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p53 transformation-related protein: Detection by monoclonal antibody in mouse and human cells

(intranuclear antigen/cellular proliferation/contact inhibition)

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ABSTRACT **A transformation-related protein of M_r 53,000, designated p53, has been detected in a range of neoplastic cell types of the mouse by using immunoprecipitation of [^{35}S]-methionine-labeled cell extracts with mouse antiserum [DeLeo, A. B., Jay, G., Appella, E., DuBois, G. C., Law, L. W. & Old, L. J. (1979) *Proc. Natl. Acad. Sci. USA* 76, 2420–2424]. We have now prepared a monoclonal antibody to p53 and have used it to study the occurrence and intracellular location of p53 by indirect immunofluorescence assays. In accordance with the results of immunoprecipitation, these tests showed p53 in all 13 transformed mouse cell lines studied. In each case, p53 was found in the nucleus. No p53 was detected in normal mouse fibroblasts, 3T3 cells, bone marrow cells, thymus cells, or embryo cells. A serologically related protein was detected in the nucleus of human cells by monoclonal antibody and was found in both normal and neoplastic cultured cells. Expression of p53 in human cells correlates with the growth characteristics of the culture, high p53 levels being associated with rapid cell proliferation and low p53 levels, with cessation of cell division. Normal and malignant human cells differ, however, with regard to the effect of confluency on p53 expression. Normal kidney epithelium and fetal brain cells, which express high p53 levels during exponential growth, show a prompt decrease in p53 associated with contact inhibition of cell division. Malignant cells, on the other hand, continue to express p53 after confluency and subsequent overgrowth of the monolayers. These results suggest that p53 may be involved in the normal regulation of cell division and that malignant transformation leads to abnormalities in the control of p53 expression.**

When transformed by viruses, chemicals, or x-ray, malignant cells of the mouse express a cellular protein with a M_r of 53,000 (1, 2). This component, designated p53, was identified in [^{35}S]methionine-labeled cell extracts by immunoprecipitation with syngeneic antisera prepared against methylcholanthrene-induced sarcoma cells. Detectable levels of p53 were not found in a broad range of normal cell types, including mouse embryo cells, adult fibroblasts, spleen cells, and bone marrow cells. Extracts of normal thymus, however, were found to express p53. Lane and Crawford (3), Linzer and Levine (4), and Melero *et al.* (5) identified proteins with related characteristics in their studies of simian viruses 40 (SV40)-transformed cells. By virtue of its affinity for SV40 tumor antigen, p53 is coprecipitated from extracts of SV40-transformed cells by anti-tumor antigen antibody. A M_r 53,000 protein has also been detected by Luka *et al.* (6) in transformed B cells of human origin but not in non-transformed B cells. In cell lines carrying the Epstein–Barr virus-directed EBNA antigen, the M_r 53,000 component forms a complex with EBNA.

In this report, we characterize a monoclonal antibody to mouse p53 by immunoprecipitation and immunofluorescence (IF) assays. This antibody identifies a serologically related component in human cells, and this has permitted us to survey normal and malignant human cells for p53 expression.

MATERIALS AND METHODS

Cells. For derivation and culture of mouse cells, see refs. 1, 7, and 8. The cultured human cells are described in refs. 9 and 10. The cytotoxic T-cell lines of mouse and human origin were provided by Michael Palladino and Alexander Knuth.

p53 Monoclonal Antibody. This antibody was prepared according to the technique of Köhler and Milstein (11) as described in ref. 12. Spleen cells for fusion came from a (C57BL/6 × BALB/c)F_1 mouse shown to have p53 precipitating antibody after hyperimmunization with BALB/c methylcholanthrene-induced sarcoma CMS4.

Indirect IF Assays. Target cells were harvested from subconfluent cultures by trypsinization, washed, and plated (200 μl, containing 0.4×10^4 cells, per well) on LAB-TEK no. 4808 tissue culture slides (VWR Scientific). At varying intervals after plating, the cells were washed in phosphate-buffered saline, fixed in methanol/acetone, 1:1 (vol/vol), at −20°C for 5 min, and air dried for 20 min. To each well was added 100 μl of mouse serum containing p53 monoclonal antibody (diluted 1:50 or 1:100) and the slides were incubated for 60 min at 24°C. After removal of mouse serum by washing, the cells were treated with a 1:15 dilution of fluorescein isothiocyanate-conjugated F$(ab')_2$ fragments of rabbit Ig anti-mouse Ig (Cappel Laboratories, Cochranville, PA) for 45 min at 24°C, washed, and examined by fluorescence microscopy (Leitz).

