BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **251,** 153–157 (1998)
ARTICLE NO. RC989418

Detection of DNA Abnormalities by Arbitrarily Primed PCR Fingerprinting: Allelic Losses in Chromosome 10q in Lung Cancers

Keita Kawakami,*,†,‡ Jun Yasuda,* Masahiko Shiraishi,* Takamasa Kayama,† Katsuhiko Doi,‡ Manuel Perucho,§ and Takao Sekiya*,[1]

Oncogene Division, National Cancer Center Research Institute, 1-1, Tsukiji 5-Chomoe, Chuo-ku, Tokyo 104-0045, Japan; †Department of Neurosurgery and ‡First Department of Physiology, Yamagata University School of Medicine, Yamagata, Japan; and §The Burnham Institute, La Jolla Cancer Research Center, 10901 North Torrey Pines Road, La Jolla, California 92037

Received September 1, 1998

DNA fingerprinting using arbitrarily primed PCR (AP-PCR) is useful for detecting cancer-specific DNA aberrations without targeting any particular genes or knowing any nucleotide sequences in advance. AP-PCR fingerprinting is an efficient method for finding loss of anonymous chromosomal regions in cancers. We analyzed DNA from 44 human non-small cell lung cancers by fingerprinting using a single primer and found a loss of signal intensity in a DNA fragment amplified from chromosome 10 (fragment F) in 15 tumors. The detailed location of the fragment F locus on chromosome 10q was determined by PCR-based analysis of radiation hybrid panels using a sequence-tagged site established for the fragment. In 12 of the 15 tumors, loss of the signal detected by AP-PCR fingerprinting was in agreement with the results obtained by analysis of allelic imbalances using 7 polymorphic CA-microsatellite DNA markers for loci around the fragment F locus ($p=0.0009$). We conclude that a hitherto unknown suppressor gene for lung cancer resides at 10q in the vicinity of fragment F.

Neoplasms are caused by the accumulation of abnormalities of oncogenes and tumor suppressor genes due to multistep DNA alterations. Among the DNA abnormalities involving relatively large regions of the chromosomes, amplifications or rearrangement of genes often activate protooncogenes to become oncogenes (1, 2). Inactivation of tumor suppressor genes is also associated with aberrations of large DNA regions, such as loss of an entire chromosome or a segment of the chromosome that contains the normal counterpart of the gene (3). Thus gain or loss of chromosomal regions may reflect aberrations of the genes involved in cancer genesis.

AP-PCR was designed to amplify multiple DNA fragments from anonymous regions of a complex genome without knowledge of the nucleotide sequences in advance (4). Amplification is semi-quantitative, and comparison of the AP-PCR products from normal and tumor DNAs in a fingerprint obtained by polyacrylamide gel electrophoresis reveals gains and losses of several chromosomal regions (5-8). We developed a method for the chromosomal assignment of most of the AP-PCR fragments by the simultaneous hybridization of AP-PCR products (SHARP) from human genomic DNA (9). Combined use of SHARP and AP-PCR provides a convenient method for molecular karyotyping of tumors (10).

We applied the SHARP and AP-PCR methods for detection of DNA aberrations in non-small cell lung carcinomas (NSCLCs) and found a DNA fragment assigned to chromosome 10 whose intensity decreased frequently in the carcinomas. Analysis of radiation hybrid panels (11, 12) using an established sequence-tagged-site (STS) based on the nucleotide sequence of the fragment revealed a more precise chromosomal location at 10q. Analysis of allelic imbalance at loci around the locus of the fragment using microsatellite polymorphic markers confirmed the allelic losses at chromosome 10q in NSCLCs and further revealed the location of a region of common loss in lung cancer.

MATERIAL AND METHODS

DNA samples. Paired DNA samples from normal and cancer tissues of 44 patients with NSCLC who underwent surgery at the National Cancer Center Hospital (Tokyo, Japan) were analyzed. The cancers included 23 adenocarcinomas, 14 squamous cell carcinomas and 7 large cell carcinomas. High-molecular-weight DNAs were isolated from these tissues (13).

