Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 3439–3442, April 1992
Medical Sciences

Immune response to p53 is dependent upon p53/HSP70 complexes in breast cancers

(heat shock protein/tumor-suppressor gene/antibody response)

ANDREW M. DAVIDOFF, J. DIRK IGLEHART, AND JEFFREY R. MARKS*

Department of Surgery, Box 3873, Duke University Medical Center, Durham, NC 27710

Communicated by Arnold J. Levine, January 21, 1992 (received for review August 22, 1991)

ABSTRACT **Overexpression of the p53 protein, resulting from gene mutations that increase protein stability, has been detected in >25% of primary human breast cancers. In addition, ≈10% of breast cancer patients have circulating antibodies to the p53 protein. In this study, the anti-p53 humoral response is correlated with the presence and type of mutant p53 protein expressed in the tumor. In a series of 60 breast cancer patients, 0 of 30 tumors with normal, low-level p53 expression induced anti-p53 antibodies, whereas 7 (23%) of 30 tumors with p53 overexpression elicited a specific anti-p53 antibody response. These 7 patients had anti-p53 antibodies that recognized wild-type p53 and a variety of mutant p53 proteins. A comparison of p53 mutations revealed that antibody-negative tumors had mutations exclusively in exons 7 and 8, whereas antibody-positive tumors had mutations primarily in exons 5 and 6. Moreover, all antibody-eliciting tumors contained complexes between p53 and a 70-kDa heat shock protein, whereas none of the antibody-negative tumors contained this complex. This study implicates a 70-kDa heat shock protein in the antigenic presentation of p53.**

Mutations in the p53 tumor-suppressor gene are found at a high frequency in a wide variety of primary human cancers (1–3). The neoplastic potential of these mutant genes has been demonstrated by their efficient transformation of rodent fibroblasts (4). In normal adult tissues and in cancers synthesizing only wild-type p53, the protein is difficult to detect. However, mutant proteins are degraded less rapidly than wild-type p53, which has a half-life of ≈20 min (5, 6). Therefore, the mutant proteins accumulate to relatively high steady-state levels that can be detected readily in primary tumors by immunoblotting or immunohistochemistry. In tumors of epithelial origin such as colorectal, lung, breast, and ovarian cancer, detection of the protein is an accurate indication of the presence of a missense mutation in the p53 gene (7–10).

Deletion of the other p53 allele in many of these tumors results in the loss of wild-type p53 expression (11). While the absence of wild-type p53 appears to be common in human cancers, a mutant p53 gene can transform, in a dominant manner, fibroblasts expressing wild-type p53 (12). Mutant p53 may accomplish this through oligomerization and inactivation of wild-type p53 (13), perhaps by driving the wild-type protein into a mutant conformation (14). The conformation of p53 has been examined by using several criteria, including conformationally sensitive monoclonal antibodies and the ability of p53 to complex with viral and cellular proteins. The wild-type protein forms stable complexes with the simian virus 40-encoded T antigen, whereas many of the mutant proteins are unable to form this complex (15). Conversely, some mutant p53 proteins are observed to complex with two cellular proteins; a 70-kDa heat shock protein (HSP70) identified in rodent fibroblasts as the constitutively expressed member of this family (hsc70) and an unidentified 90-kDa protein (4, 16–18). Mutants that fail to complex with hsc70 still have an extended half-life yet are more weakly transforming *in vitro* than mutants that complex with hsc70 (4).

Breast cancer is typical of human carcinomas with respect to p53 involvement. In a series of >200 primary cancers, 27% showed high-level expression of the p53 protein in the majority of malignant cells, indicative of gene mutation (19). Approximately 10% of breast cancer patients have circulating antibodies directed against the p53 protein (20). In this study, p53 immunogenicity was examined in breast cancer patients with particular regard to the type of p53 mutant being expressed by tumor cells and the ability of these proteins to bind HSP70. Complexes with HSP70 are of particular interest in light of the studies implicating HSPs in protein translocation across intracellular membranes and in antigen presentation (21–23).