[^{35}S]Methionine Labeling, Immunoprecipitation, NaDodSO$_4$/Polyacrylamide Gel Electrophoresis, and Tryptic Peptide Analysis. See refs. 1 and 13.

RESULTS

Selection of a Hybridoma Line Producing p53 Monoclonal Antibody. Supernatants from wells containing hybrids between NS-1 myeloma cells and spleen cells from mice producing p53 antibody were screened for p53-precipitating activity by using [^{35}S]methionine-labeled Meth A sarcoma extracts. Cells from wells with p53 reactivity were cloned; one line (200-47), secreting an IgG2a, continued to produce p53 antibody after repeated cloning. The sera of *nu/nu* (Swiss) mice and normal (BALB/c × C57BL/6)F_1 mice growing the 200-47 clone precipitated a M_r 53,000 component from Meth A cells (Fig. 1). Tryptic peptide maps of the p53 components precipitated by conventional p53 antiserum and by p53 monoclonal antibody were identical (Fig. 2).

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Abbreviations: SV40, simian virus 40; IF, immunofluorescence; MuSV, murine sarcoma virus; TCGF, T-cell growth factor.

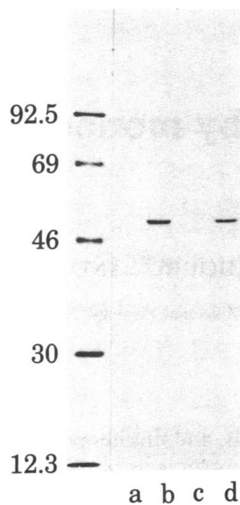

FIG. 1. Autoradiogram of [^{35}S]methionine-labeled proteins immunoprecipitated from extracts of BALB/c Meth A sarcoma and analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis. Lanes: a and c, normal mouse serum; b, conventional p53 antiserum [(BALB/c × C57BL/6)F_1 anti-BALB/c sarcoma CMS4]; d, p53 monoclonal antibody [serum of *nu/nu* (Swiss) mouse growing hybridoma clone 200-47]. M_r markers are shown at left, × 10^{-3}.

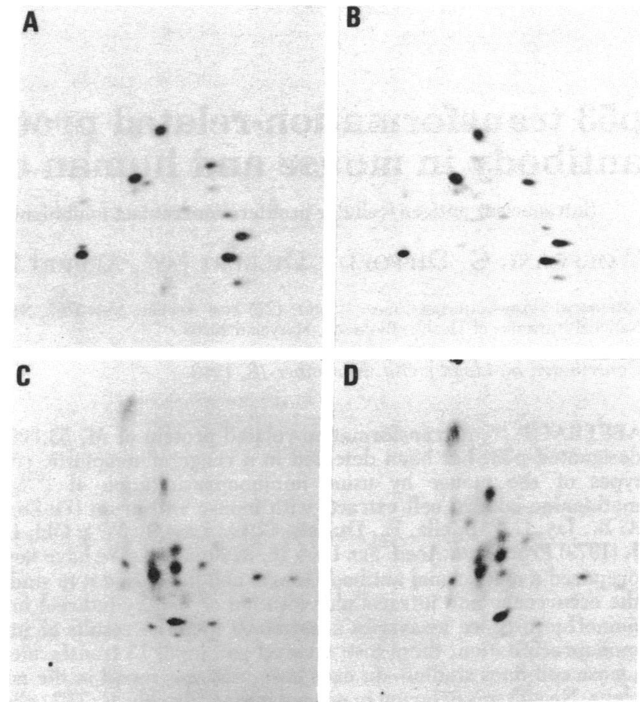

FIG. 2. Tryptic peptide maps of p53 from [^{35}S]methionine-labeled Meth A extracts (*A* and *B*) and [^{3}H]leucine-labeled Meth A extracts (*C* and *D*) identified by p53 monoclonal antibody (*A* and *C*) or conventional p53 antibody (*B* and *D*).