[1] The corresponding author: Takao Sekiya, Ph.D., Oncogene Division, National Cancer Center Research Institute, 1-1, Tsukiji 5-Chomoe, Chuo-ku, Tokyo 104-0045, Japan. Fax: +81-3-5565-9535. E-mail: tsekiya@ncc.go.jp.

0006-291X/98 $25.00

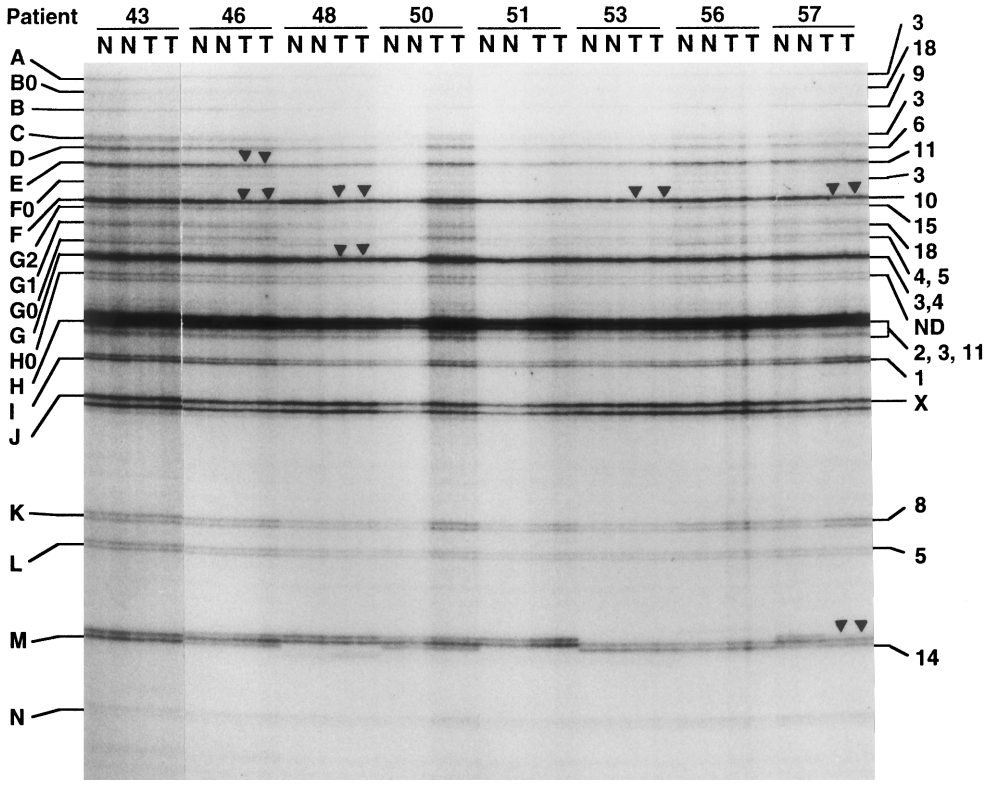

FIG. 1. AP-PCR fingerprinting analysis of DNA samples from patients with NSCLC using Blue primer. N and T indicate normal and cancer samples, respectively. The letters on the left side of the gel indicate the name of the DNA fragments. Numbers on the right side of the gel indicate the chromosome from which the DNA fragments are amplified, determined by the SHARP method. (ND: not determined) Losses of signals of DNA fragments are indicated by downward arrowheads. The experiment was performed in duplicate, with two DNA samples from normal and tumor tissue.

