Advances in Brief

p53 Gene Mutations Occur in Combination with 17p Allelic Deletions as Late Events in Colorectal Tumorigenesis[1]

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Abstract

Coordinate loss of one copy of the p53 gene and mutation of the remaining copy occur in colorectal carcinomas and in many other human malignancies. However, the prevalence of p53 gene mutations in carcinomas which maintain both parental copies of p53 has not previously been evaluated. Moreover, it is not known whether p53 gene mutations are limited to malignant tumors or whether they can also occur in benign neoplasms. To answer these questions, a total of 58 colorectal tumors have been examined; in each tumor, allelic losses were assessed using restriction fragment length polymorphisms and p53 gene mutations were assessed by sequencing cloned polymerase chain reaction products. The following conclusions emerged: (*a*) p53 gene mutations occurred but were relatively rare in adenomas, regardless of size and whether the adenomas were derived from patients with familial adenomatous polyposis; (*b*) In carcinomas as well as in adenomas, p53 gene mutations were infrequently observed in tumors which contain both copies of chromosome 17p (17% of 30 tumors), while tumors which lost one copy of chromosome 17p usually had a mutation in the remaining p53 allele (86% of 28 tumors); (*c*) p53 gene mutations were found at similar frequencies in primary tumor samples and in cell lines derived from tumors. These and other data suggest that the rate limiting step in p53 inactivation is point mutation and that once a mutation occurs, loss of the remaining wild-type allele rapidly follows. Both mutations and allelic losses generally occur near the transition from benign to malignant growth, and the p53 gene may play a causal role in this progression.

Introduction

The role of the p53 gene in tumorigenesis has evolved considerably in the last decade. The gene product was originally discovered through its association with DNA tumor virus antigens in murine cells (1–3). The gene was subsequently cloned and shown to transform rodent cells *in vitro*, either alone or in cooperation with an activated *ras* gene (4–6). These transfection experiments, together with the demonstration that p53 gene expression was increased in a variety of chemically, virally, and spontaneously transformed human and rodent tumors cells (7–11), all suggested that the p53 gene was an oncogene, much like *myc* or *ras*, and that it could exert its oncogenic effects if the normal ("wild-type") gene was expressed at a high level.

Three convergent lines of evidence have more recently suggested a different role for p53 in neoplasia. First, it was found that in mouse erythroleukemias induced by Friend virus, the integration of the viral genome often resulted in structural alterations of the p53 gene (12, 13). Structural alterations of

p53 genes were also seen in a human leukemia cell line and in a subset of human osteosarcomas (14, 15). Although these structural alterations could potentially "activate" the p53 gene by removing negative growth regulatory components (such as occurs in several oncogenes, *e.g.*, Refs. 16 and 17), the data were also consistent with the idea that these structural alterations inactivated the gene product.

The second line of evidence came from the study of allelic losses in human tumors. Examination of several kinds of tumors (including those of the colon, lung, breast, brain, bladder, and ovary) showed that one copy of the short arm of chromosome 17 was frequently lost (18–26). Detailed study of chromosome 17p in colorectal cancers demonstrated that 17p13.1 was consistently lost in every tumor that lost any part of chromosome 17p (27). According to Knudson's model of suppressor gene action, such allelic losses are thought to indicate the presence of a target suppressor gene within the lost region (28). In such cases, it is presumed that one copy of the gene is deleted through a gross chromosomal event (mitotic recombination, abnormal chromosomal segregation, etc.), while the remaining allele is inactivated by a more localized mutation (missense or nonsense point mutation, small deletion, splice site mutation, etc.) (29). The p53 gene had been previously mapped to 17p13.1 (30) and thus became a candidate for the "target" of the allelic losses. To test the possibility that p53 was the target, the remaining p53 allele of tumors was sequenced, first in colorectal tumors and subsequently in many other tumor types. In nearly all cases, the remaining allele was found to be mutated (27, 31–36). These studies thus indicated that p53 gene mutations/allelic losses were characteristic of those expected to occur in tumor suppressor genes and implied that p53 was more like the retinoblastoma gene than like *myc* or *ras*.

The third line of evidence was functional. It was observed that the p53 genes capable of transformation of rodent cells were in fact not normal but had sustained mutations either *in vivo* or *in vitro* (37, 38). At the time of the original transfection experiments, it was not realized that the cloned p53 genes being used were in fact mutant. Moreover, it was shown that wild-type murine p53 genes actually inhibited, rather than promoted, transformation of rodent cells (39, 40). Finally, it has been recently shown that human wild-type p53 genes (but not mutant human p53 genes) can suppress the growth of human carcinoma cells *in vitro* (41–43).

Thus, accumulating evidence favors the idea that the wild-type p53 gene exerts a restraining influence on cell growth and that mutations of the gene inactivate this restraining capacity. Because p53 proteins oligomerize *in vivo* (44), mutant p53 proteins are thought to bind to and inactivate the wild-type

protein present in cells, thus acting as "dominant negative" mutations (45) when the wild-type allele is still present in the cell.