MATERIALS AND METHODS

Tissue. Pieces from breast biopsies and mastectomies performed at Duke University Medical Center were collected after surgical removal, immediately flash frozen, and stored at −120°C. Specimens from 60 patients whose tumors were diagnosed as primary invasive breast carcinoma by a member of the Department of Pathology were studied. Thirty of these tumors had been shown, by immunohistochemical analysis in a previous study (19), to overexpress the p53 protein, while 30 expressed normal, low levels of the protein.

Cell Lines. The established cell lines HBL-100, BT-20, BT-474, T-47D, and MDA-MB-468 were obtained from the American Type Culture Collection. HBL-100 is a simian virus 40-transformed human breast epithelial cell line that expresses wild-type p53 (24); BT-20, BT-474, T-47D, and MDA-MB-468 are human breast cancer-derived cell lines that express p53 proteins with mutations at codons 132, 285, 194, and 273, respectively (11, 25). The I3 cell line, obtained from C. A. Finlay (Department of Molecular Biology, Princeton University), is a rat embryo fibroblast line immortalized by the murine mutant p53 clone LTRcG-val^{135} (12). Cells were grown at 37°C in RPMI 1640 supplemented with bovine insulin (10 μg/ml), glutamine (300 μg/ml), and 10% fetal bovine serum. Normal human mammary epithelial cells (HMECs) were collected following reduction mammoplasties and maintained in short-term culture by the methods of Band and Sager (26).

Antibodies. Serum from patients with primary invasive breast cancer was obtained at the time of diagnosis and stored at −20°C. PAb1801 (Ab-2, Oncogene Sciences, Mineola, NY) is a murine anti-p53 monoclonal antibody that reacts specifically with human p53 at an epitope between amino acids 32 and 79 (27). PAb421 is a murine anti-p53 monoclonal antibody that

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

Abbreviations: HSP, heat shock protein; HMEC, human mammary epithelial cell.

*To whom correspondence should be addressed.

reacts with an epitope of mammalian p53 between amino acids 370 and 378 (28) and is produced by a hybridoma cell line obtained from A. J. Levine (Department of Molecular Biology, Princeton University). HSP72/73 (Ab-1, Oncogene Sciences) is a murine monoclonal antibody that reacts with HSP70 in mammalian cells (clone W27, E. Harlow). Rabbit antiserum directed against human C-terminal epitopes representing the last 21 amino acids of at least the HSC70 and HSP70 members of the HSP family (17) was obtained from P. W. Hinds (Department of Molecular Biology, Whitehead Institute). TA-1, an anti-HER2/neu murine IgG1 monoclonal antibody (DuPont), normal human serum, and serum from 15 patients with anti-nuclear antibodies associated with autoimmune disorders (obtained from J. D. Keene, Department of Microbiology, Duke University) were used as control antibodies.

Sequence Analysis. Sequencing of the highly conserved region of the p53 gene from mRNA was performed as described (9). Briefly, 1 μg of total RNA was used as a template for first-strand cDNA synthesis by murine leukemia virus reverse transcriptase (Bethesda Research Laboratories) using an antisense oligonucleotide from p53 exon 10 as a primer. Exons 4–10 were then amplified by the polymerase chain reaction (PCR) with an oligonucleotide primer from exon 4 and *Taq* DNA polymerase (Promega). The 712-base-pair product of this reaction was purified by gel electrophoresis, reamplified, purified again by filtration through a Sepharose CL-6B (Pharmacia) spin column, ethanol-precipitated, and resolubilized in water. This material was the template for dideoxy sequencing with Sequenase 2.0 (United States Biochemical). Oligonucleotides flanking each of the exons 5, 6, 7, and 8 were used to prime the reactions, which were performed by first boiling the primer/template mix, labeling on ice for 10 min with [α-^{32}P]dATP, and then running the termination reactions at 45°C for 10 min. The products were electrophoresed in a polyacrylamide gel, which was then soaked in 10% acetic acid/12% methanol, dried, and set with Kodak XAR film.

