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Advances in Brief

p53 Mutations in Nonastrocytic Human Brain Tumors[1]

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Abstract

Genomic DNA from 51 primary human brain tumors was screened for the presence of mutations in the tumor suppressor gene, *p53*, using the polymerase chain reaction and single strand conformation polymorphism analysis, followed by direct DNA sequencing. Mutations leading to an amino acid change were found in 2 of 17 (12%) oligodendrogliomas and 2 of 19 (11%) medulloblastomas but none of 15 ependymomas. Sites of mutations were in exon 5 (codon 141), exon 6 (codon 193 and 213), and exon 7 (codon 246). In addition, there were silent mutations in exon 6 (codon 213) in one oligodendroglioma and in one ependymoma. This study points to the possible role of the *p53* tumor suppressor gene in some central nervous system neoplasms of divergent histogenesis.

Introduction

As in other organ sites, both transforming and suppressor genes are involved in the pathogenesis of nervous system tumors (1, 2). Protooncogene activation by gene amplification has been identified in a variety of neural tumors, *e.g.*, N-*myc* in neuroblastomas (3, 4), c-*myb* (5) and epidermal growth factor receptor gene (6, 7) in gliomas, and c-*myc* in medulloblastomas (8, 9). Information on the involvement of other transforming genes in the pathogenesis of human brain tumors is scarce; in particular, there is no evidence of *ras* mutations in CNS[3] neoplasms (2). In contrast, there is increasing evidence that tumor suppressor genes play an important role in neurocarcinogenesis (2).

Loss of heterozygosity and chromosomal abnormalities have been observed in human brain tumors with variable histology. Thomas and Raffel (10) reported on the loss of heterozygosity in chromosomes 6q, 16q, and 17p in 22, 13, and 26% of primitive neuroectodermal tumors, respectively. A loss of heterozygosity was also found in chromosomes 13, 17, and 22 in 14, 22–50, and 19%, respectively, of astrocytic gliomas of various malignancy (11–13), but deletions on chromosome 10 appeared to be specific (97%) for glioblastomas (13). Translocation involving chromosomes 9, 17, and 22 and the loss of chromosomes 17 and 22 were observed in ependymomas (14, 15). Chromosomal abnormalities in chromosomes 1 and 17 and the loss of chromosome 17p also have been found in medulloblastomas (14, 16, 17). These cytogenetic data suggest that there may be several different tumor suppressor genes and that the loss or impaired function of any one, including the *p53* gene, may constitute important genetic events in the development of human brain tumors. One of the most common genetic abnormality in human brain tumors is the loss of heterozygosity for chromosome 17, *i.e.*, the chromosome on which the *p53* gene resides, but there are only limited data on *p53* mutations in CNS neoplasms (18).[4]

In the present study, we screened 51 primary nonastrocytic human brain tumors, *i.e.*, oligodendrogliomas, ependymomas, and medulloblastomas, for mutations in exons 5 to 8 of the *p53* gene. Genomic DNAs from frozen tissues of these tumors were amplified by PCR and analyzed for mutations using the SSCP assay (19). Mutations in positive samples were identified by direct sequencing of the PCR-amplified DNA.

Materials and Methods

Brain Tumor Samples. Primary brain tumor biopsies from 51 patients were collected during standard neurosurgical procedures, frozen in liquid nitrogen, and kept at −80°C until DNA extraction. These neoplasms comprised 17 oligodendrogliomas, 15 ependymomas, and 19 medulloblastomas. The age of patients ranged from 2 to 63 years (mean, 43 years) for oligodendrogliomas, 3 to 70 years (mean, 27 years) for ependymomas, and 10 months to 34 years (mean, 12 years) for medulloblastomas. Histopathological diagnosis and tumor grading were established according to the guidelines of the World Health Organization (20). Of 17 oligodendrogliomas, 8 were of grade II (isomorphous), and 9 were of grade III (anaplastic oligodendroglioma); of 15 ependymomas, 2 were of grade I (myxopapillary ependymoma of the cauda equina), 9 were of grade II, and 4 were of grade III (anaplastic ependymoma). All medulloblastomas were WHO grade IV.