These studies stimulate many questions, and in this communication we address those relating to the timing and frequency of p53 gene mutations during tumorigenesis. As noted above, most of the carcinomas with 17p allelic deletions studied to date have had p53 gene mutations in the remaining alleles, but the frequency of such mutations in tumors without allelic deletions has not been examined in detail. Moreover, all previous studies on this topic have involved malignant tumors. It is not known whether p53 gene mutations can occur prior to malignancy. One possibility, for example, is that p53 gene mutations occur early in tumorigenesis and that allelic losses of the remaining p53 gene occur only in malignant tumors. These issues were addressed by examination of a panel of benign and malignant colorectal tumors in which chromosome 17p allelic losses and p53 gene mutations were both assessed.

Materials and Methods

Tissue and DNA Preparation. Areas rich in neoplastic cells from primary tumor samples, removed surgically, were carefully separated from areas relatively devoid of neoplastic cells by a cryostat sectioning technique. DNA was directly prepared from these cryostat sections as described previously (19). DNA from cell lines and xenografts (46–49) was purified in an identical fashion.

Allelic Deletion Analysis. To measure allelic deletions, two highly informative probes mapping near p53 were used [pYNZ22.1 (50) and 144-D6 (51)], both after HinfI digestion (31). In the few cases in which these two probes were not informative, additional probes from chromosome 17p were analyzed as described (27).

p53 Gene Mutations. Exons 5–9 of the p53 gene were amplified by the PCR[3] from 500 ng of genomic DNA. PCR was performed in 50-μl reaction volumes using 350 ng each of the primers 5'-GTAGGAATT-CACTTGTGCCCTGACTT-3' from intron 4 and 5'-CATCGA-ATTCTGGAAACTTTCCACTTGAT-3' from intron 9. EcoRI sites were incorporated at the 5' ends of the oligomers to facilitate cloning of PCR products. The concentration of nucleotides, dimethyl sulfoxide, and magnesium were as described previously (52). Thirty-five cycles consisting of 30 s at 95°C, 3 min at 58°C, and 2 min at 70°C were performed. PCR products were purified with phenol-chloroform, cleaved with EcoRI, and separated in a 1.2% agarose gel. The 1.8-kilobase PCR product was purified from the agarose by centrifugation through a Costar Spin-X column (53) and ligated into the EcoRI site of Bluescript SK (Stratagene). Following phenol-chloroform extraction and ethanol precipitation, the ligation reaction was used to transform XL-1 Blue (Stratagene) cells by electroporation. Pools of greater than 100 clones were used to prepare plasmid DNA for double-stranded sequencing. The primers used for sequencing were 5'-GACTTT-CAACTCTGTCTC-3' and 5'-CTGGGGACCCTGGGCAAC-3' for exon 5, 5'-GAGACGACAGGGCTGGTT-3' and 5'-CCACTGA-CAACCACCCTT-3' for exon 6, 5'-CCAAGGCGCACTGGCCTC-3' and 5'-GAGGCAAGCAGAGGCTGG-3' for exon 7, 5'-CCTTACTGC-CTCTTGCTTC-3' and 5'-TGAATCTGAGGCATAACTGC-3' for exon 8, and 5'-TTATGCCTCAGATTCACTTTT-3' for exon 9.

Results

Allelic deletions and p53 gene mutations were assessed as described in "Materials and Methods." In total, we have evaluated 58 colorectal tumors (25 adenomas and 33 carcinomas), including 11 carcinomas and 1 adenoma from earlier work (Table 1).

Previous studies have demonstrated that chromosome 17p

[3] The abbreviation used is: PCR, polymerase chain reaction.