Immunoprecipitation. Cell lines were metabolically labeled with [^{35}S]methionine (50 μCi/ml; Amersham; 1 μCi = 37 kBq) for 2 hr. At the end of the labeling period, cells were scraped, homogenized in lysis buffer (50 mM Tris, pH 8.0/5 mM EDTA/150 mM NaCl/0.5% Nonidet P-40/1 mM phenylmethylsulfonyl fluoride), and then sonicated for 10 sec at 5 W/sec. The supernatants were collected following centrifugation at 100,000 × *g* and preadsorbed with protein G-Sepharose (Pharmacia) for 1 hr at 4°C. Supernatants were collected following centrifugation at 12,000 × *g*, and incorporation was quantitated by trichloroacetic acid precipitation, followed by scintillation counting. Immunoprecipitation of p53 was performed from 5 × 10^6 acid-precipitable cpm from each cell line. The lysates were incubated at 4°C for 1 hr with 1 μl of human serum or 0.1 μg of a monoclonal antibody. The immune complexes were then bound with 20 μl of protein G-Sepharose (4°C, 2 hr). The Sepharose was washed three times with lysis buffer, and the samples were denatured by boiling for 5 min in an equal volume of 100 mM Tris, pH 6.8/4% SDS/0.2% bromophenol blue/20% glycerol/50 mM 2-mercaptoethanol and loaded onto an SDS/10% polyacrylamide gel. After electrophoresis, the protein was transferred to a nitrocellulose membrane (Schleicher & Schuell) by electroblotting at 400 mA for 2 hr. The membrane was dried and set with Kodak XAR film overnight.

Immunoblotting. Protein extraction from unlabeled cell pellets and frozen tissues was performed as described above except that tissues were homogenized with a Polytron (Brinkmann). Unlabeled total protein (250 μg) (2 mg for HMECs and NIH 3T3 cells) was incubated with patient sera or monoclonal antibodies, and the recovered protein was electrophoresed in an SDS/polyacrylamide gel and then transferred to a nitrocellulose membrane as described above. The membrane was then treated in a blocking solution [3% bovine serum albumin (BSA)/0.2% Tween 20/0.02% NaN_3/1 mM NaI/phosphate-buffered saline (PBS)] overnight and probed for 90 min at 37°C with PAb421 supernatant diluted 1:3 in 5% BSA. After washing with PBS, the blot was incubated with biotinylated goat anti-mouse F(ab')$_2$ (Tago) in 5 ml of 5% BSA for 1 hr. After a final wash in PBS, antibody binding was visualized with an avidin-conjugated immunoperoxidase detection system (Vector Laboratories).

Coimmunoprecipitation of p53 and HSP70 in samples from patients with primary invasive breast cancer was performed by first reacting protein extracts (250 μg) from tumor tissues with either an anti-p53 antibody or an anti-HSP70 antibody. Four immunoprecipitation reactions, two with each antibody, were performed on protein extracts from each tissue. To detect HSP70 in the complex, immunoprecipitation was performed with 0.1 μg of the murine monoclonal antibodies PAb1801 and HSP72/73. To detect complexed p53, immunoprecipitation was performed with one of the human anti-p53 antisera, s11, and the rabbit anti-HSP70 antiserum. These immunoprecipitates were electrophoresed and blotted as described above. Probing for HSP70 was performed with 10 μl of rabbit anti-HSP70 serum in 5% BSA. Probing for p53 was performed with 1.0 μg of PAb1801. Heterologous species antisera were used for these immunoprecipitations because the mouse monoclonals themselves are detected as ≈50-kDa proteins with the biotinylated goat anti-mouse IgG detection system. Binding was detected with biotinylated goat anti-rabbit (Vector Laboratories) or anti-mouse as appropriate; visualization was again performed with an immunoperoxidase detection system.

RESULTS

Detection of Anti-p53 Antibodies. Sera collected in the perioperative period from 30 patients whose breast cancer expressed high levels of p53 and from 30 patients whose tumor expressed no detectable p53 protein were screened for anti-p53 antibodies. One microliter of each serum was reacted with metabolically labeled protein lysate from the established human breast cancer cell line BT-20. This cell line expresses high levels of a p53 protein harboring a mutation at codon 132 (25). Sera from 7 patients with tumors that overexpressed p53 contained antibodies that immunoprecipitated a 53-kDa protein which comigrated with p53 immunoprecipitated with the monoclonal antibody PAb1801 (Fig. 1). Dilutional analysis of these sera showed >10-fold variation in their reactivity, with the strongest ones comparable to the immunoglobulin affinity-purified pAB1801. No band corresponding to a protein of this molecular mass was immunoprecipitated by any of the sera from patients with p53-negative tumors. In addition, no 53-kDa protein was recovered from the BT-20 lysate with sera from 15 patients with autoimmune disorders who were known to have anti-nuclear antibodies.