After identification of tumor tissue on cryostat sections stained with hematoxylin and eosin, samples were homogenized and digested with RNase A, RNase T_1, and proteinase K. DNA was then extracted by phenol and chloroform and precipitated with ethanol.

PCR-SSCP Analysis. For prescreening the samples for mutations in the *p53* gene, PCR-SSCP analysis was performed according to a slight modification of the method of Orita *et al.* (19). Briefly, PCR was performed with 200 ng of genomic DNA, 2.5 pmol of each primer, 50 μM concentrations of deoxynucleoside triphosphates, 1 μCi of [α-^{32}P] dCTP (Amersham; specific activity, 3000 Ci/mmol), 10 mM Tris (pH 8.8), 50 mM KCl, 1 mM $MgCl_2$, and 0.5 unit Taq polymerase (Perkin-Elmer Cetus) in a final volume of 10 μl. After addition of 10 μl of mineral oil (Sigma), 35 cycles of denaturation (95°C) for 50 s, annealing (63°C for exons 5, 6, and 7; 58°C for exon 8) for 50 s, and extension (72°C) for 70 s were done using an automated DNA Thermal Cycler (Perkin-Elmer Cetus). Primer sequences were as follows: 5′-TTCCTCTTCCTGCAGTACTC (A), and 5′-ACCCTGGGCAACCAGCCCTGT (B) for exon 5, 5′-ACAGGGCTGGTTGCCCAGGGT (C), and 5′-AGTTGCAAACCAGACCTCAG (D) for exon 6; 5′-GTGTTGTCTCCTAGGTTGGC (E) and 5′-GTCAGAGGCAAGCAGAGGCT (F) for exon 7; and 5′-TATCCTGTAGTAGTGGTAATC (G) and 5′-AAGTGAATCTGAGGCATAAC (H) for exon 8 (21, 22). Primers A, D, E, and G were designed from the respective intron/exon border sequence and primers B, C, F, and H from intron sequences.

The reaction mixture (1.5 μl) was mixed with 2 μl of 0.1 M NaOH and 9 μl of sequencing stop solution (USB). Samples were heated at 95°C for 10 min, chilled on ice, and immediately loaded onto a 6% polyacrylamide nondenaturing gel containing 10% glycerol. Gels were run at 7 W for 13–15 h at room temperature. Gels were fixed in 10%

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[3] The abbreviations used are: CNS, central nervous system; PCR, polymerase chain reaction; SSCP, single strand conformation polymorphism.

[4] R. H. Frankel, W. Bayona, M. Koslow, and E. W. Newcomb. *p53* mutations in human malignant gliomas: comparison of loss of heterozygosity with mutation frequency, submitted for publication.

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acetic acid and dried at 80°C. Autoradiography was performed with an intensifying screen for 5–48 h and the patterns of single and double-stranded DNA were checked for differences. Positive controls consisted of samples with confirmed point mutations for each exon 5–8.

Direct Sequencing of PCR Products. PCR was performed with 1 μg of genomic DNA, 12.5 pmol of each primers, 200 μM concentrations of deoxynucleoside triphosphates, 10 mM Tris (pH 8.8), 50 mM KCl, 1 mM $MgCl_2$, and 2.5 units Taq polymerase in a total volume 100 μl. Thirty-five to 40 cycles of denaturation (95°C) for 1 min, annealing (63°C for exons 5, 6, and 7; 58°C for exon 8) for 1 min, and extension (72°C) for 1 min and 30 s were done. After amplification, 70 μl of the PCR reaction were electophoresed on a 6% polyacrylamide gel. The amplified bands were cut out, eluted in 0.5 M ammonium acetate and 1 mM EDTA at 37°C overnight, and precipitated with ethanol. Dried DNA was resuspended in 12 μl of distilled water.