Table 1 *p53 gene mutations and 17p allelic losses in colorectal tumors*

Tumor	Type[a]	No. 17p alleles	Source[c]	Codon	Mutation	Amino acid
AA	A	2	L		None	
AN	A	2	L		None	
BH	A	2	L		None	
S48A	A	2	P		None	
S83 1-5	A	2	P		None	
S100B	A	2	P		None	
S110C	A	2	P		None	
S127A	A	2	P		None	
S137A	A	2	P		None	
S172A	A	2	P		None	
S178A	A	2	P		None	
S181C	A	2	P		None	
S181D	A	2	P		None	
S184B	A	2	P		None	
S184C	A	2	P	175	CGC→CAC	Arg→His
S185A	A	2	P		None	
S192A	A	2	P		None	
VACO 235[b]	A	2	L		None	
VACO 330	A	2	L	248	CGG→TGG	Arg→Trp
RG	A	1	L	282	CGG→TGG	Arg→Trp
S60A	A	1	P	175	CGC→CAC	Arg→His
S102A	A	1	P		None	
S168A	A	1	P	175	CGC→CAC	Arg→His
S181A	A	1	P		None	
S184G	A	1	P	273	CGT→TGT	Arg→Cys
CX2	C	2	X	282	CGG→TGG	Arg→Trp
				283	CGC→TGC	Arg→Cys
CX7[b]	C	2	X, P	281	GAC→GGC	Asp→Gly
CX9	C	2	X		None	
CX11	C	2	X	213	CGA→TGA	Arg→Stop
CX14	C	2	X		None	
CX18	C	2	X		None	
CX19[b]	C	2	X		None	
CX21	C	2	X		None	
CX28	C	2	X		None	
LoVo	C	2	L		None	
RKO[b]	C	2	L		None	
CX1[b]	C	1	X, P	175	CGC→CAC	Arg→His
CX3[b]	C	1	X, P	143	GTG→GCG	Val→Ala
CX4	C	1	X	239	AAC→AGC	Asn→Ser
CX5	C	1	X	248	CGG→TGG	Arg→Trp
CX6[b]	C	1	X	132	AAG→AAC	Lys→Asn
				133	ATG→TTG	Met→Leu
CX20[b]	C	1	X, P	175	CGC→CAC	Arg→His
CX22[b]	C	1	X	175	CGC→CAC	Arg→His
CX26[b]	C	1	X	141	TGC→TAC	Cys→Tyr
CX27	C	1	L	248	CGG→CAG	Arg→Gln
SW480[b]	C	1	L, P	273	CGT→CAT	Arg→His
				309	CCC→TCC	Pro→Ser
JW	C	1	L		None	
SW837[b]	C	1	L	248	CGG→TGG	Arg→Trp
S22A	C	1	P		None	
S67A	C	1	P	211	ACT→GCT	Thr→Ala
S98A	C	1	P	248	CGG→CAG	Arg→Gln
S109A	C	1	P	220	TAT→TGT	Tyr→Cys
S154A	C	1	P	248	CGG→TGG	Arg→Trp
S168A	C	1	P	175	CGC→CAC	Arg→His
S174A	C	1	P	286	GAA→AAA	Glu→Lys
S177A	C	1	P	175	CGC→CAC	Arg→His
S184A	C	1	P	135	TGC→TAC	Cys→Tyr
S190A	C	1	P	196	CGA→TGA	Arg→Stop

[a] A, adenoma; C, carcinoma.
[b] Tumor lines and xenografts studied previously for p53 mutations (9, 13, 27).
[c] P, primary tumor; L, tumor cell line maintained in culture; X, tumor xenograft maintained in nude mice.

allelic deletions are uncommon in adenomas, occurring in less than 10% of the total (19). We studied 19 adenomas which retained both alleles of chromosome 17p. These included 7 adenomas less than 1 cm and 12 adenomas greater than 1 cm. Eight of the adenomas were from familial adenomatous polyposis patients, and 11 were sporadic tumors. Only 2 of the 19 tumors had p53 gene mutations (Table 1). Both of these tumors contained one wild-type allele and one mutant allele.

We next selected examples of the relatively rare class of

adenomas that had allelic losses of chromosome 17p markers; in our collection of 66 adenomas removed at surgery for other colorectal pathology (usually a concurrent carcinoma), only 7 had such an allelic deletion of chromosome 17. In 6 such tumors analyzed, 4 had p53 gene mutations (Table 1).

We also examined carcinomas to determine the relative frequency of p53 gene mutations and their correlations with allelic loss. The results were similar to those found in adenomas. Twenty of 22 one allele carcinomas and 3 of 11 two allele carcinomas analyzed contained p53 gene mutations (Tables 1 and 2). One of the two allele carcinomas (CX2) contained two mutations, one in each of the two alleles. The other 2 two allele tumors (CX7, CX11) were found to contain one wild-type allele and one mutant allele.

There was little difference in p53 mutation frequency between primary tumors and cell lines (maintained in plastic dishes or in nude mice as xenografts). In primary tumors with one allele, for example, 12 of 15 had p53 gene mutations, while 12 of 13 cell lines with one allele had such mutations. Also, the mutations identified in the tumor cell lines were always (5 of 5 cases) identified in the primary tumors from which they were derived when this could be evaluated (Table 1).

One tumor was found in which the timing of p53 gene mutations during tumorigenesis could be directly assessed. This specimen contained a carcinomatous region within an adenoma from which it apparently arose. Cryostat sectioning allowed us to separate the two areas of the tumor. This tumor was derived from a familial adenomatous polyposis patient, so that the tumor presumably contained an inherited mutation of the familial adenomatous polyposis gene. As shown in Fig. 1, two somatic alterations were additionally present in the adenoma region: an allelic loss of the DCC gene on chromosome 18q; and a *ras* gene point mutation. The same two alterations were also present in the carcinoma region, strongly suggesting that the carcinoma actually arose from the adenoma and did not simply develop from an adjacent, independently transformed cell. Both copies of chromosome 17p were present in the adenoma, but the carcinoma had sustained an allelic deletion. Sequence analysis of the p53 gene in the carcinoma sections revealed a point mutation, resulting in the substitution of tyrosine for cysteine at codon 135. This mutation was not found in either allele of the adenoma. Allelic loss and p53 gene mutation therefore both occurred near the transition from adenoma to carcinoma in this tumor.

Discussion

The p53 gene mutations found in this study clustered within the four "hot spots" (Fig. 2) defined in earlier studies (31).