AP-PCR fingerprinting and SHARP analysis. The mixture for AP-PCR (50 ml) contained 20 ng of template DNA, 125 mM each of 4 deoxynucleoside triphosphates, 0.15 μl of [α-^{32}P]dCTP (Amersham, 3000 Ci/mmol/ml), 10 mM Tris-HCl (pH 8.2), 50 mM KCl, 4.5 mM $MgCl_2$, 0.01% gelatin, 0.6 units of Taq polymerase (Perkin Elmer Cetus, Branchburg) and 10 mM of an arbitrary primer (Blue, 5′-CCGAATTCGCAAAGCTCTGA-3′) (9). The reaction was started by heating at 94°C for 5 min to denature the DNA template and then followed by 5 cycles of incubation in a low-stringent condition (94°C for 30 sec, 50°C for 1 min and 72°C for 1.5 min), then 25 cycles of incubation in a high-stringent condition (94°C for 15 sec, 60°C for 15 sec and 72°C for 1 min) and finally by incubation at 72°C for 5 min. A fingerprint was obtained by electrophoresis of the AP-PCR products in a 5 % polyacrylamide gel containing 7 M urea, followed by exposure of the dried gel to an X-ray film for 12-24 h. SHARP analysis was performed as described previously (9)

Analysis of radiation hybrid panels. A small piece of the dried gel of a fingerprint was excised at the position of a normal sample corresponding to fragment F. DNA was eluted from the gel piece by putting it into 100 μl of deionized water. The eluted DNA was amplified by PCR using the same single primer as in the high-stringency condition. The PCR product was cloned using the pCR2.1 vector (Invitrogen, San Diego). Six independent clones were sequenced by cycle sequencing. Based on the nucleotide sequence of fragment F, we designed a PCR primer set specific for amplification of the fragment. Two publicly available radiation hybrid panels, GeneBridge 4 radiation hybrid (GeneBridge 4) panel and Stanford Human Genome Center G3 radiation hybrid (Stanford G3) panel (Research Genetics, Huntsville, Alabama, USA) were analyzed by PCR using the primer set.

CA-microsatellite analysis of chromosome 10q. PCR primer sets for 7 CA-microsatellite polymorphic markers of the D10S187, D10S221, D10S542, D10S209, D10S216, D10S575 and D10S214 loci were obtained from Research Genetics (Huntsville, Alabama, USA). The allelic status at these loci of DNA samples from 44 patients was analyzed. PCR products using radioisotope-labeled primer sets were separated by electrophoresis in 5 % denaturing polyacrylamide gel. Dried gels were exposed to X-ray films for 4 hours.

RESULTS

Analysis of DNA from NSCLCs by AP-PCR Fingerprinting

Paired DNA samples from the normal and tumor tissues of patients with NSCLC were analyzed by AP-PCR fingerprinting using an arbitrary primer, Blue (9). Fig. 1 shows typical fingerprints. Comparison of the fingerprints from normal and tumor DNAs revealed tumor specific gain or loss of signal intensities in several DNA fragments. As observed in cases 46, 48 and 53, a tumor specific loss of signal intensity of fragment F was found in 15 of 44 cases analyzed (34 %). By SHARP analysis, fragment F was shown to be derived from chromosome 10 (9).

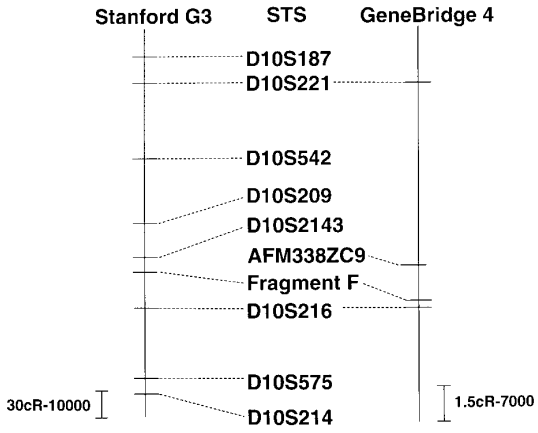

FIG. 2. Radiation hybrid mapping of fragment F using a Stanford Human Genome Center G3 panel (Stanford G3) and GeneBridge 4 panel provided by Whitehead Institute Center for Genome Research (GeneBridge 4).