Sanger dideoxynucleotide sequencing was performed using [α-^{35}S] dATP and primers for amplification (23). The template-primer mixture (4 μl DNA and 10 pmol primer) was heated at 95°C for 5 min and immediately placed in liquid nitrogen. An aliquot containing 20 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 25 mM NaCl, 0.1 M dithiothreitol, 10% dimethyl sulfoxide, and 5 μCi [α-^{35}S]dATP was added to each of the four termination mixtures and incubated at 37°C for 10 min with 2 units of Sequenase version 2.0 (USB). Samples were mixed with 4 μl stop solution, heated at 80°C for 2 min, and immediately loaded onto a 6% polyacrylamide-7 M urea gel. Gels were fixed with 10% acetic acid and 10% methanol, dried, and autoradiographed for 1–7 days. When mutations were detected, they were further confirmed by sequencing the opposite strand.

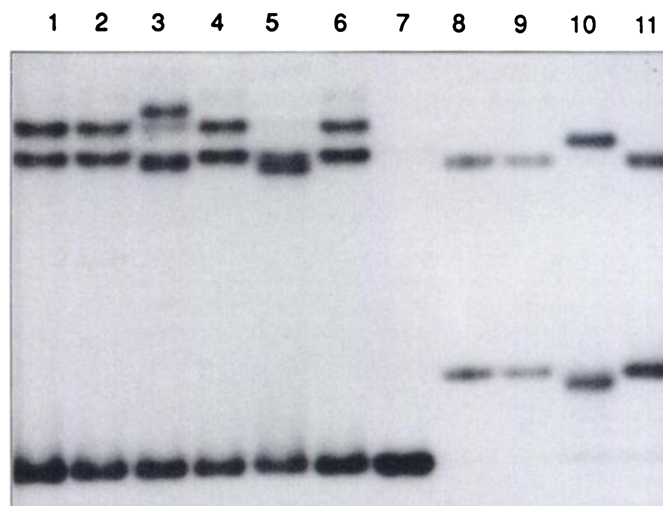

Fig. 1. SSCP autoradiographs of *p53* exons 6 (*Lanes 1–7*) and 7 (*Lanes 8–11*). Genomic brain tumor DNA was amplified by PCR in the presence of [α-P^{32}] dCTP, denatured, and run on a 6% acrylamide gel containing 10% glycerol. Samples with point mutations differ in their mobility from single strand fragments of normal brain DNA. Of 5 medulloblastomas (*Lanes 1–5*), two (*Lanes 3* and *5*) show a mutation. *Lanes 6* and *7* are from denatured and double-stranded normal brain DNA, respectively. *Lanes 8–11* (exon 7) show normal (wild-type) sequences for two oligodendrogliomas (*Lanes 8* and *9*), a mutated sequence in another oligodendroglioma (*Lane 10*) and again a wild-type sequence in normal brain DNA (*Lane 11*).

Results

SSCP analysis revealed 6 tumor samples suggestive of a mutation (Fig. 1). Sequence analysis showed single missense point mutations in 3 samples (Table 1): 2 oligodendrogliomas (grade II; 28-year-old female and a 32-year-old male patient); and 1 medulloblastoma (grade IV; 18-year-old female patient) at codons 141, 246, and 193, respectively. A deletion of the first base of codon 213 was identified in one of the medulloblastomas (grade IV; 8-year-old boy) (Table 1). Point mutations were present in exon 5–7, *i.e.*, in highly conserved regions for which mutations have frequently been reported (18, 24).[4] However, no clustering of mutations in specific codons was observed. Further, all point mutations were found in G or A. The spectrum of mutations which we observed in our panel of nonastrocytic brain tumors, mainly G to A transitions, is consistent with the reports of brain tumors studied by others (24, 25). In all cases but one, normal (wild type) bases were found together with mutated bases; in one oligodendroglioma (grade II, 32-year-old male patient), only the mutated base was detectable, presumably due to a loss of the normal allele. Typical DNA sequence autoradiographs are shown in Fig. 2.