Reactions of p53 Monoclonal Antibody with Normal and Transformed Mouse Cells in Indirect IF Tests. Fig. 3 illustrates the results of IF tests with SV40-transformed 3T3 cells and nontransformed 3T3 cells. Strong nuclear fluorescence was seen in SV40-transformed cells; no fluorescence was found in nontransformed 3T3 cells. Various other cell types were tested for reactivity with the p53 monoclonal antibody by IF (Table 1). Table 1 also summarizes the results of p53 tests using immunoprecipitation with conventional antiserum or monoclonal antibody. All transformed cell lines examined showed p53 reactivity by IF and immunoprecipitation. Cell lines tested included six independently derived methylcholanthrene-induced sarcomas of BALB/c or C57BL/6 origin, BALB/c 3T3 lines transformed by SV40, Moloney or Kirsten murine sarcoma virus (MuSV), a line of spontaneously transformed BALB/c fibroblasts (CSF), and three x-ray-induced leukemias [ERLD (C57BL/6), RADA1 (A strain), and RL♂1 (BALB/c)]. In each case, fluorescence was confined to the nucleus and the reactions

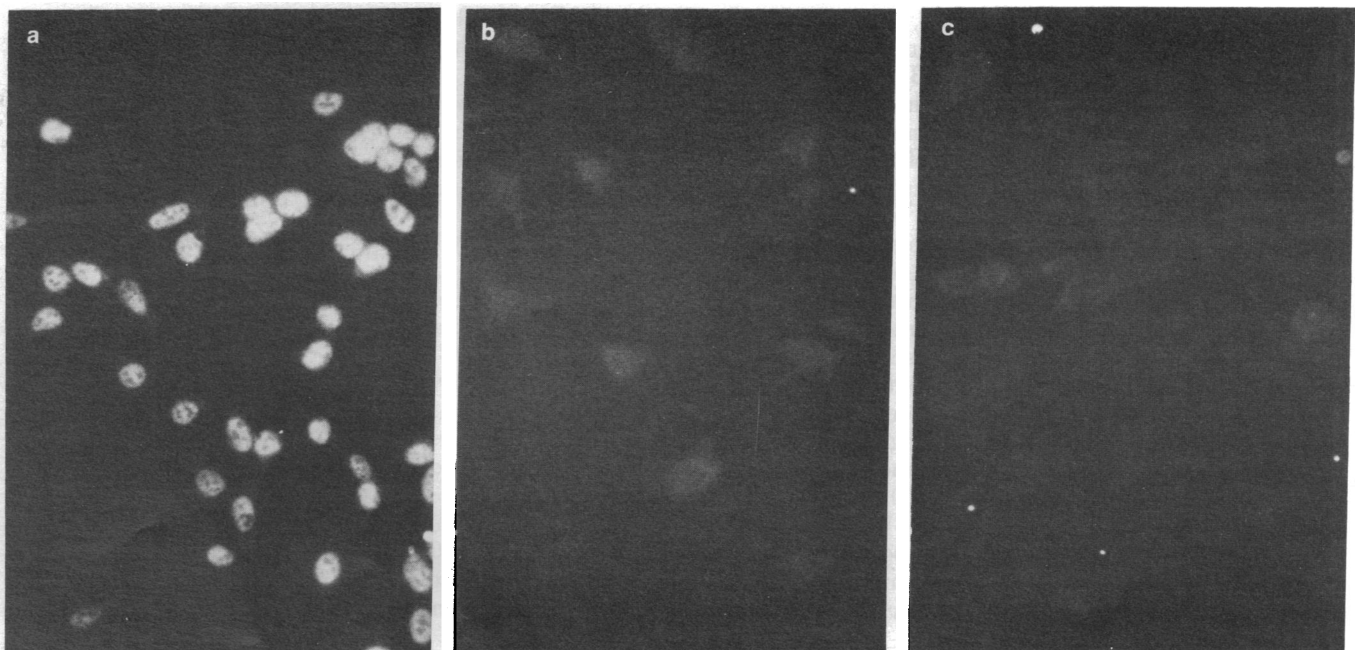

FIG. 3. Reactions of p53 monoclonal antibody in indirect IF tests. (*a*) With SV40-transformed 3T3 cells. (×180.) (*b*) 3T3 nontransformed cells. (×340.) (*c*) SV40-transformed 3T3 cells treated with Lyt-2.2 monoclonal antibody (IgG2a) as control. (×340.)