Mapping of Fragment F on Chromosome 10

DNA fragment F was cloned and its nucleotide sequence was determined. No homology of the nucleotide sequence to any known ones was detected by a BLAST search (14). On the basis of the nucleotide sequence, a STS specific for the fragment was established. Using the STS, PCR-based analyses of the two radiation hybrid panels, the GeneBridge 4 and the Stanford G3 panels, were performed. The results revealed that the fragment F locus was 1.5 centiRays-7000 telomeric from the AFM338ZC9 locus (the GeneBridge 4 panel) and 17.5 centiRays-10000 telomeric from the D10S2143 locus (the Stanford G3 panel) on chromosome 10q (Fig. 2). Around the fragment F locus, at least two loci, the D10S221 and D10S216 loci, were characterized on both radiation hybrid maps and the two loci flanked the region containing the AFM338ZC9, D10S2143 and fragment F loci (Fig. 2). These results localize fragment F at 10q 24-q25.

Allelic Losses of Loci around the Fragment F Locus

To verify the alterations in DNA copy number detected by AP-PCR fingerprinting, the allelic status of the region giving the reduced signal intensities of fragment F was analyzed using 7 STSs carrying CA-microsatellites for D10S187, D10S221, D10S542, D10S209, D10S216, D10S575 and D10S214 loci (Fig. 2). Representative results with the STSs for the D10S209 and D10S216 loci are shown in Fig. 3, together with a part of the AP-PCR fingerprints containing fragment F. In tumors 57, 113, 138 and 204 that showed a loss of signal intensity of fragment F, allelic loss at both or either one of the two loci was observed. However, in tumor 60 loss of the fragment F signal was not accompanied by allelic losses at any of the two adjacent loci. On the other hand, in tumors 56 and 180 with allelic losses at the adjacent loci, loss of the fragment F signal was not observed.

Of 44 tumor samples analyzed, 26 (62%) showed DNA aberrations with both AP-PCR fingerprinting and allelic imbalance analysis or either one of these analyses (Fig. 4). AP-PCR fingerprinting revealed reduced signal intensities of fragment F in 15 tumors, of which 12 were accompanied with allelic loss at least at one of the flanking loci analyzed. These are therefore defined as positives. On the other hand, the remaining 3 tumors 53, 60 (Fig. 3) and 149 did not exhibit allelic losses at the loci flanking the fragment F locus by microallelotyping.

The fingerprinting also showed gain of the signal intensities of fragment F in 2 tumors, 180 and 202, although the significance of these results is not clear. In particular, in tumor 180 the allelic imbalances observed at two loci adjacent to the fragment F locus suggest that the result on fragment F in the fingerprinting could be an example of a false negative for allelic loss. Similarly, in other 9 of the 24 tumors, no aberration of fragment F was observed in the fingerprints. However, in 6 of the 9 tumors, 51, 56, 131, 171, 208 and 130, allelic loss at both or one of the two loci adjacent to the fragment F was detected. We assumed that some of these tumors were false negatives regarding loss of the fragment F signals. False negatives for loss in AP-PCR fingerprinting are frequently due to the loss of one allele and reduplication of the other (10). In the remaining 3 tumors, 196, 163 and 178, the allelic imbalances detected by microallelotyping were at 10q loci located in regions farther away from the fragment F locus and might not be related to the allelic status of