Table 2 *Comparison of mutations with malignancy and allelic loss*

|		Frequency of mutations	P^a
A.	1 allele tumors	24/28 (86)[b]	<0.001
	2 allele tumors	5/30 (17)	
B.	1 allele adenomas	4/6 (67)	<0.02
	2 allele adenomas	2/19 (11)	
C.	1 allele carcinomas	20/22 (91)	<0.001
	2 allele carcinomas	3/11 (27)	
D.	1 allele adenomas	4/6 (67)	>0.1
	1 allele carcinomas	20/22 (91)	
E.	2 allele adenomas	2/19 (11)	>0.1
	2 allele carcinomas	3/11 (27)	

[a] Fisher exact test.
[b] Numbers in parentheses, percentage.

These hot spots correspond to subsets of the four most highly conserved regions of the gene (54). There were 5 mutations in hot spot A (codons 132–145), 8 mutations in hot spot B (codons 171–179), 7 mutations in hot spot C (codons 239–248), and 7 mutations in hot spot D (codons 272–286). The preponderance of mutations in these regions and their evolutionary conservation are consistent with the idea that these four parts of the gene product represent important functional domains.

All of the mutations we observed in colorectal tumors were point mutations, resulting in single base pair changes. Of 32 such mutations, 2 resulted in nonsense codons and the remainder resulted in amino acid substitutions. At the DNA level, the most common change was a C to T transition in the coding or noncoding strand, accounting for 25 of the 32 mutations. Of these 25, 20 occurred at CG sites, far in excess of the representation of this dinucleotide in the genome (55). CG sites are known to be preferential targets for mammalian point mutations, presumably due to the spontaneous deamination of methylated cytosine residues (56). In the p53 gene, some of the CG sites have recently been shown to be methylated *in vivo* (57). An important, though speculative, conclusion from these observations is that the majority of p53 gene mutations in colorectal tumors occur as a result of cellular mistakes; there is no need to invoke carcinogenic insults to explain the pattern of mutations observed. A further discussion of this point can be found in Ref. 58.

Based on the data presented in Tables 1 and 2, alteration of the p53 gene appears to be a late step in the progression of colorectal tumorigenesis. Allelic deletions of chromosome 17p are rarely seen before the carcinoma stage (19), and the presence of p53 gene mutations correlates strongly with the presence of such allelic deletions. Of the one allele tumors examined, 20 of 22 (91%) carcinomas and 4 of 6 (67%) adenomas contained p53 gene mutations (Table 2). The frequency of these mutations was much lower in the two allele tumors, however, with only 3 of 11 (27%) carcinomas and 2 of 19 (11%) adenomas containing p53 gene mutations (Table 2). The positions of mutations in the two allele tumors were similar to those found in one allele tumors. Greater sensitivity is required to detect mutations in surgical samples of two allele tumors because a mutation must be seen above the signal contributed by a second allele as well as above the signal from the two wild-type alleles contributed by contaminating normal tissue. We consider it unlikely, however, that there were many undetected mutations in the two allele tumors due to inadequate sensitivity of our assay, for the following reasons: (a) all of the DNA samples from the primary tumors were purified from an enriched population of neoplastic cells prepared by cryostat sectioning (19). These populations contained a maximum of 30% nonneoplastic cell nuclei; (b) we examined 16 cell lines with two alleles, thus precluding any increased background from contaminating normal tissue; a p53 gene mutation was detected in only 4 of these lines.

Four of 28 (14%) of the tumors which had lost one allele did not contain a mutation in the remaining p53 allele (Table 2). There are two potential explanations for the lack of mutation in these tumors: (a) the remaining allele may have contained a point mutation which inactivated the gene but was outside the previously defined region found to contain the great majority of p53 gene mutations in human tumors (27, 33–37). For example, p53 splice junction mutations have recently been observed in two lung tumors (35), and one of these would not have been detected in our assay. Similarly, a mutation in the regulatory region of the gene could have resulted in decreased

ADENOMA ➡ CARCINOMA
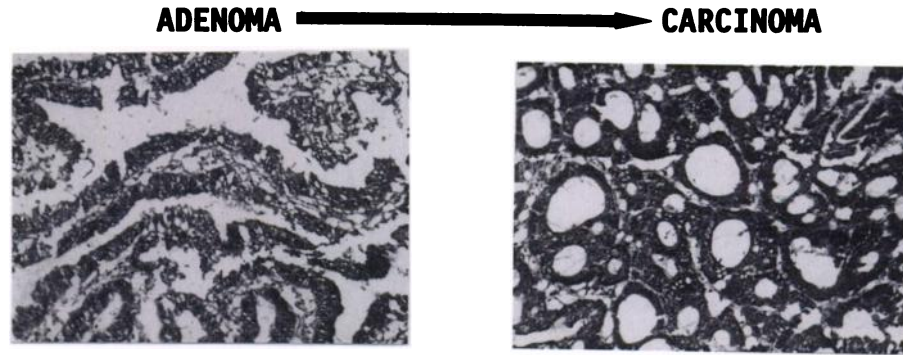
		ADENOMA	CARCINOMA
5q21:	ALLELE 1	MUTANT	MUTANT
	ALLELE 2	PRESENT	PRESENT
K-RAS:	ALLELE 1	ASP^{12}	ASP^{12}
	ALLELE 2	WILD-TYPE	WILD-TYPE
DCC:	ALLELE 1	DELETED	DELETED
	ALLELE 2	PRESENT	PRESENT
p53:	ALLELE 1	WILD-TYPE	TYR^{135}
	ALLELE 2	WILD-TYPE	DELETED