Table 1. Indirect IF and immunoprecipitation assays for p53 in normal and malignant mouse cells

Target cells: Transformed cells	Transforming agent	IF*	Immunoprecipitation of ^{35}S-labeled extracts†
3T3 WT	SV40	+++	++ (c,m)
11A	Moloney MuSV	++	+ (c)
K234	Kirsten MuSV	+	+ (c)
Meth A sarcoma	Methylcholanthrene	+++	++ (c,m)
CMS3,CMS5,CI-4 sarcoma	Methylcholanthrene	++	+ (c,m)
CMS4 sarcoma	Methylcholanthrene	+	+ (c,m)
B6MS2 sarcoma	Methylcholanthrene	+	
CTF sarcoma	Spontaneous	+	+ (c)
ERLD, RADA1 leukemias	X-ray	+	
RL♂1 leukemia	X-ray		+ (c)
Nontransformed cells			
BALB/c 3T3		−	− (c,m)
5 independently derived cultures of BALB/c embryo cells (passage 2)		−	− (c,m)
5 independently derived cultures of BALB/c adult lung fibroblasts (passage 2)		−	− (c)
Cytotoxic C57BL/6 T-cell line (TCGF-dependent)		−	− (m)
BALB/c bone marrow		−‡	− (c)
BALB/c thymus		−§	++ (c,m)

* Scoring of nuclear fluorescence with p53 monoclonal antibody [1:100 dilution of sera from *nu/nu* (Swiss) mice growing hybridoma clone 200-47]: +++, strong; ++, intermediate; +, weak; −, none.

† Sources of p53 antibody: c, conventional p53 antiserum [(BALB/c × C57BL/6)F_1 anti-BALB/c sarcoma CMS4]; m, p53 monoclonal antibody. Scoring of p53 precipitation reaction: ++, strongly positive; +, positive; −, no precipitation.

‡ Imprints of bone marrow cells from the femur.

§ Imprints and frozen sections.

generally showed a patchy rather than homogeneous distribution. The strength of IF reactions varied considerably, with SV40-transformed cells showing the strongest fluorescence, certain methylcholanthrene-induced tumors showing intermediate fluorescence, and leukemias showing the weakest reaction.

No p53 reactivity could be observed in the following nontransformed cell types by IF or immunoprecipitation: fibroblasts cultured from adult BALB/c lungs (passage 2); cells cultured from 10- to 15-day-old BALB/c embryos (passage 2); BALB/c 3T3 cells; a C57BL/6 cytotoxic T-cell line growing in the presence of T-cell growth factor (TCGF); and BALB/c bone marrow cells (not cultured). No IF-positive cells were observed in imprints or frozen sections of normal adult BALB/c thymus. This is in contrast to the detection of p53 in extracts of thymus by immunoprecipitation tests with conventional and monoclonal antibodies.

Reactions of p53 Monoclonal Antibody with Normal and Transformed Human Cells in Indirect IF Tests. Table 2 summarizes the results of IF tests with the p53 monoclonal antibody on a panel of human cell types. Both normal and malignant human cells were found to react with p53 antibody. Fig. 4 *a–c* illustrates IF reactions with SK-MEL-64 and SK-MEL-28, two established lines of malignant melanoma. As in the mouse, fluorescence was confined to the nucleus and usually occurred in irregular patches rather than as homogeneous staining throughout the nucleus. Fig. 4*d* illustrates the similar pattern of fluorescence in rapidly growing early passages of normal kidney epithelium. Eighty to 90% of these cells showed strong nuclear fluorescence. After reaching confluence, cultures of normal kidney cells showed strong contact inhibition; associated with cessation of cell division, p53 nuclear fluorescence disappeared (Fig. 4*e*). Accompanying this loss of nuclear fluorescence, confluent cultures of kidney epithelium developed a weak, diffuse cytoplasmic fluorescence. Cultures of fetal brain also showed strong nuclear fluorescence with p53 monoclonal antibody during the active growth phase of the cells, followed by loss of nuclear fluorescence and the development of cytoplasmic fluorescence after the cultures reached confluence. Malignant