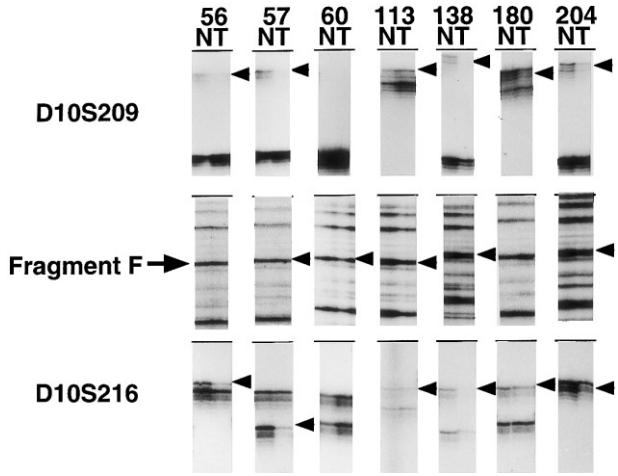

FIG. 3. Allelic status of loci adjacent to the fragment F locus. The heterozygosity of the D10S209 and D10S216 loci was analyzed using microsatellite DNA markers. The results are compared with those of AP-PCR analysis in which an arrow indicates the position of fragment F. N and T indicate DNA from normal and cancer samples from patients with NSCLC, respectively. Allelic losses and loss of signal are indicated by arrowheads.

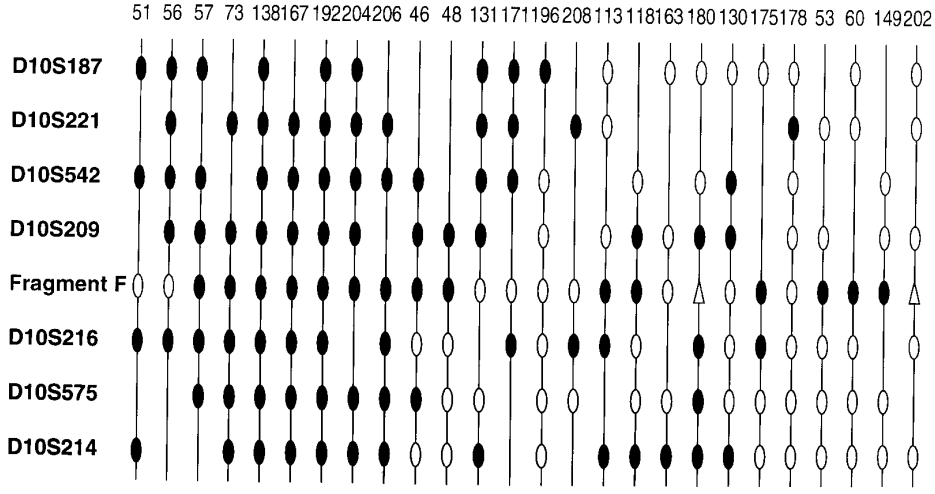

FIG. 4. Allelic imbalances of loci around the fragment F locus. Numbers of the DNA samples are indicated on the top of the figure. Solid circles indicate allelic imbalances (losses) detected by microallelotyping or signal losses of fragment F by AP-PCR fingerprinting. Open circles indicate retention of heterozygosity or no changes of signal intensity of the fragment F. Open triangles indicate signal gains of fragment F. Vertical lines indicate noninformative results.

the fragment F locus. Thus in spite of the presence of possible false negatives and positives, the positive results obtained by the AP-PCR fingerprinting were in good agreement with those from the allelic imbalance analysis ($p=0.0009$, Fisher's exact test).

DISCUSSION

AP-PCR fingerprinting has previously been applied to the analysis of genetic aberrations in human cancers (5, 6, 8, 10, 15, 16) and semi-quantitative amplification of DNA fragments by AP-PCR can detect tumor specific changes of copy numbers of anonymous regions of the genome. The SHARP method can easily assign chromosomal origins to the AP-PCR products from human DNA without cloning and sequencing (9). Determination of the chromosomal origin of DNA fragments in AP-PCR fingerprints by this simple method is thus helpful for evaluating the significance of the abnormalities detected and for making decisions of further analysis of the corresponding regions.

Radiation hybrid mapping systems established by the Whitehead-MIT Center for Genome Research (11) (http://www.genome.wi.mit.edu) and the Stanford Human Genome Center (http://www-shgc.stanford.edu) can be used for further characterizing the chromosomal regions containing the cloned AP-PCR fragments which show abnormalities in human cancers. In this study, fragment F indicating loss of signal intensities in lung tumor DNA was detected by AP-PCR fingerprinting and assigned to chromosome 10 by the SHARP method and 10q by radiation hybrid mapping.