A silent point mutation was found at codon 213 (CGA → CGG/Arg → Arg) in one oligodendroglioma (grade II; 2-year-old boy) and one ependymoma (grade III; 12-year-boy). This polymorphism in codon 213 has also been reported to be present in 2% of lymphoid malignancies (21), in 2.5% of glioblastomas,[4] and in 11% of the Italian population.[5]

Discussion

The *p53* gene encodes a M_r 53,000 nuclear phosphoprotein which is considered to play an essential role in the regulation of cell proliferation (26). The wild-type *p53* gene appears to act as a tumor suppressor gene whereas a mutant *p53* gene can promote transformation by inactivation of the normal function of *p53* in a dominant negative fashion (27, 28). Loss or impaired function of the *p53* gene appears to play an important role in the development of a wide range of human neoplasms (18, 21, 24, 25, 29–31). Tumors frequently exhibit either a loss of both alleles of the *p53* gene, the loss of one *p53* allele with an associated point mutation, insertion or deletion of the remaining allele, or an inactivation of the *p53* gene in one allele but a normal (wild-type) sequence in the remaining allele. The transforming activity resulting from a point mutation, insertion, or deletion in one *p53* allele without the loss of remaining wild type is considered to be due to one of the following mechanisms: (*a*) the mutant *p53* protein may compete with the wild type protein for interaction with target molecules and thus inhibit wild type function (32); (*b*) the mutant *p53* protein may complex with the wild type protein to produce an inactive oligomeric complex (33, 34); (*c*) the mutant *p53* protein affects the conformational phenotype of the wild type *p53* protein thus inhibiting its normal function (35). Milner and Medcalf (35) tested 5 mutant *p53* genes and found that 4 of them had a common, dominant effect on the phenotype of cotranslated wild type human *p53*, driving the latter into the mutant phenotypic form. Interestingly, the only exception was a mutant (*p53-trp*^{248}) previously identified as germ line mutation in patients with the cancer predisposing Li-Fraumeni syndrome (36, 37) and showed the lack of dominant negative effect. It might thus be concluded that most *p53* point mutations could have dominant negative effects even in the presence of one remaining wild type allele.

The molecular mechanisms involved in the development of human gliomas are still poorly understood. Astrocytomas have been examined for the presence of *p53* point mutations, particularly because a significant number of these neoplasms show a partial loss of the short arm of chromosome 17, *i.e.*, the site of the *p53* gene (18).[4] Nigro *et al.* (18) reported that 4 of the 5

[5] A. Serra, G. L. Gaidano, D. Revello, A. Guerrasio, P. Ballerini, R. Dalla-Favera, and G. A. Saglio. New taq I polymorphism in *p53* gene, submitted for publication.

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Table 1 *Missense mutations and a deletion in p53 gene in human nonastrocytic brain tumors*

Tumor (Incidence of mutation)	Exon	Codon	Mutation	Amino acid substitution
Oligodendroglioma (2/17 = 12%)	5	141	TGC→TAC	Cys→Tyr
	7	246[a]	ATG→ATA	Met→Ile
Medulloblastoma (2/19 = 11%)	6	193	CAT→CCT	His→Pro
	6	213	First base deleted	Frame-shift

[a] Wild type sequence at the mutation site could not be detected.

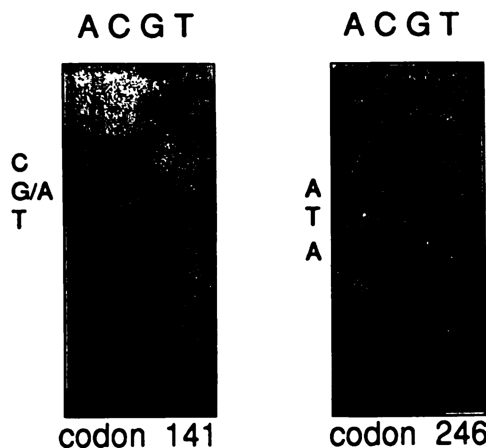

Fig. 2. DNA sequence autoradiographs of *p53* mutations in human brain tumors. CGT → CAT transition at codon 141 in an oligodendroglioma (*left*). ATG → ATA transition at codon 246 in an oligodendroglioma (*right*). In this case, the wild-type sequence was not detectable, suggesting a loss of the normal allele.

glioblastomas with a single 17p allele contained *p53* point mutations. Similarly, Frankel *et al.*[4] found that 9 of 27 malignant gliomas (33%) with two 17p alleles and 7 of 11 (64%) of glioblastomas with only one 17p allele contained *p53* point mutations. In the present study, we have analyzed three different histological types of nonastrocytic brain tumors, oligodendrogliomas, medulloblastomas, and ependymomas for genetic events which may play a role in their development.