Fig. 1. Rapid selection for p53 gene mutation and allelic loss. A single tumor containing a region of carcinoma arising within an adenoma was carefully sectioned to separate the two areas. The tumor occurred in a patient with familial adenomatous polyposis, so there was presumably a mutation in one 5121 allele (61, 62). Both sections of tumor were identical for K-*ras* and DCC alterations. Both p53 alleles were intact in the adenoma, while the carcinoma had sustained a point mutation and an allelic loss of the p53 gene.
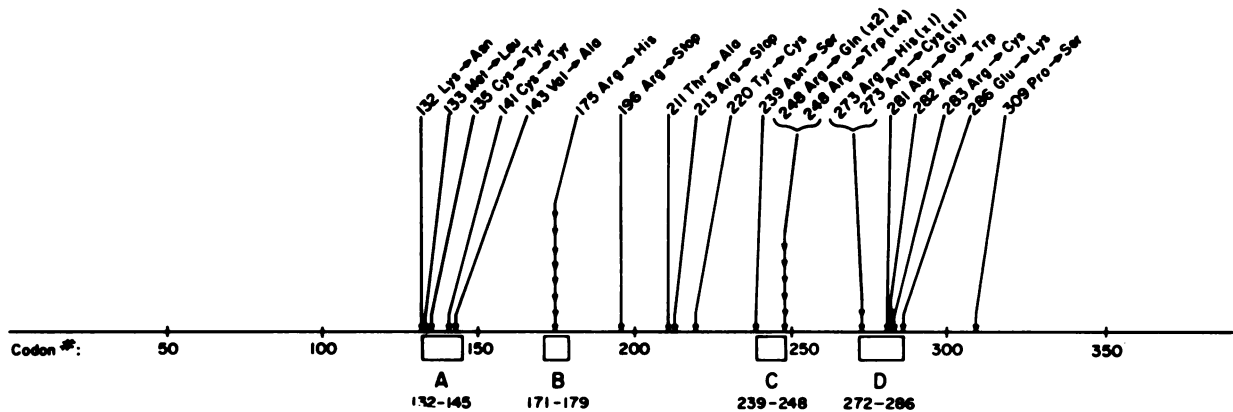

Fig. 2. p53 point mutations cluster in four "hot spots". Each point mutation listed in Table 1 is represented by an *arrowhead*. The *boxes* denote the four hot spots.

or altered transcriptional or translational control of the gene but would not have involved the coding region. Such a mutation would not have been detected; (*b*) it is possible that the p53 gene was not involved in the formation of these tumors. The loss of one copy of chromosome 17 may indicate the presence of a different tumor suppressor gene located on the same chromosomal arm, or a "random" chromosomal loss associated with the increased aneuploidy found in tumors, possibly in connection with an abnormal mitotic event (59, 60).

Allelic loss and p53 gene mutation were usually observed together in our study. It therefore could not be determined if one event consistently preceded the other. We believe, however, that point mutation precedes allelic loss in most cases. A mutation in the p53 gene probably decreases the level of functional protein through a "dominant negative" effect (45). Oligomerization of mutant and wild-type protein may prevent the wild-type protein from interacting with other cellular factors critical for its normal function (44). We suggest that point mutation would thereby create a stronger growth advantage for the cell than that resulting from the simple quantitative decrease in normal protein which would occur if one of two copies of the p53 gene was lost in the absence of point mutation. Once a

p53 gene point mutation occurs, however, it seems that loss of the remaining allele usually follows rapidly. The tumor illustrated in Fig. 1 provides a good example of the close temporal relationship between p53 gene mutation and allelic loss.

This study demonstrates that the p53 gene usually remains wild type throughout the progression from normal mucosa to adenoma development. At some point in tumorigenesis, however, expression of wild-type p53 may become rate limiting for cell growth, perhaps because of other genetic alterations that have accumulated. This hypothesis is supported by *in vitro* studies in which transfection with the wild-type p53 gene suppressed the growth of colorectal carcinoma but not adenoma cells (41). Selection for p53 point mutations and allelic deletions might occur very rapidly at this point and are likely to provide an important contribution to further tumor progression.

