to compare the frequency of INK4a/ARF alterations in pure germinomas and nongerminomatous germ cell tumors.

## Results

To determine the variation in the ratio of the PCR products of the *INK4a/ARF* genes to those of the *APRT* gene in normal DNA, we studied 60 genomic DNA samples from peripheral lymphocytes. The mean ratio and SD of the coamplified *INK4a-ARF*:*APRT* were 0.99 and 0.18, respectively. Cases exhibiting *INK4a-ARF*:*APRT* values lower than 4 SD below this mean ratio, *i.e.,* $<0.27$, were considered to have *INK4a/ARF* gene deletions (Fig. 1). The deletions were considered to be homozygous because it was known that cases carrying hemizygous deletions should have INK4a-ARF values higher than half of the mean ratio, *i.e.,* $>0.45$, or 3 SD below the mean value.

Homozygous deletions of the *INK4a/ARF* genes were detected in 9 (90%) of 10 patients with pure germinomas, and 5 (45%) of 11 patients with nongerminomatous germ cell tumors. Among the patients who carried no homozygous deletions, only one showed an altered migration pattern of SSCP. DNA sequencing confirmed an altered nucleotide sequence resulting in a frameshift mutation in this patient (Table 2).

Although there was no statistical correlation between the frequency of *INK4a/ARF* gene alterations and tumor types (pure germinomas and nongerminomatous germ cell tumors), pure germinomas tended to carry the *INK4a/ARF* gene alterations more frequently than the nongerminomatous germ cell tumors ($P = 0.09$).

## Discussion

Little is known about the molecular mechanisms occurring in ICGTs. Although *TP53* gene mutations are rare, some subsets of ICGTs carry the *MDM2* gene amplification (7). This suggests that the disruption of the balance of p53-mdm2 interaction might play an important role in the tumorigenesis of ICGTs.

In this series, 9 (90%) of 10 pure germinomas and 6 (55%) of 11 nongerminomatous germ cell tumors exhibited *INK4a/ARF* gene alterations. The alterations in the *ARF* gene after the p53 degradation via the stabilized mdm2 could abrogate the normal balance of each molecule in the ARF-mdm2-p53 pathway. This could break down the

Table 1 *Oligonucleotide primers used for the analyses of the INK4a/ARF and APRT genes*

| Gene | Primers | Orientation | Sequence |
|---|---|---|---|
| *INK4a/ARF* | ex1B-1f | Forward | TCCCAGTCTGCAGTTAAGGG |
| | ex1B-1r | Reverse | ACCACGAAAACCCTCACTCG |
| | ex1B-2f | Forward | TGCGCAGGTTCTTGGTGACC |
| | ex1B-2r | Reverse | GACTTTTCGAGGGCCTTTCC |
| | ex1A-f | Forward | GGGAGCAGCATGGAGCCG |
| | ex1A-r | Reverse | AGTCGCCCGCCATCCCCT |
| | ex2-1f | Forward | AGCTTCCTTTCCGTCATGC |
| | ex2-1r | Reverse | GCAGCACCACCAGCGTG |
| | ex2-2f | Forward | AGCCCAACTGCGCCGAC |
| | ex2-2r | Reverse | CCAGGTCCACGGGCAGA |
| | ex2-3f | Forward | TGGACGTGCGCGATGC |
| | ex2-3r | Reverse | GGAAGCTCTCAGGGTACAAATTC |
| | ex3-f | Forward | CCGGTAGGGACGGCAAGAGA |
| | ex3-r | Reverse | CTGTAGGACCCTCGGTGACTGATGA |
| *APRT* | APRT-f | Forward | CAGGGAACACATTCCTTTGC |
| | APRT-r | Reverse | TGGGAAAGCTGTTTACTGCG |

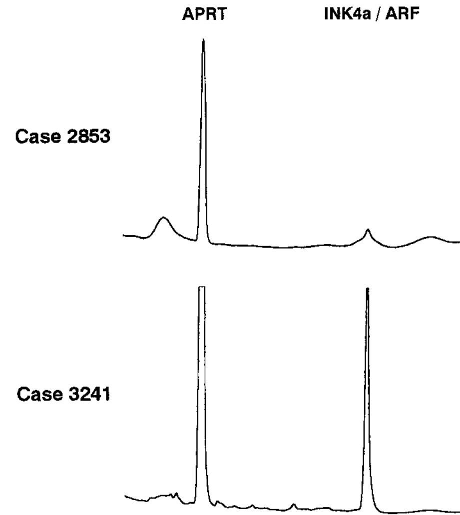


Fig. 1. Differential PCR-analysis for homozygous deletions of the *INK4a/ARF* genes. Representative cases with or without the *INK4a/ARF* gene homozygous deletions. *Top*, a pure germinoma with the *INK4a/ARF* gene homozygous deletion. *Bottom*, a pure germinoma with no evidence for deletion of the genes.