Oligodendrogliomas are glial tumors, typically located in the cerebral hemispheres and usually manifesting in adults, with a peak incidence at 30–40 years. Low grade oligodendrogliomas grow slowly but progression into a malignant phenotype (anaplastic oligodendroglioma) is occasionally observed. There are no reports of oncogene activation, loss of heterozygosity of specific chromosomal regions, or inactivation of tumor suppressor genes in this type of neoplasm. In the present study, we found *p53* missense mutations in 2 of 17 (12%) oligodendrogliomas.

Medulloblastomas are malignant, embryonal cerebellar tumors with a peak incidence between 7 and 12 years of age. In addition to allelic loss in 17p, c-*myc* amplification has been reported to occur in this childhood tumor (8, 9). In the present analysis of 19 medulloblastomas, we found *p53* missense mutations and a deletion at an incidence of 11%.

Ependymomas develop from the neuroepithelial lining of the ventricles and the central canal of the spinal cord and are predominantly composed of neoplastic ependymal cells. Ependymomas of the cerebral hemisphere occur predominantly during childhood and adolescence. They grow slowly, usually intraventricularly, and are well delineated from adjacent brain structures. Ependymomas have been frequently reported to exhibit a loss of one allele in chromosome 22 in addition to the loss of heterozygosity in chromosome 17p, suggesting the presence of tumor suppressor genes in both chromosomal locations which may be important for the development of ependymomas (14, 15). In our survey of 15 ependymomas, no mutations were found in *p53*.

These results demonstrate that *p53* mutations occur in oligodendrogliomas, *i.e.*, a clinically important type of nonastrocytic brain tumor in adults and in medulloblastomas, the most frequent malignant pediatric CNS neoplasm. The incidence of point mutations in oligodendrogliomas and medulloblastomas in this present study is similar to that reported in other type of human cancers, *i.e.*, peripheral T-cell lymphoma (8%) (21) and B-cell chronic lymphocytic leukemia (15%) (21). However, the incidence of *p53* mutations was greater in glioblastomas (40–80%) (18),[4] *i.e.*, an astrocytic glioma type considerably more malignant than ependymomas and oligodendrogliomas. This difference in frequency may thus reflect the extent to which gliomas progress from differentiated lesions to highly malignant tumors with frank anaplasia and poor clinical prognosis.