Immunoblotting was performed to confirm that each of these seven sera recognized the p53 protein. Unlabeled protein extracts were immunoprecipitated with each sera, electrophoresed, transferred to a solid support, and then probed with PAb421. The specific recovery of a 53-kDa protein detected by the p53 monoclonal antibody in each case confirmed the identity of the immunoprecipitated protein (Fig. 2). Immunoprecipitation from different established human and murine cell lines revealed that each of the antisera recognized a wide range of p53 proteins, both mutant and wild-type (Fig. 2).

p53 Mutations Correlate with Immune Response. Several possibilities could explain why only a subset of breast cancers expressing mutant p53 proteins elicit p53 antibodies. The amount of mutant protein may be highly variable and, after cell death, attain immunogenicity only in those tumors with

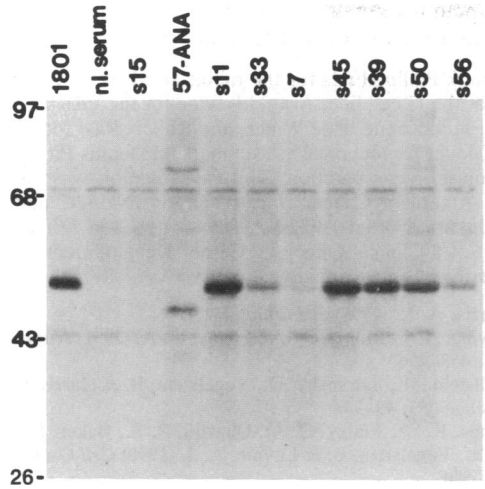

FIG. 1. Screening of sera from patients with breast cancer for anti-p53 antibodies by immunoprecipitation of metabolically labeled protein extracts from the breast cancer cell line BT-20 with PAb1801 (1801), normal human serum (nl serum), serum from a breast cancer patient whose tumor did not overexpress p53 protein (s15), serum from a patient with other (undefined) anti-nuclear antibodies (57-ANA), and serum from breast cancer patients whose tumors expressed high levels of mutant p53 protein (s11, s33, s7, s45, s39, s50, and s56). Molecular size markers (kDa) are at left.

high levels. However, by immunohistochemistry no major differences in the levels of p53 expression were observed in these tumors (data not shown). Alternatively, only certain mutant p53 proteins may be immunogenic even though the antibodies themselves are not mutant-specific. To test this, direct sequencing of PCR-amplified p53 cDNA was performed from 15 breast cancers overexpressing the protein. In each case, a mutation was found in a highly conserved region of the gene that altered the coding sequence of the protein (Table 1). A generally consistent clustering of these mutations was observed. Each patient who had not mounted an antibody response to p53 had a tumor that contained a mutation in exon 7 or 8 of the p53 gene. Conversely, five of seven patients who were antibody-positive had tumors with mutations in exon 5 or 6. The two exceptions were patients

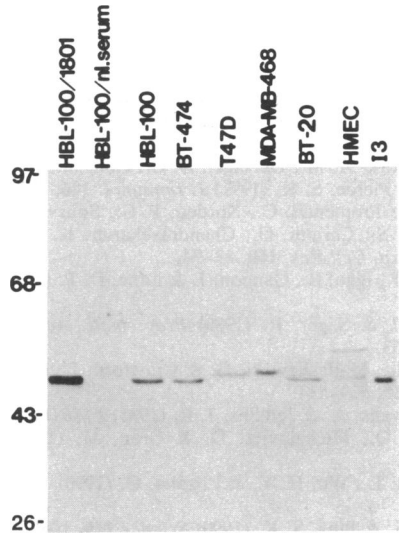

FIG. 2. Recovery of wild-type p53 and a variety of mutant p53 proteins with serum from breast cancer patient s11. All seven antibody-positive sera recognized this panel of diverse p53 proteins, in addition to wild-type murine p53 from NIH 3T3 cells (data not shown).