The low copy number of fragment F detected by AP-PCR fingerprinting was in good accordance with allelic imbalances of chromosome 10q detected using polymorphic dinucleotide DNA markers around the fragment F locus. A few tumors were found to show reduced signal intensities of fragment F but without allelic imbalances at the adjacent loci. These could be false positive results, or tumors with a very localized region of loss. Tumors with allelic imbalances at the loci adjacent to the fragment F locus but without loss of the signal intensities of fragment F might bring about underestimation of the real frequency of loss. In concert with microallelotyping, however, AP-PCR fingerprinting may reveal the existence of novel regions of frequent chromosomal loss in defined cancers.

There have been a few reports on allelic imbalances of chromosome 10q in NSCLC showing relatively frequent losses of chromosome 10q in squamous cell carcinomas and less frequent losses in adenocarcinomas (17-21). Allelic losses of loci on 10q have also been reported in malignant gliomas (22-24), prostate cancers (25-27) and melanomas (28-30). A candidate tumor suppressor gene on 10q23.1, *PTEN*, has been cloned (31, 32) and its mutations have been observed in about 20% of these tumors. Although the fragment F locus is included in the regions deleted in these tumors, the locus might not coincide with the PTEN locus. Our results of the frequent loss of fragment F locus on 10q24-q25 in NSCLC with similar frequency either in squamous cell carcinomas or in adenocarcinomas suggest the presence of other tumor suppressor gene(s) involved in NSCLC at this chromosomal region.

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in-Aid from the Ministry of Health and Welfare for the 2nd Term Comprehensive 10-year Strategy for Cancer Control, Japan, and a Research Grant

on Human Genome and Gene Therapy from the Ministry of Health and Welfare. M.P. was a visiting scholar of the Foundation for the Promotion of Cancer Research.