Table 2 *INK4a/ARF genes alterations in intracranial germ cell tumors*

| Case no. | Age/Sex | Site | Diagnosis | Alteration |
|---|---|---|---|---|
| 2665 | 16/M | P[a] | PG | HD |
| 2832 | 16/F | S | PG | HD |
| 2853 | 21/M | S | PG | HD |
| 2962 | 16/M | P | PG | HD |
| 2985 | 20/M | P | PG | HD |
| 3241 | 19/M | P | PG | WT |
| 3408 | 60/M | P | PG | HD |
| 3437 | 18/M | P | PG | HD |
| 3477 | 19/M | P | PG | HD |
| 3573 | 7/M | P | PG | HD |
| 3516 | 11/M | P | MT | WT |
| 3378 | 15/M | P | MT | HD |
| 2626 | 25/M | P | MT | WT |
| 2633 | 15/M | P | MT | WT |
| 2630 | 4/M | P | IT | HD |
| 3384 | 5/M | P | IT | HD |
| 3413 | 14/M | P | IT | WT |
| 2577 | 10/F | P | YS | HD |
| 3475 | 18/M | P | YS | INK4a: c226 insT / D76X<br>ARF: c293 insT / R98fs → 160X |
| 2591 | 12/M | H | CH | HD |
| 3234 | 7/M | P | CH | WT |

[a] P, pineal region; S, supra sellar; H, hypothalamus; PG, pure germinoma; MT, mature teratoma; IT, inmature teratoma; YS, york sac tumor; CH, choriocarcinoma; HD, homozygous deletion; WT, wild type.

$G_1$-S phase checkpoint and subsequently lead to cell proliferation. Thus, the high frequency of *ARF* gene alterations in ICGTs could confirm the mechanism of ICGTs tumorigenesis suggested above. Furthermore, another important pathogenesis of ICGTs in addition to the *ARF* alterations might be the *INK4a* alterations.

The higher frequency of *INK4a/ARF* alterations in pure germinomas (90%) compared with that in nongerminomatous germ cell tumors (55%) may help explain why pure germinomas are more sensitive to chemotherapy and/or radiotherapy than nongerminomatous germ cell tumors. In an earlier report (25), *INK4a* gene transfection and forced expression of $p16^{INK4a}$ in the $p16^{INK4a}$-deficient cell line were found to increase the radiosensitivity of the cells. This is opposed to our findings, which suggested that pure germinoma, the more radiosensitive neoplasm, carried *INK4a* gene alterations. On the other hand, the abrogation of p53 function was found to increase the response to radiation in some types of neoplasms (26). This was consistent with our findings, which showed that the *ARF* alterations and subsequent abrogations of p53 function were more frequent in



Downloaded from http://aacrjournals.org/cancerres/article-pdf/60/8/2113/2483904/ch08000002113.pdf by guest on 07 October 2024

pure germinomas. In ICGTs, disorder in ARF-p53 pathway may overcome the INK4a-Rb pathway abrogation at the point of radiosensitivity.

ICGTs and TGTs may share similar cellular origins. Both types of tumors have the same molecular genetic abnormalities in *TP53* gene and *MDM2* genes (7, 27, 28). However, deletions or mutations in *INK4a/ARF* genes are rare in TGTs (29, 30). In contrast, in our study, a high frequency of *INK4a/ARF* gene deletions was demonstrated in ICGTs. In *ARF*-deficient mice, tumors of the nervous system are likely to emerge (21). The difference in the status of the *ARF* gene between ICGTs and TGTs may affect the site of germ cell tumor development.

The silencing of the *INK4a* gene by methylation at the promoter site has been reported in a variety of tumors (31). Recently, it has also been shown that the *ARF* gene is silenced by methylation at the *ARF*-specific promoter site in some types of cell lines, although not in natural tumors (32, 33). In our series of 21 ICGTs, cases without deletions or mutations in *INK4a/ARF* may carry a promoter site methylation. In the future, in addition to the analysis for genetic alterations, additional examinations for gene modifications of this type will be required to evaluate the biological features of $p16^{INK4a}$ and $p14^{ARF}$ in, and their influences on, ICGTs.

## Acknowledgments

We thank A. Imamura for her technical assistance.