Table 2. Indirect IF assays of normal and malignant cultured human cells with p53 monoclonal antibody

Target cells*	IF†
Malignant cell lines	
Melanoma:	
SK-MEL-28	++
SK-MEL-37	++
SK-MEL-64	+++
Renal cancer:	
SK-RC-2	++
SK-RC-6	++
SK-RC-9	++
Breast cancer:	
BT-20	++
MCF-7	++
Burkitt lymphoma:	
Daudi	+
Normal cells	
Kidney epithelium (passages 1, 2):	
1	+++
2	+++
3	++
4	++
Fetal brain (passage 3)	++
Skin fibroblasts 1 (passage 1)	+
Skin fibroblasts 2, 3, 4 (passages 7, 10, 13)	−
Cytotoxic T-cell line (TCGF-dependent)	−

* Cells tested for p53 during exponential growth phase (see text for discussion).

† See Table 1 for scoring of IF reactions.

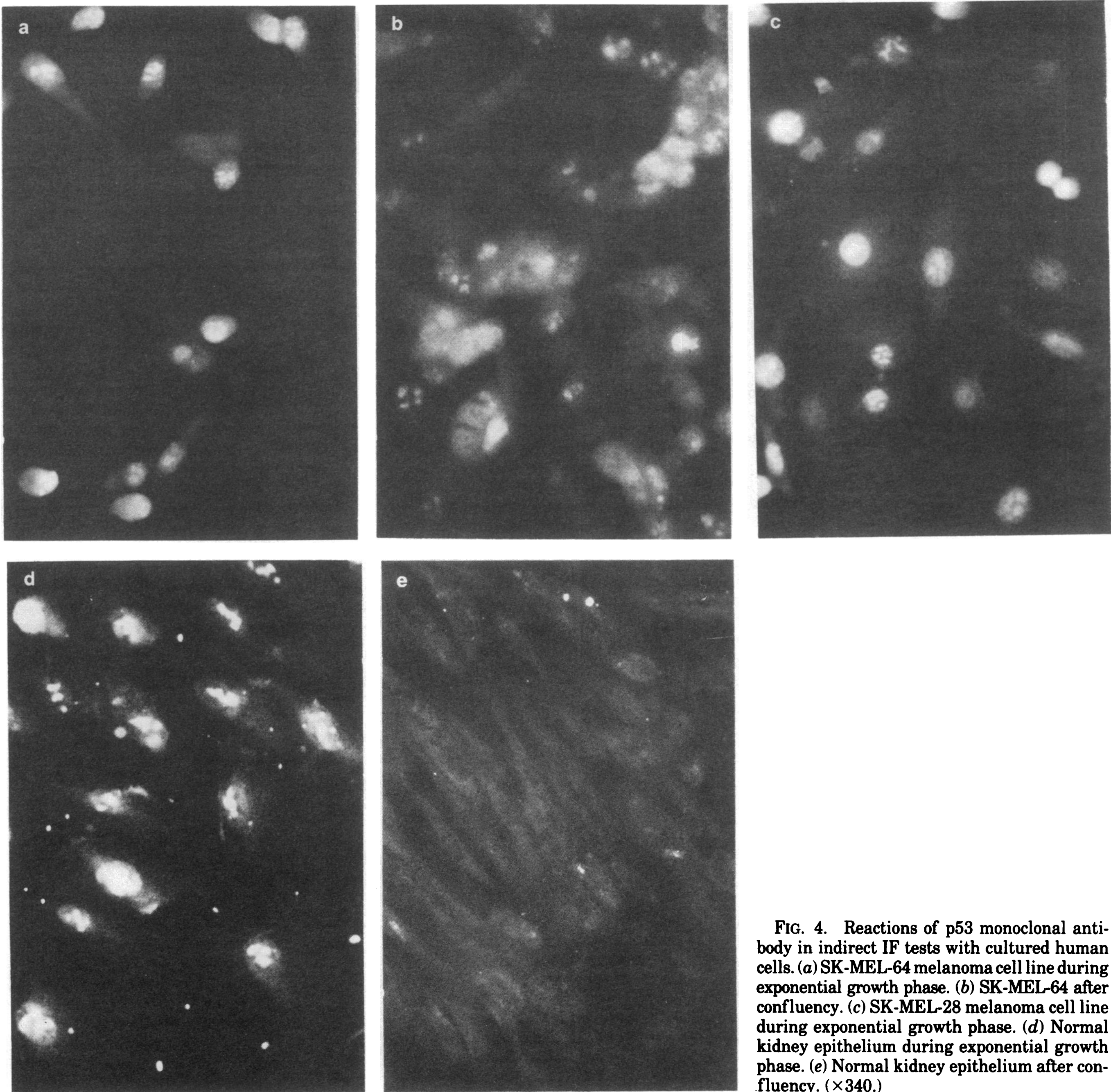

FIG. 4. Reactions of p53 monoclonal antibody in indirect IF tests with cultured human cells. (*a*) SK-MEL-64 melanoma cell line during exponential growth phase. (*b*) SK-MEL-64 after confluency. (*c*) SK-MEL-28 melanoma cell line during exponential growth phase. (*d*) Normal kidney epithelium during exponential growth phase. (*e*) Normal kidney epithelium after confluency. (×340.)

cells, such as SK-MEL-64 and renal cancers SK-RC-2 and SK-RC-9, which do not show contact inhibition of cell division, behaved quite differently in this regard. Nuclear fluorescence did not disappear after the cultures reached confluence (Fig. 4*b*). Rather, p53 expression persisted as the cells continued to divide and pile up. However, conditions that lead to reduced growth rates of cultures of malignant cells can cause p53 levels to fall. SK-MEL-64 melanoma cells growing optimally in 15% fetal calf serum showed strong nuclear fluorescence. Replacing the medium in such cultures with media containing less fetal calf serum (≤7.5%) caused a rapid decrease in cell division and an 80–90% reduction in numbers of cells expressing p53.

DISCUSSION

The molecular weight and tryptic peptide map of the mouse p53 component detected by the monoclonal antibody characterized in this report correspond precisely to the component defined in our original study with hyperimmune antisera. As measured by immunoprecipitation of labeled extracts, the monoclonal antibody and the conventional antibody show the same pattern of p53 expression in various normal and