References

1. Lane, D. P., and Crawford, L. V. T antigen is bound to a host protein in SV40-transformed cells. Nature (Lond.), *278:* 261–263, 1979.
2. Linzer, D. I. H., and Levine, A. J. Characterization of a 54K dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. Cell, *17:* 43–52, 1979.
3. DeLeo, A. B., Jay, G., Appella, E., Dubois, G. C., Law, L. W., and Old, L. J. Detection of a transformation-related antigen in chemically induced sarcomas and other transformed cells of the mouse. Proc. Natl. Acad. Sci. USA, *76:* 2420–2424, 1979.
4. Eliyahu, D., Raz, A., Gruss, P., Givol, D., and Oren, M. Participation of p53 cellular tumour antigen in transformation of normal embryonic cells. Nature (Lond.), *312:* 646–649, 1984.
5. Parada, L. F., Land, H., Weinberg, R. A., Wolf, D., and Rotter, V. Cooperation between gene encoding p53 tumour antigen and ras in cellular transformation. Nature (Lond.), *312:* 649–651, 1984.
6. Jenkins, J. R., Rudge, K., and Currie, G. A. Cellular immortalization by a cDNA clone encoding the transformation-associated phosphoprotein p53. Nature (Lond.), *312:* 651–654, 1984.
7. Crawford, L. V., Pim, D. C., Gurney, E. G., Goodfellow, P., and Taylor-Papadimitriou, J. Detection of a common feature in several human tumor cell lines—a 53,000-dalton protein. Proc. Natl. Acad. Sci. USA, *78:* 41–45, 1981.
8. Benchimol, S., Pim, D., and Crawford, L. Radioimmunoassay of the cellular protein p53 in mouse and human cell lines. EMBO J., *1:* 1055–1062, 1982.
9. Rotter, V. p53, a transformation-related cellular-encoded protein, can be used as a biochemical marker for detection of primary mouse tumor cells. Proc. Natl. Acad. Sci. USA, *80:* 2613–2617, 1983.
10. Thomas, R., Kaplan, L., Reich, N., Lane, D. P., and Levine, A. J. Characterization of human p53 antigens employing primate specific monoclonal antibodies. Virology, *131:* 502–517, 1983.
11. Koeffler, H. P., Miller, C., Nicolson, M. A., Ranyard, J., and Bosselman, R. A. Increased expression of p53 protein in human leukemia cells. Proc. Natl. Acad. Sci. USA, *83:* 4035–4039, 1986.
12. Mowat, M. A., Cheng, A., Kimura, N., Bernstein, A., and Benchimol, S. Rearrangements of the cellular p53 gene in erythroleukemic cells transformed by Friend virus. Nature (Lond.), *314:* 633–636, 1985.
13. Munroe, D. G., Rovinski, B., Bernstein, A., and Benchimol, S. Loss of a highly conserved domain on p53 as a result of gene deletion during Friend virus-induced erythroleukemia. Oncogene, *2:* 621–624, 1988.
14. Wolf, D. and Rotter, V. Major deletions in the gene encoding the p53 tumor antigen cause lack of p53 expression in HL-60 cells. Proc. Natl. Acad. Sci. USA, *82:* 790–794, 1985.
15. Masuda, H., Miller, C., Koeffler, H. P., Battifora, H., and Cline, M. J. Rearrangement of the p53 gene in human osteogenic sarcomas. Proc. Natl. Acad. Sci. USA, *84:* 7716–7719, 1987.
16. Downward, J., Yarden, Y., Mayes, E., Scrace, G., Totty, N., Stockwell, P., Ullrich, A., Schlessinger, J., and Waterfield, M.D. Close similarity of epidermal growth factor receptor and v-erb-B oncogene protein sequences. Nature (Lond.), *307:* 521–527, 1984.
17. Shore, S. K., Bogart, S. L., and Reddy, E. P. Activation of murine c-abl protooncogene: effect of a point mutation on oncogenic activation. Proc. Natl. Acad. Sci. USA, *87:* 6502–6506, 1990.
18. Fearon, E. R., Hamilton, S. R., and Vogelstein, B. Clonal analysis of human colorectal tumors. Science (Washington DC), *238:* 193–197, 1987.
19. Vogelstein, B., Fearon, E. R., Hamilton, S. R., Kern, S. E., Preisinger, A. C., Leppert, M., Nakamura, Y., White, R., Smits, A. M. M., and Bos, J. L. Genetic alterations during colorectal tumor development. N. Engl. J. Med., *319:* 525–532, 1988.
20. Yokota, J., Wada, M., Shimosato, Y., Terada, M., and Sugimura, T. Loss of heterozygosity on chromosomes 3, 1, and 17 in small-cell carcinoma and on
21. chromosome 3 in adenocarcinoma of the lung. Proc. Natl. Acad. Sci. USA, *84:* 9252–9256, 1987.
21. James, C. D., Carlbom, E., Dumanski, J. P., Hansen, M., Nordensjold, M., Collins, V. P., and Cavenee, W. K. Clonal genomic alterations in glioma malignancy stages. Cancer Res., *48:* 5546–5551, 1988.
22. Muleris, M., Salmon, R. J., Zafrani, B., Girodet, J., and Dutrillaux, B. Consistent deficiencies of chromosome 18 and of the short arm of chromosome 17 in eleven cases of human large bowel cancer: a possible recessive determinism. Ann. Genet., *28:* 206–213, 1985.