## References


1. Jellinger, K. Primary germ cell tumors. Acta Neuropathol., *25:* 291–306, 1973.
2. Hoffman, H., Otsubo, H., Hendrick, B., Humphreys, R., Drake, J., Becker, L., Greenberg, M., and Jenkin, D. Intracranial germ-cell tumors in children. J. Neurosurg., *74:* 545–551, 1991.
3. Araki, C., and Matsumoto, S. Statistical reevaluation of pinealoma and related tumors in Japan. J. Neurosurg., *30:* 146–149, 1969.
4. Bjornsson, J., Scheithauer, B. W., Okazaki, H., and Leech, R. W. Intracranial germ cell tumors: pathobiological and immunohistochemical aspects of 70 cases. J. Neuropathol. Exp. Neurol., *44:* 32–46, 1985.
5. Brodeur, G., Howarth, C., Pratt, C., and Caces, J., and Husto, H. O. Malignant germ cell tumors in 57 children and adolescents. Cancer (Phila.), *48:* 1890–1898, 1981.
6. Jennings, M. T., Gelman, R., and Hochberg, F. Intracranial germ-cell tumors: natural history and pathogenesis. J. Neurosurg., *63:* 155–167, 1985.
7. Iwato, M., Tachibana, O., Tohma, Y., Nitta, H., Hayashi, Y., and Yamashita, J. Molecular analysis for p53 and mdm2 in intracranial germ cell tumors. Acta Neuropathol., *99:* 21–25, 2000.
8. Kamb, A., Gruis, N., Weaver-Feldhaus, J., Liu, Q., Harshman, K., Tavtigian, S., Stockert, E., Day, R., III, Johnson, B., and Skolnick, M. A cell cycle regulator potentially involved in genesis of many tumor types. Science (Washington DC), *264:* 436–440, 1994.
9. Cairns, P., Polascik, T., Eby, Y., Tokino, K., Califano, J., Merlo, A., Mao, L., Herath, J., Jenkins, R., Westra, W., Rutter, J., Buckler, A., Gabrielson, E., Tockman, M., Cho, K., Hedrick, L., Bova, G., Isaacs, W., Koch, W., Schwab, D., and Sidransky, D. Frequency of homozygous deletion at *p16/CDKN2* in primary human tumours. Nat. Genet., *11:* 210–212, 1995.
10. Sherr, C. J. Tumor surveillance via the ARF-p53 pathway. Genes Dev., *12:* 2984–2991, 1998.
11. Mao, L., Merlo, A., Bedi, G., Shapiro, G., Edwards, C., Rollins, B., and Sidransky, D. A novel $p16^{INK4A}$ transcript. Cancer Res., *55:* 2995–2997, 1995.
12. Stone, S., Jiang, P., Dayananth, P., Tavtigian, S., Katcher, H., Parry, D., Peters, G., and Kamb, A. Complex structure and regulation of the *P16* (*MTS*) locus. Cancer Res., *55:* 2988–2994, 1995.
13. Medema, R., Herrera, R., Lam, F., and Weinberg, R. Growth suppression by $p16^{Ink4}$ requires functional retinoblastoma protein. Proc. Natl. Acad. Sci. USA, *92:* 6289–6293, 1995.
14. Otterson, G., Kratzke, R., Coxon, A., Kim, Y., and Kaye, F. Absence of $p16^{INK4}$ protein is restricted to the subset of lung cancer lines that retains wildtype RB. Oncogene, *9:* 3375–3378, 1994.
15. Sherr, C., and Roberts, J. Inhibitors of mammalian $G_1$ cyclin-dependent kinases. Genes Dev., *9:* 1149–1163, 1995.
16. Pomerantz, J., Schreiber-Agus, N., Liegéois, N., Silverman, A., Alland, L., Chin, L., Potes, J., Chen, K., Orlow, I., Lee, H., Cordon-Cardo, C., and DePinho, R. The INK4a tumor suppressor gene product, $p19^{ARF}$, interacts with MDM2 and neutralizes MDM2's inhibition of p53. Cell, *92:* 713–723, 1998.
17. Honda, R., and Yasuda, H. Association of p19(ARF) with Mdm2 inhibits ubiquitin ligase activity of Mdm2 for tumor suppressor p53. EMBO J., *18:* 22–27, 1999.
18. Kamijo, T., Weber, J., Zambetti, G., Zindy, F., Roussel, M., and Sherr, C. Functional and physical interactions of the ARF tumor suppressor with p53 and Mdm2. Proc. Natl. Acad. Sci. USA, *95:* 8292–8297, 1998.