malignant mouse cells. The monoclonal antibody also permits clear-cut detection of p53 by indirect IF, and this provides a way to measure p53 in individual cells. Expression of p53 appears to be generally restricted to the nucleus, and different cell types show considerable variation in the level of detectable p53. In the mouse, SV40-transformed 3T3 cells and certain methylcholanthrene-induced tumors, such as Meth A, express high levels of p53 whereas leukemia cells express much lower amounts. The predominantly transformation-related pattern of p53 in the mouse as originally described by using immunoprecipitation analysis is also seen with IF. Cultures of embryo cells, adult lung fibroblasts, nontransformed 3T3 cells, and T cells growing in the

presence of TCGF are negative for p53 expression. No p53 could be detected by IF in frozen sections of normal thymus or in thymic imprints, despite the fact that p53 is demonstrable in extracts of thymus by immunoprecipitation. This may be due to low levels of p53 throughout the thymus or high levels in a restricted cell population that was not identified in our IF studies.

An additional benefit of the p53 monoclonal antibody is that it detects a serologically related component in certain human cells, and recent characterization of the crossreacting antigen in SK-MEL-64 melanoma shows that it has an M_r of approximately 55,000. IF tests reveal the presence of this component in various cell lines derived from human cancer and, similar to our observations with mouse tumors, the antigen usually is found in the nucleus and varies in strength of expression in the different cell lines. Human p53 is also easily detected in certain cultured normal cells, such as rapidly dividing kidney epithelium and cells derived from fetal brain. In both normal and malignant cells, expression of p53 was strongly influenced by culture conditions. For instance, levels of p53 that were characteristically high in SK-MEL-64 cells growing optimally in medium supplemented with 15% fetal calf serum show a rapid fall in media supplemented with lower concentrations of serum, and this decrease in p53 expression is associated with a corresponding reduction in cell division in such cultures.