23. Delattre, P., Olschwang, S., Law, D. J., Melot, T., Remvikos, Y., Salmon, R. J., Sastre, X., Validire, P., Feinberg, A. P., and Thomas, G. Multiple genetic alterations distinguish distal from proximal colorectal cancer. Lancet, *2:* 353–356, 1989.
24. Mackay, J., Steel, C. M., Elder, P. A., Forrest, A. P. M., and Evans, H. J. Allele loss on short arm of chromosome 17 in breast cancer. Lancet, *2:* 1384–1385, 1988.
25. Devilee, P., van den Broek, M., Kuipers-Dijkshoorn, N., Lolluri, R., Khan, P. M., Pearson, P. L., and Cornelisse, D. J. At least four different chromosomal regions are involved in loss of heterozygosity in human breast carcinoma. Genomics, *5:* 554–560, 1989.
26. Tsai, Y. C., Nichols, P. W., Hiti, A. L., Skinner, D. G., and Jones, P. A. Allelic losses of chromosome 9, 11, and 17 in human bladder cancer. Cancer Res., *50:* 44–47, 1990.
27. Baker, S. J., Fearon, E. R., Nigro, J. M., Hamilton, S. R., Preisinger, A. C., Jessup, J. M., van Tuinen, P., Ledbetter, D. H., Barker, D. F., Nakamura, Y., White, R., and Vogelstein, B. Chromosome 17 deletions and p53 gene mutations in colorectal carcinomas. Science (Washington DC), *244:* 217–221, 1989.
28. Knudson, A. G. Hereditary cancer, oncogenes, and anti-oncogenes. Cancer Res., *45:* 1437–1443, 1985.
29. Hansen, M. F. and Cavenee, W. K. Genetics of cancer predisposition. Cancer Res., *47:* 5518–5527, 1987.
30. vanTuinen, P., Dobyns, W. B., Rich, D. C., Summers, K. M., Robinson, T. J., Nakamura, Y., and Ledbetter, D. H. Molecular detection of microscopic and submicroscopic deletions associated with Miller-Dieker syndrome. Am. J. Hum. Genet., *43:* 587–596, 1988.
31. 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., Modali, R., Harris, C. C., and Vogelstein, B. Mutations in the p53 gene occur in diverse human tumour types. Nature (Lond.), *342:* 705–708, 1989.
32. Menon, A. G., Anderson, K. M., Riccardi, V. M., Chung, R. Y., Whaley, J. M., Yandell, D. W., Farmer, G. E., Freiman, R. N., Lee, J. K., Li, F. P., Barker, D. F., Ledbetter, D. H., Kleider, A., Martuza, R. L., Gusella, J. F., and Seizinger, B. R. Chromosome 17 deletions and p53 gene mutations associated with the formation of malignant neurofibrosarcomas in von Recklinghausen neurofibromatosis. Proc. Natl. Acad. Sci. USA, *87:* 5435–5439, 1990.
33. Iggo, R., Gather, K., Bartek, J., Lane, D., and Harris, A. L. Increased expression of mutant forms of p53 oncogene in primary lung cancer. Lancet, *335:* 675–679, 1990.
34. Takahashi, T., Nau, M. M., Chiba, I., Birrer, J. 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), *246:* 491–494, 1989.
35. Takahashi, T., Damico, D., Chiba, I., Buchhage, D. L., Minna, J. D. Identification of intronic point mutations as an alternative mechanism for p53 inactivation in lung cancer. J. Clin. Invest., *86:* 363–369, 1990.
36. Mulligan, L. M., Matlashewski, G. J., Scrable, H. J., and Cavenee, W. K. Mechanisms of p53 loss in human sarcomas. Proc. Natl. Acad. Sci. USA, *87:* 5863–5867, 1990.
37. Eliyahu, D., Goldfinger, N., Pinhasi-Kimhi, O., Shaulsky, G., Skurnik, Y., Arai, N., Rotter, V., and Oren, M. Meth A fibrosarcoma cells express two transforming mutant p53 species. Oncogene, *3:* 313–321, 1988.
38. Hinds, P., Finlay, C., and Levine, A. J. Mutation is required to activate the p53 gene for cooperation with the ras oncogene and transformation. J. Virol., *63:* 739–746, 1989.
39. Finlay, C. A., Hinds, P. W., and Levine, A. J. The p53 proto-oncogene can act as a suppressor of transformation. Cell, *57:* 1083–1093, 1989.
40. Eliyahu, D., Michalovitz, D., Eliyahu, S., Pinhasi-Kimi, O., and Oren, M. Wild-type p53 can inhibit oncogene-mediated focus formation. Proc. Natl. Acad. Sci. USA, *86:* 8763–8767, 1989.
41. Baker, S. J., Markowitz, S., Fearon, E. R., Willson, J. K. V., and Vogelstein, B. Suppression of human colorectal carcinoma cell growth by wild-type p53. Science (Washington DC), *249:* 912–915, 1990.
42. Mercer, W. E., Shields, M. T., Amin, M., Sauve, G., Appella, E., Ramano, J. W., and Ullrich, S. J. Negative growth regulation in a glioblastoma tumor cell line that conditionally express human wild-type p53. Proc. Natl. Acad. Sci. USA, *87:* 6166–6170, 1990.
43. Diller, L., Kassel, J., Nelson, C. E., Gryka, M. A., Litwak, G., Gebhardt, M., Bressac, B., Ozturk, M., Baker, S. J., Vogelstein, B., and Friend, S. H. p53 functions as a cell-cycle control gene in osteosarcomas. Mol. Cell. Biol., in press, 1990.
44. Kraiss, S., Quaiser, A., Oren, M., and Montenarh, M. Oligomerization of oncoprotein p53. J. Virol., *62:* 4737–4744, 1988.
45. Herskowitz, I. Functional inactivation of genes by dominant negative muta-