Table 1. Characterization of mutant p53 proteins with respect to the site of gene mutation, immunogenicity, and the ability to complex with HSP70 in 15 breast cancers

Patient	Mutation: Sequence (amino acid)	Mutation: Codon (exon)	Anti-p53 antibodies	HSP binding
7	TAT (Y) → TGT (C)	205 (6)	+	+
11	TAC (Y) → TGC (C)	163 (5)	+	+
33	TGT (C) → CGT (R)	275 (8)	+	+
39	CGC (R) → CAC (H)	175 (5)	+	+
45	TGT (C) → CGT (R)	238 (7)	+	+
50	Deletion	174–181 (5)	+	+
56	CGC (R) → CAC (H)	175 (5)	+	+
3	CCT (P) → GCT (A)	278 (8)	–	–
12	CGT (R) → TGT (C)	273 (8)	–	–
13	CGG (R) → CAG (Q)	248 (7)	–	–
17	CGG (R) → CTG (L)	282 (8)	–	–
24	ATC (I) → AAC (N)	254 (7)	–	–
43	ATG (M) → ATA (I)	237 (7)	–	ND
55	TAC (Y) → TGC (C)	234 (7)	–	–
63	GGC (G) → GAC (D)	245 (7)	–	–

ND, not done (insufficient tissue).

33 and 45. Their tumors contained mutations at codons 275 (exon 8) and 238 (exon 7), and yet the patients mounted strong immune responses. In both cases, the mutations changed a cysteine residue that might be predicted to alter the long-range tertiary structure of the protein.

Mutant Proteins That Induce an Antibody Response Form a Complex with HSP70. A similar clustering is evident in the human and murine mutant p53 proteins that have been analyzed for HSP binding in tissue culture cells. p53 proteins containing mutations in exon 5 bind to a HSP70, while several proteins with mutations located in exon 8 fail to complex (4, 29). Given the possible function of an HSP70 in antigen presentation, it was of interest to determine whether the immunogenic p53 mutants formed complexes with this HSP *in vivo*. Protein extracts from 14 of the 15 tumors containing p53 mutations were immunoprecipitated with anti-p53 and anti-HSP antibodies. After blotting, separate membranes were probed with anti-p53 and anti-HSP antibodies in order to detect coprecipitation of these proteins. Tumor tissue from all seven patients with circulating p53 antibodies contained coprecipitating p53 and HSP70 (Fig. 3). Conversely, no p53/HSP complexes were detected in tissues from the seven patients lacking detectable p53 antibodies. Tissues that did not contain p53/HSP70 complexes had levels of both p53 and HSP70, as assessed by immunoblotting and immunohistochemistry, similar to those in tissues that did contain the complex, so it does not appear to be an absence of either component that explains the lack of the complex. Since there

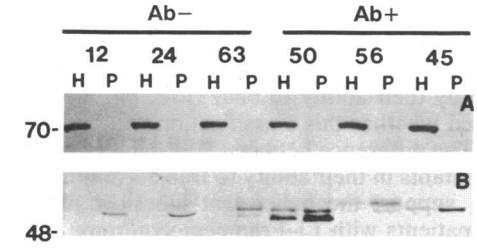

FIG. 3. Coimmunoprecipitation of p53 and HSP70 only in tumors from patients with anti-p53 antibodies. Protein extracts from tumor tissues were immunoprecipitated with an anti-p53 antibody (P) or anti-HSP70 antibody (H) and then probed with rabbit anti-HSP70 antiserum (*A*) and PAb1801 (*B*). Ab–, anti-p53 antibody-negative patients 12, 24, and 63; Ab+, anti-p53 antibody-positive patients 50, 56, and 45.

are several species of HSP70 (i.e., hsp, hsc, and hsx) that are not distinguished by these antisera, it is possible that tissues which lack the complex do not synthesize the appropriate member of the heat shock family. Different mutant p53 proteins have been shown, in rodent fibroblasts, to have different abilities to complex with hsc70 (4, 29), suggesting that a property intrinsic to p53 is responsible for complex formation in these breast cancers.