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References

1. Seemayer, T. A., and Cavenee, W. K. Molecular mechanisms of oncogenesis. Lab. Invest., *60:* 585–599, 1989.
2. Bigner, S. H., and Vogelstein, B. Cytogenetics and molecular genetics of malignant gliomas and medulloblastoma. Brain Pathol., *1:* 12–18, 1990.
3. Schwab, M., Alitaro, K., Klempnauer, K. H., Varmus, H. E., Bishop, J. M., Gilbert, F., Brodeur, G., Goldstein, M., and Trent, J. Amplified DNA with limited homology to *myc* cellular oncogene is shared by human neuroblastoma cell line and a neuroblastoma tumor. Nature (Lond.), *305:* 245–248, 1983.
4. Schwab, M. Amplification of the *MYCN* oncogene and deletion of putative tumour suppressor gene in human neuroblastomas. Brain Pathol., *1:* 41–46, 1990.
5. Welter, C., Henn, W., Theisinger, B., Fischer, H., Zang, K. D., and Blin, N. The cellular *myb* oncogene is amplified, rearranged and activated in human glioblastoma cell lines. Cancer Lett., *52:* 57–62, 1990.
6. Ekstrand, A. J., James, C. D., Cavenee, W. K., Seliger, B., Pettersson, R. F., and Collins, V. P. Genes for epidermal growth factor receptor, transforming growth factor α, and epidermal growth factor and their expression in human gliomas *in vivo*. Cancer Res., *15:* 2164–2172, 1991.
7. Bigner, S. H., Burger, P. C., Wong, A. J., Werner, M. H., Hamilton, S. R., Muhlabier, L. H., Vogelstein, B., and Bigner, D. D. Gene amplification in malignant human gliomas: clinical and histopathologic aspects. J. Neuropathol. Exp. Neurol., *47:* 191–205, 1988.
8. Bigner, S. H., Friedman, H. S., Vogelstein, B., Oakes, W. J., and Bigner, D. D. Amplification of the c-*myc* gene in human medulloblastoma cell lines and xenografts. Cancer Res., *50:* 2347–2350, 1990.
9. MacGregor, D. N., and Ziff, E. B. Elevated c-*myc* expression in childhood medulloblastomas. Pediatr. Res., *28:* 63–68, 1990.
10. Thomas, G. A., and Raffel, C. Loss of heterozygosity on 6q, 16q, and 17p in human central nervous system primitive neuroectodermal tumors. Cancer Res., *51:* 639–643, 1991.
11. El-Azouzi, M., Chung, R. Y., Farmer, G. E., Martuza, R. L., Black, P. M., Rouleau, G. A., Hettlich, C., Hedley-Whyte, E. T., Zervas, N. T., Panagopouls, K., Nakamura, Y., Gusella, J. F., and Seizinger, B. R. Loss of distinct regions on the short arm of chromosome 17 associated with tumorigenesis of human astrocytomas. Proc. Natl. Acad. Sci. USA, *86:* 7186–7190, 1989.
12. Fults, D., Tippets, R. H., Thomas, G. A., Nakamura, Y., and White, R. Loss of heterozygosity for loci on chromosome 17p in human malignant astrocytoma. Cancer Res., *49:* 6572–6577, 1989.
13. James, C. D., Carlbom, E., Dumanski, J. P., Hausen, M., Nordenskjold, M.,