tions. Nature (Lond.), *329:* 219–222, 1987.

46. Willson, J. K. V., Bittner, G., Oberley, T. D., Meisner, L., and Weese, J. L. Cell culture of human colon adenomas and carcinomas. Cancer Res., *47:* 2704–2713, 1987.

47. Jessup, J. M., Gizvazzi, R., Campbell, D., Cleary, K., Morikawa, K., and Fidler, I. J. Growth potential of human colorectal carcinomas in nude mice: association with the preoperative serum concentration of carcinoembryonic antigen in patients. Cancer Res. *48:* 1689–1692, 1988.

48. Paraskeva, C., Finerty, S., Mountford, R., and Powell, S. C. Specific cytogenetic abnormalities in two new human colorectal adenoma-derived epithelial cell lines. Cancer Res. *49:* 1282–1286, 1989.

49. Leibovitz, A., Stinson, J. C., McCombs, W. B., McCoy, C. E., Mazur, K. C., and Mabry, N. D. Classification of human colorectal adenocarcinoma cell lines. Cancer Res., *36:* 4562–4569, 1976.

50. Nakamura, Y., Ballard, L., Leppert, M., O'Connell, P., Lathrop, G. M., Lalouel, J. M., and White, R. Isolation and mapping of a polymorphic DNA sequence (pYNZ22) on chromosome 17p [D17S30]. Nucleic Acids Res., *16:* 5707, 1988.

51. Kondoleon, S., Vissing, H., Luo, X. Y., Nagenis, R. E., Kellogg, J., and Litt, M. A hypervariable RFLP on chromosome 17p13 is defined by an arbitrary single copy probe p144-D6 [HGM9NoD17S34]. Nucleic Acids Res., *15:* 10605, 1987.

52. Kogan, S. C., Doherty, M. D., and Gitschier, J. An improved method for prenatal diagnosis of genetic diseases by analysis of amplified DNA sequences. N. Engl. J. Med., *317:* 985–990, 1987.

53. Vogelstein, B. Rapid purification of DNA from agarose gels by centrifugation through a disposable plastic column. Anal. Biochem., *160:* 115–118, 1987.

54. Soussi, T., deFromentel, C. C., Mechali, M., May, P., and Kress, M. Cloning and characterization of a cDNA from *Xenopus laevis* coding for a protein homologous to human and murine p53. Oncogene, *1:* 71–78, 1987.

55. Bird, A. P. DNA methylation and the frequency of CpG in animal DNA. Nucleic Acids Res., *8:* 1499–1504, 1980.

56. Sved, J., and Bird, A. The expected equilibrium of the CpG dinucleotide in vertebrate genomes under a mutation model. Proc. Natl. Acad. Sci. USA, *87:* 4692–4696, 1990.

57. Rideout, W. M., Coetzee, G. A., Olumi, A. F., and Jones, P. A. 5-Methylcytosine as an endogenous mutagen in the human LDL receptor and p53 genes. Science (Washington DC), *249:* 1288–1290, 1990.

58. Sommer, S. S. Mutagen test. Nature (Lond.), *346:* 22–23, 1990.

59. Vogelstein, B., Fearon, E. R., Kern, S. E., Hamilton, S. R., Preisinger, A. C., Nakamura, Y., and White, R. Allelotype of colorectal carcinomas. Science (Washington DC), *244:* 207–211, 1989.

60. Fearon, E. R., and Vogelstein, B. A genetic model for colorectal tumorigenesis. Cell, *61:* 759–757, 1990.

61. Leppert, M., Dobbs, M., Scambler, P., O'Connell, P., Nakamura, Y., Stuaffer, D., Woodward, S., Burt, R., Hughes, J., Gardner, E., Lathrop, M., Wasmuth, J., Lalouel, J.-M., and White, R. The gene for familial polyposis coli maps to the long arm of chromosome 5. Science (Washington DC), *238:* 1411–1413, 1987.

62. Bodmer, W. F., Bailey, C. J., Bodmer, J., Bussey, J. J. R., Ellis, A., Gorman, P., Lucibello, F. C., Murday, V. A., Rider, S. H., Scambler, P., Sheer, D., Solomon, E., and Spurr, N. K. Localization of the gene for familial adenomatous polyposis on chromosome 5. Nature (Lond.), *328:* 614–616, 1987.