REFERENCES

1. Bishop, J. M. (1991) *Cell* **64,** 235–48.
2. Hunter, T. (1991) *Cell* **64,** 249–70.
3. Cavenee, W. K., Dryja, T. P., Phillips, R. A,, Benedict, W. F., Godbout, R., Gallie, B. L., Murphree, A. L., Strong, L. C., and White, R. L. (1983) *Nature* **305,** 779–84.
4. Welsh, J., and McClelland, M. (1990) *Nucleic Acids Res.* **18,** 7213–7218.
5. Peinado, M. A., Malkhosyan, S., Velazquez, A., and Perucho, M. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 10065–10069.
6. Kohno, T., Morishita, K., Takano, H., Shapiro, D. N., and Yokota, J. (1994) *Oncogene* **9,** 103–108.
7. Achille, A., Biasi, M. O., Zamboni, G., Bogina, G., Magalini, A. R., Pederzoli, P., Perucho, M., and Scarpa, A. (1996) *Cancer Res.* **56,** 3808–13.
8. Okazaki, T., Takita, J., Kohno, T., Handa, H., and Yokota, J. (1996) *Human Genet.* **98,** 253–8.
9. Yasuda, J., Navarro, J. M., Malkhosyan, S., Velazquez, A., Arribas, R., Sekiya, T., and Perucho, M. (1996) *Genomics* **34,** 1–8.
10. Malkhosyan, S., Yasuda, J., Soto, J. L., Sekiya, T., Yokota, J., and Perucho, M. (1998). *Proc. Natl. Acad. Sci. U.S.A.*, in press.
11. Hudson, T. J., Stein, L. D., and other 49 authors. (1995) *Science* **270,** 1945–1954.
12. Johnson, R. L., Rothman, A. L., Xie, J., Goodrich, L. V., Bare, J. W., Bonifas, J. M., Quinn, A. G., Myers, R. M., Cox, D. R., Epstein, E., Jr., and Scott, M. P. (1996) *Science* **272,** 1668–71.
13. Shiraishi, M., Morinaga, S., Noguchi, M., Shimosato, Y., and Sekiya, T. (1987) *Jpn. J. Cancer Res. (Gann)* **78,** 1302–1308.
14. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) *Biology* **215,** 403–10.
15. Ionov, Y., Peinado, M. A., Malkhosyan, S., Shibata, D., and Perucho, M. (1993) *Nature* **363,** 558–561.
16. Yasuda, J., Kashiwabara, H., Kawakami, K., Uematsu, K., Sugano, K., Perucho, M., and Sekiya, T. (1996) *Biol. Chem. (Hoppe Seyler)* **377,** 563–570.
17. Testa, J. R., Siegfried, J. M., Liu, Z., Hunt, J. D., Feder, M. M., Litwin, S., Zhou, J.-Y., Taguchi, T., and Keller, S. M. (1994) *Genes Chromosom. Cancer* **11,** 178–194.
18. Sato, S., Nakamura, Y., and Tsuchiya, E. (1994) *Cancer Res.* **54,** 5652–5655.
19. Johansson, M., Jin, Y., Mandahl, N., Hambraeus, G., Johansson, L., Mitelman, F., and Heim, S. (1995) *Cancer Genet. Cytogenet.* **81,** 46–55.
20. Petersen, S., Wolf, G., Bochkmühl, U., Gellert, K., Dietel, M., and Petersen, I. (1998) *British J. Cancer* **77,** 270–276.
21. Virmani, A. K., Fong, K. M., Kodagoda, D., McIntire, D., Hung, J., Tonk, V., Minna, J. D., and Gazdar, A. F. (1998) *Genes Chromosom. Cancer* **21,** 308–319.
22. Rasheed, B. K., McLendon, R. E., Friedman, H. S., Friedman, A. H., Fuchs, H. E., Bigner, D. D., and Bigner, S. H. (1995) *Oncogene* **10,** 2243–2246.
23. Albarosa, R., Colombo, B. M., Roz, L., Magnani, I., Pollo, B., Cirenei, N., Giani, C., Conti, A. M., DiDonato, S., and Finocchiaro, G. (1996) *Am. J. Hum. Genet.* **58,** 1260–1267.
24. Sonoda, Y., Murakami, Y., Tominaga, T., Kayama, T., Yoshimoto, T., and Sekiya, T. (1996) *Jpn. J. Cancer Res.* **87,** 363–367.
25. Gray, I. C., Phillips, S. M., Lee, S. J., Neoptolemos, J. P., Weissenbach, J., and Spurr, N. K. (1995) *Cancer Res.* **55,** 4800–4803.
26. Ittmann, M. (1996) *Cancer Res.* **56,** 2143–2147.
27. Trybus, T. M., Burgess, A. C., Wojno, K. J., Glover, T. W., and Macoska, J. A. (1996) *Cancer Res.* **56,** 2263–7.
28. Isshiki, K., Elder, D. E., Guerry, D., and Linnenbach, A. J. (1993) *Genes, Chromosomes Cancer* **8,** 178–84.
29. Healy, E., Rehman, I., Angu, B., and Rees, J. L. (1995) *Genes, Chromosomes Cancer* **12,** 152–156.
30. Healy, E., Belgaid, C. E., Takata, M., Vahlquist, A., Rehman, I., Rigby, H., and Rees, J. L. (1996) *Cancer Res.* **56,** 589–593.
31. Li, J., Yen, C., Liaw, D., Podsypanina, K., Bose, S., Wang, S. I., Puc, J., Miliaresis, C., Rodgers, L., McCombie, R., Bigner, S. H., Giovanella, B. Ittmann, M., Tycko, B., Hibshoosh, H., Wigler, M. H., and Parsons, R. (1997) *Science* **275,** 1876–1878.
32. Steck, P. A., Pershouse, M. A., Jasser, S. A., Yung, W. K., Lin, H., Ligon, A. H., Langford, L. A., Baumgard, M. L., Hattier, T., Davis, T., Frye, C., Hu, R., Swedlund, B., Teng, D. H., and Tavtigian, S. V. (1997) *Nature Genet.* **15,** 356–3562.