DISCUSSION

These results suggest that mutant p53 proteins which complex with HSP70 in primary breast cancers induce a p53-specific humoral response. The pathway through which this immune response is elicited is not clear; however, several interesting possibilities are raised. Antibodies found in these patients recognize both wild-type p53 and a variety of different mutant species. Therefore, it is unlikely that an intrinsic antigenic conformation of p53 is responsible for the response. Additionally, steady-state levels of the different p53 mutants in the tumor cells do not appear to be a primary determinant for antigenicity. Based upon the activities of HSP70 species, two mechanisms may be envisioned for presentation of mutant p53 proteins to the immune system. (*i*) In yeast, HSP70 species have been shown to facilitate secretory protein translocation that may be independent of signal-recognition particle (21, 22). We have been unable to detect p53 protein in the serum of either antibody-positive or antibody-negative patients by immunoblotting (data not shown). Particularly in the antibody-positive patients, if the protein were secreted, it would most likely be rapidly cleared by the high-titer antibody. (*ii*) A 72/74-kDa peptide-binding protein that plays a role in antigen processing and presentation was recently shown to be a constitutively expressed member of the HSP70 family (23). Association of p53 with this protein could present p53 to the immune system in the tumor cell itself. If this is the case, the immune response may potentially have an impact on the growth and spread of the tumor. It remains to be determined whether, in addition to the humoral response, a cytotoxic cellular response is also present in these patients.

Our findings suggest a method for discriminating two classes of p53 mutations in human tumors. Several lines of evidence support the idea that this may be a functionally important way to categorize mutations. p53 mutations that bind HSP are more potent dominant transforming genes in conjunction with *ras* (4). It is possible that the p53/HSP complex facilitates p53-mediated transformation by sequestration of the wild-type tumor-suppressor protein, perhaps more avidly than mutant p53 proteins that fail to complex with HSP. There also appears to be a difference in the ability of mutant p53 proteins to function in transcriptional activation. A GAL4–p53 fusion protein containing a mutation at codon 273 (human) retains this function, whereas a mutation at codon 135 (murine) destroys the ability to transactivate a GAL4 target sequence (30, 31).

While the biologic activity of different mutant p53 genes can be assessed *in vitro*, parallel experiments are difficult to carry out *in vivo*. p53 mutations found in cancers can now be classified by their ability to bind HSP, but it remains to be determined whether this classification will be clinically significant. The difference between HSP-binding and -nonbinding p53 mutants in their ability to transform fibroblasts may, however, support the belief that inherited p53 mutations found in patients with Li–Fraumeni syndrome result in less potent oncogenes. These mutations appear to cluster around codon 250 of the p53 gene (32, 33) and would therefore be predicted not to bind HSP and, perhaps, to be less oncogenic. Patient 63 from this study contained a mutation identical to one found in a Li–Fraumeni family (codon 245, Gly → Asp) and did not bind HSP in the breast cancer. This might explain why many of these patients can reach young adulthood before manifesting the characteristic malignancies of the syndrome despite having germ-line p53 mutations.

We thank Philip Hinds for the rabbit anti-HSP70 antisera; Cathy Finlay for the I3 cell line; Arnold Levine for the PAb421 hybridoma cell line; Jack Keene, Eric Winer, and Robert Bast for patient sera; Nancy Glover for technical assistance; and Dennis Pittman for help with manuscript preparation. This investigation was supported in part by American Cancer Society Grant IN-158C and National Cancer Institute Grant 1 F32 CA08899-01 (to A.M.D.). J.D.I. is the recipient of a Clinical Oncology Career Development Award from the American Cancer Society.