This relationship of cell division and p53 expression is also strikingly evident in the case of cultured normal kidney epithelium. These cells, which grow vigorously during their initial two or three subcultures *in vitro*, are subject to strong contact inhibition upon reaching confluence. Expression of nuclear p53 correlates directly with the growth characteristics of these cultures—i.e., high levels during the period of rapid cell division and low to undetectable levels in confluent cultures. With its loss from the nucleus, p53 becomes demonstrable in the cytoplasm of contact-inhibited cultures of normal kidney and fetal brain, possibly indicating that p53 continues to be synthesized under these conditions but is not transported to the nucleus. In contrast to these findings with normal cells, in malignant lines, such as SK-MEL-64, p53 levels tend to remain high after cultures reach confluence and cells begin to overgrow the monolayer.

These observations suggest that p53 may be involved in the regulation of cell division, possibly as one of the factors initiating DNA synthesis. In normal cells, p53 would be under the control of signals that normally regulate cell division, as shown by the decrease in p53 levels in the nucleus coincidental with density-dependent inhibition of cell growth. To probe possible causal associations between p53 and cell division, it will be important to know if p53 is synthesized at a specific point in the cell cycle or if its synthesis is independent of the cell cycle. Comparable questions will need to be asked about p53 transport and turnover. In malignant cells, p53 expression and cell division are not regulated by contact inhibition (although they continue to be influenced by growth stimuli in the culture medium). In addition, especially in the case of the mouse, levels of p53 are generally higher in neoplastic cells than in normal cells. The nature of the regulatory defect that leads to persistent high levels of p53 in transformed cells is a key issue for future study. In the case of cells transformed by viruses, following the model of SV40 in which tumor antigen forms a complex with p53 antigen, transforming protein may interact with p53 in such a way as to impede the rate of p53 breakdown or alter the regulatory circuit controlling p53 synthesis among several possibilities. In the case of cells transformed by other means, endogenous products may be found with p53-binding activity similar to virus-encoded transforming proteins.

Note Added in Proof. p53 monoclonal antibodies have now been described by two other groups (14, 15).

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1. DeLeo, A. B., Jay, G., Appella, E., DuBois, G. C., Law, L. W. & Old, L. J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2420–2424.
2. Jay, G., DeLeo, A. B., Appella, E., DuBois, G. C., Law, L. W., Khoury, G. & Old, L. J. (1980) *Cold Spring Harbor Symp. Quant. Biol.* **44**, 659–664.
3. Lane, D. P. & Crawford, L. V. (1979) *Nature (London)* **278**, 261–263.
4. Linzer, D. I. & Levine, A. J. (1979) *Cell* **17**, 43–52.
5. Melero, J. A., Tur, S. & Carroll, R. B. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 97–101.
6. Luka, J., Jörnvall, H. & Klein, G. (1980) *J. Virol.* **35**, 592–602.
7. DeLeo, A. B., Shiku, H., Takahashi, T., John, M. & Old, L. J. (1977) *J. Exp. Med.* **146**, 720–734.
8. Old, L. J. & Stockert, E. (1977) *Annu. Rev. Genet.* **17**, 127–160.
9. Carey, T. E., Takahashi, T., Resnick, L. A., Oettgen, H. F. & Old, L. J. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 3278–3282.
10. Ueda, R., Shiku, H., Pfreundschuh, M., Takahashi, T., Li, L. T. C., Whitmore, W. F., Oettgen, H. F. & Old, L. J. (1979) *J. Exp. Med.* **150**, 564–579.
11. Köhler, G. & Milstein, C. (1975) *Nature (London)* **256**, 495–497.
12. Dippold, W. G., Lloyd, K. O., Li, L. T. C., Ikeda, H., Oettgen, H. F. & Old, L. J. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6114–6118.
13. Jay, G., Ferrini, U., Robinson, E. A., Khoury, G. & Appella, E. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 6562–6566.
14. Gurney, E. G., Harrison, R. O. & Fenno, J. (1980) *J. Virol.* **34**, 752–763.
15. Rotter, V., Witte, O. N., Coffman, R. & Baltimore, D. (1980) *J. Virol.* **36**, 547–555.