19. Stott, F., Bates, S., James, M., McConnell, B., Starborg, M., Brookes, S., Palmero, I., Ryan, K., Hara, E., Vousden, K., and Peters, G. The alternative product from the human *CDKN2A* locus, $p14^{ARF}$, participates in a regulatory feedback loop with p53 and MDM2. EMBO J., *17:* 5001–5014, 1998.
20. Kamijo, T., Zindy, F., Roussel, M., Quelle, D., Downing, J., Ashmun, R., Grosveld, G., and Sherr, C. Tumor suppression at the mouse *INK4a* locus mediated by the alternative reading frame product $p19^{ARF}$. Cell, *91:* 649–659, 1997.
21. Kamijo, T., Bodner, S., van de Kamp, E., Randle, D. H., and Sherr, C. J. Tumor spectrum in ARF-deficient mice. Cancer Res., *59:* 2217–2222, 1999.
22. Hayashi, Y., Ueki, K., Waha, A., Wiestler, O. D., Louis, D. N., and von Deimling, A. Association of *EGFR* gene amplification and *CDKN2* (*p16/MTS1*) gene deletion in glioblastoma multiforme. Brain Pathol., *7:* 871–875, 1997.
23. Jen, J., Harper, J., Bigner, S., Bigner, D., Papadopoulos, N., Markowitz, S., Willson, J., Kinzler, K., and Vogelstein, B. Deletion of *p16* and *p15* genes in brain tumors. Cancer Res., *54:* 6353–6358, 1994.
24. Sugano, K., Nakashima, Y., Yamaguchi, K., Fukayama, N., Maekawa, M., Ohkura, H., Kakizoe, T., and Sekiya, T. Sensitive detection of loss of heterozygosity in the *TP53* gene in pancreatic adenocarcinoma by fluorescence-based single-strand conformation polymorphism analysis using blunt-end DNA fragments. Genes Chromosomes Cancer, *15:* 157–164, 1996.
25. Miyakoshi, J., Kitagawa, K., Yamagishi, N., Ohtsu, S., Day, R. S., III, and Takebe, H. Increased radiosensitivity of p16-deleted human glioma cells after transfection with wild-type *p16* gene. Jpn. J. Cancer Res., *88:* 34–38, 1997.
26. Tada, M., Matsumoto, R., Iggo, R. D., Shirato, H., Sawamura, Y., and Shinohe, Y. Selective sensitivity to radiation of cerebral glioblastomas harboring p53 mutations. Cancer Res., *58:* 1793–1797, 1998.
27. Riou, G., Barrois, M., Prost, S., Terrier, M. J., Theodore, C., and Levine, A. J. The *p53* and *mdm-2* genes in human testicular germ-cell tumors. Mol. Carcinog., *12:* 124–131, 1995.
28. Guillou, L., Estreicher, A., Chaubert, P., Hurlimann, J., Kurt, A-M., Metthez, G., Iggo, R., Gray, A. C., Jichlinski, P., Leisinger, H-J., and Benhattar, J. Germ cell tumors of the testis overexpress wild-type p53. Am. J. Pathol., *149:* 1221–1228, 1996.
29. Chaubert, P., Guillou, L., Kurt, A., Bertholet, M., Metthez, G., Leisinger, H., Bosman, F., and Shaw, P. Frequent *p16INK4(MTS1)* gene inactivation in testicular germ cell tumors. Am. J. Pathol., *151:* 859–865, 1997.
30. Hatta, Y., Hirama, T., Takeuchi, S., Lee, E., Pham, E., Miller, C., Strohmeyer, T., Wilczynski, S., Melmed, S., and Koeffler, H. Alterations of the *p16*(*MTS1*) gene in testicular, ovarian, and endometrial malignancies. J. Urol., *154:* 1954–1957, 1995.
31. Merlo, A., Herman, J., Mao, L., Lee, D., Gabrielson, E., Burger, P., Baylin, S., and Sidransky, D. 5′CpG island methylation is associated with transcriptional silencing of the tumour suppressor *p16/CDKN2/MTS1* in human cancers. Nat. Med., *1:* 686–692, 1995.
32. Robertson, K. D., and Jones, P. A. The human ARF cell cycle regulatory gene promoter is a CpG island which can be silenced by DNA methylation and down-regulated by wild-type p53. Mol. Cell. Biol., *18:* 6457–6473, 1998.
33. Haber, D. Splicing into senescence: the curious case of p16 and $p19^{ARF}$. Cell, *91:* 555–558, 1997.




Downloaded from http://aacrjournals.org/cancerres/article-pdf/60/8/2113/2483904/ch080002113.pdf by guest on 07 October 2024