1. Harris, A. L. (1990) *J. Pathol.* **162,** 5–6.
2. Levine, A. J., Momand, J. & Finlay, C. A. (1991) *Nature (London)* **351,** 453–456.
3. Hollstein, M., Sidransky, D., Vogelstein, B. & Harris, C. C. (1991) *Science* **253,** 49–53.
4. Hinds, P. W., Finlay, C. A., Quartin, R. S., Baker, S. J., Fearon, E. R., Vogelstein, B. & Levine, A. J. (1990) *Cell Growth Differ.* **1,** 571–580.
5. Reich, N. C., Oren, M. & Levine, A. J. (1983) *Mol. Cell. Biol.* **3,** 2143–2150.
6. Finlay, C. A., Hinds, P. W., Tan, T. H., Eliyahu, D., Oren, M. & Levine, A. J. (1988) *Mol. Cell. Biol.* **8,** 531–539.
7. Rodrigues, N. R., Rowan, A., Smith, M. E., Kerr, I. B., Bodmer, W. F., Gannon, J. V. & Lane, D. P. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 7555–7559.
8. Iggo, R., Gatter, K., Bartek, J., Lane, D. & Harris, A. L. (1990) *Lancet* **335,** 675–679.
9. Davidoff, A. M., Humphrey, P. A., Iglehart, J. D. & Marks, J. R. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 5006–5010.
10. Marks, J. R., Davidoff, A. M., Kerns, B. J., Humphrey, P. A., Pence, J. C., Dodge, R. K., Clarke-Pearson, D. L., Bast, R. C. & Berchuck, A. (1991) *Cancer Res.* **51,** 2979–2984.
11. 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. (1989) *Nature (London)* **342,** 705–708.
12. Hinds, P., Finlay, C. & Levine, A. J. (1989) *J. Virol.* **63,** 739–746.
13. Kraiss, S., Quaiser, A., Oren, M. & Montenarh, M. (1988) *J. Virol.* **62,** 4737–4744.
14. Milner, J. & Medcalf, E. A. (1991) *Cell* **65,** 765–774.
15. Braithwaite, A. W., Sturzbecher, H. W., Addison, C., Palmer, C., Rudge, K. & Jenkins, J. R. (1987) *Nature (London)* **329,** 458–460.
16. Pinhasi-Kimhi, O., Michalovitz, D., Ben Zeev, A. & Oren, M. (1986) *Nature (London)* **320,** 182–184.
17. Hinds, P. W., Finlay, C. A., Frey, A. B. & Levine, A. J. (1987) *Mol. Cell. Biol.* **7,** 2863–2869.
18. Sturzbecher, H. W., Chumakov, P., Welch, W. J. & Jenkins, J. R. (1987) *Oncogene* **1,** 201–211.
19. Davidoff, A. M., Herndon, J. E., Kerns, B. J., Pence, J. C., Iglehart, J. D. & Marks, J. R. (1991) *Surgery* **110,** 259–264.
20. Crawford, L. V., Pim, D. C. & Bulbrook, R. D. (1982) *Int. J. Cancer* **30,** 403–408.
21. Deshaies, R. J., Koch, B. D., Werner-Washburne, M., Craig, E. A. & Schekman, R. (1988) *Nature (London)* **332,** 800–805.
22. Chirico, W. J., Waters, M. G. & Blobel, G. (1988) *Nature (London)* **332,** 805–810.
23. VanBuskirk, A. M., DeNagel, D. C., Guagliardi, L. E., Brodsky, F. M. & Pierce, S. K. (1991) *J. Immunol.* **146,** 500–506.
24. Caron de Fromentel, C., Nardeu, P. C., Soussi, T., Lavialle, C., Estrade, S., Carloni, G., Chandrasekaran, K. & Cassingena, R. (1985) *Exp. Cell Res.* **160,** 83–94.
25. Bartek, J., Iggo, R., Gannon, J. & Lane, D. P. (1990) *Oncogene* **5,** 893–899.
26. Band, V. & Sager, R. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 1249–1253.
27. Banks, L., Matlashewski, G. & Crawford, L. (1986) *Eur. J. Biochem.* **159,** 529–534.
28. Wade-Evans, A. & Jenkins, J. R. (1985) *EMBO J.* **4,** 699–706.
29. Halevy, O., Michalovitz, D. & Oren, M. (1990) *Science* **250,** 113–116.
30. Raycroft, L., Wu, H. Y. & Lozano, G. (1990) *Science* **249,** 1049–1051.
31. Fields, S. & Jang, S. K. (1990) *Science* **249,** 1046–1049.
32. Srivastava, S., Zou, Z. Q., Pirollo, K., Blattner, W. & Chang, E. H. (1990) *Nature (London)* **348,** 747–749.
33. Malkin, D., Li, F. P., Strong, L. C., Fraumeni, J. F., Jr., Nelson, C. E., Kim, D. H., Kassel, J., Gryka, M. A., Bischoff, F. Z. & Tainsky, M. A. (1990) *Science* **250,** 1233–1238.