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Collins, V. P., and Cavenee, W. K. Clonal genomic alterations in glioma malignancy stages. Cancer Res., *48:* 5546–5551, 1988.
14. James, C. D., He, L., Carlbom, E., Mikkelsen, T., Ridderheim, P. A., Cavanee, W. K., and Collins, V. P. Loss of genetic information in central nervous system tumors common to children and young adults. Genes Chromosomes Cancer, *2:* 94–102, 1990.
15. Stratton, M. R., Darling, J., Lantos, P. L., Cooper, C. S., and Reenes, B. R. Cytogenetic abnormalities in human ependymomas. Int. J. Cancer, *15:* 579–581, 1989.
16. Cogen, P. H., Daneshaar, L., Metzger, A. K., and Edwards, M. S. Deletion mapping of the medulloblastoma locus on chromosome 17p. Genomics, *8:* 279–285, 1990.
17. Bigner, S. H., Mark, J., Friedman, H. S., Biegel, J. A., and Bigner, D. D. Structual chromosomal abnormalities in human medulloblastoma. Cancer Genet. Cytogenet., *30:* 91–101, 1988.
18. Nigro, J. M., Baker, S. J., Preisinger, A. C., Jessup, J. M., Hostetter, R., Cleary, K., Bigner, S. H., Davidson, N., Baylin, S., Devilee, P., Glover, T., Collins, F. S., Weston, A., Mondali, R., Harris, C. C., and Vogelstein, B. Mutations in the *p53* gene occur in diverse human tumor types. Nature (Lond.), *342:* 705–708, 1989.
19. Orita, M., Suzuki, Y., Sekiya, T., and Hayashi, K. Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics, *5:* 874–879, 1989.
20. Kleihues, P., Burger, O. C., and Scheithauer, B. W. (eds.). Histological Typing of Tumors of the Central Nervous System. Berlin: Springer-Verlag, 1992.
21. Gaidano, G., Ballerini, P., Gong, J. Z., Inghirami, G., Neri, A., Newcomb, E. W., Magrath, I. T., Knowles, D. M., and Dalla-Favera, R. *p53* mutations in human lymphoid malignancies: association with Burkitt's lymphoma and chronic lymphocytic leukemia. Proc. Natl. Acad. Sci. USA, **88:** 5413–5417, 1991.
22. Buchman, V. L., Chumakov, P. M., Ninkina, N. N., Samarina, O. P., and Georgiev, G. P. A variation in the structure of the protein-coding region of the human *p53* gene. Gene, *70:* 245–252, 1988.
23. Sanger, F., Nicklen, S., and Coulson, A. DNA sequencing with chain-terminating inhibitor. Proc. Natl. Acad. Sci. USA, *74:* 5363–5467, 1977.
24. Hollstein, M. C., Sidransky, D., Vogelstein, B., and Harris, C. C. *p53* mutations in human cancers. Science (Washington DC), *253:* 49–53, 1991.
25. Levine, A. J., Momand, J., and Finlay, C. A. The *p53* tumor suppressor gene. Nature (Lond.), *351:* 453–456, 1991.
26. Boyd, J. A., and Barrett, J. C. Tumor suppressor genes: possible functions in the negative regulation of cell proliferation. Mol. Carcinog., *3:* 325–329, 1990.
27. Finlay, C., Hinds, P., and Levine, A. J. The *p53* protooncogene can act as a suppressor of transformation. Cell, *57:* 1083–1093, 1989.
28. Eiyahu, D., Michalovitz, D., Eiyahu, S., Pinhash-Kimhi, O., and Oren, M. Plasmids encoding wild type p53 can inhibit oncogenic-mediated transformation. Proc. Natl. Acad. Sci. USA, *86:* 8763–8767, 1989.
29. Baker, S. J., Preisinger, A. C., Jessup, J. M., Paraskeva, C., Markowitz, S., Willson, J. K. V., Hamilton, S., and Vogelstein, B. *p53* gene mutations occur in combination with 17p allele deletions as late events in colorectal tumorigenesis. Cancer Res., *50:* 7717–7722, 1990.
30. Chiba, I., Takahashi, T., Nau, M. M., D'Amico, D., Curiel, D. T., Mitsudomi, T., Buchhagen, D. L., Carbone, D., Piantadosi, S., Koga, H., Reissman, P. T., Slamon, D. J., Holmes, E. C., and Minna, J. D. Mutations in the *p53* gene are frequent in primary, resected non-small cell lung cancer. Oncogene, *5:* 1603–1610, 1990.
31. Takahashi, T., Nau, M. M., Chiba, I., Birrer, M. J., Rosenberg, R. K., Vinocour, M., Levitt, M., Pass H., Gazdar, A. F., and Minna, J. D. *p53*: a frequent target for genetic abnormalities in lung cancer. Science (Washington DC), *250:* 491–494, 1990.
32. Herskowitz, I. Functional inactivation of genes by dominant negative mutations. Nature (Lond.), *329:* 219–222, 1987.
33. Kraiss, S., Quaiser, A., Oren, M., and Montenarh, M. Oligomerization of oncoprotein p53. J. Virol., *62:* 4737–4744, 1988.
34. Milner, J., Medcalf, E. A., and Cook, A. C. The tumor suppressor *p53*: analysis of wild type and mutant *p53* complexes. Mol. Cell. Biol., *11:* 12–19, 1991.
35. Milner, J., and Medcalf, E. A. Cotranslation of activated mutant *p53* with wild type drives the wild-type p53 protein into the mutant conformation. Cell, *65:* 765–774, 1991.
36. Malkin, D., Li, F. P., Strong, L. C., Fraumeni, J. F., Nelson, C., Kim, D. H., Kassel, J., Gryka, M. A., Bishoff, F. Z., Tainsky, M. A., and Friend, S. H. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science (Washington DC), *250:* 1222–1238, 1990.
37. Srivastava, S., Zou, Z., Pirollo, K., Blattner, W., and Chang, E. H. Germline transmission of a mutated *p53* gene in a cancer-prone family with Li-Fraumeni syndrome. Nature (Lond.), *248:* 747–749, 1990.

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