Proc. Natl. Acad. Sci. USA
Vol. 78, No. 1, pp. 41–45, January 1981
Biochemistry

Detection of a common feature in several human tumor cell lines—a 53,000-dalton protein

(simian virus 40 tumor antigen-associated host protein/phosphoprotein/monoclonal antibody/immunoprecipitation)

L. V. CRAWFORD*, D. C. PIM*, E. G. GURNEY†, P. GOODFELLOW*, AND J. TAYLOR-PAPADIMITRIOU*

*Imperial Cancer Research Fund, P.O. Box 123, Lincoln's Inn Fields, London WC2A 3PX, England; and †Department of Biology, University of Utah, Salt Lake City, Utah 84112

Communicated by J. D. Watson, August 22, 1980

ABSTRACT **Human cell lines, whether derived from spontaneous tumors or transformed *in vitro* with simian virus 40, were found to contain a 53,000-dalton phosphoprotein (pp53) in contrast to normal human cells in which this protein was not detected. Isoelectric focusing showed that pp53 comprised several species in both simian virus 40-transformed and tumor cells. Comparison of the pp53 species from the various cell lines by partial proteolysis showed that they were similar but not identical. Among the 13 tumor cell lines examined, only 1 line, HeLa, did not contain detectable pp53. All the other tumor cell lines contained pp53, and it is suggested that this protein may be associated with their transformed state.**

Human tumor cell lines *in vitro* are more remarkable for their diversity than for their uniformity. In spite of this diversity, they may share some common, distinctive feature(s) related to their changed growth control properties. Although such features may not in themselves be sufficient to explain the malignancy of the tumor cells, they may be necessary for the maintenance of their transformed state and, as such, would be of considerable importance.

In mouse cells transformed by simian virus 40 (SV40), a complex has been described comprising the virus-coded large T (tumor) antigen in association with a host protein, pp53 (1). This host protein, called pp53 because of its polypeptide size of 53,000 daltons, is also found in other mouse cells that have been transformed by agents other than SV40 [e.g., polyoma virus (1)] and in teratocarcinoma cell lines such as F9 and PCC4 (2). A phosphoprotein of similar size, p53, has been found to be present in a variety of transformed mouse cells and absent in their untransformed parents (3). Although there is no direct proof that this is the same protein as pp53, it seems likely that it is. This raises the possibility that the presence of a 53,000-dalton host protein that is absent or present at much reduced levels in normal cells may be a frequent accompaniment to the transformed phenotype in mouse cells.

The isolation of a hybridoma producing a pp53-reactive monoclonal antibody (4) allowed us to search for an analogous situation in human cells. Although this monoclonal antibody was produced in mice against SV40-transformed mouse cells, it reacts strongly with human pp53 (4). This is fortunate because mouse antisera react much more weakly with human pp53 than with mouse pp53. Here we report the presence of pp53 in human cells transformed by SV40 and in cell lines derived from a variety of human tumors.

Because many cellular proteins have monomer molecular weights between 50,000 and 60,000, it was essential to show that the proteins in the human tumor cell lines were indeed closely similar to that in the SV40-transformed cell lines. The criteria used to establish this similarity were molecular weight, reactivity with the monoclonal antibody from clone 122, presence of phosphate and methionine in the protein, isoelectric focusing behavior, association with SV40 large T antigen after infection with SV40, and similarity of partial proteolysis products.

MATERIALS AND METHODS

Cell Cultures. The cells examined were 2 SV40-transformed cell lines (SV80 and SV A31 E7), 12 permanent cell lines derived from human tumors, and 3 types of normal human cells. The isolation and characterization of all the cell lines except MG have already been described (5–16). The teratocarcinoma line MG was isolated from a lung metastasis of a testicular teratocarcinoma in an adult man. Cells from the pleural effusion were plated out and maintained on gelatinized dishes. The cultures used were uncloned cells at the eighth passage, 10 weeks from the initial isolation. Further details of the characterization of this line will be published elsewhere. The normal cells were cell cultures rather than cell lines, being precrisis, and were actively growing at the time of labeling. The cells were grown in the following media: RPMI 1640 medium with 15% (vol/vol) fetal calf serum (lines Daudi, Bristol 7, Tera 1, Tera 2, NALM 1, BeWo, Jar, and MG), RPMI 1640 medium plus 5% calf serum, 10% fetal calf serum, 10 μg of insulin per ml, and 20 μg of hydrocortisone per ml (line T47D), Dulbecco's modified Eagle's medium with 10% fetal calf serum (lines Hs 578T, SK BR3, HeLa D98, FS, and LN75), Dulbecco's modified Eagle's medium with 15% fetal calf serum, 10 μg of insulin per ml, and 20 μg of hydrocortisone per ml (line BT 20), or RPMI 1640 medium with 15% fetal calf serum, 20% human serum, 5 μg of hydrocortisone per ml, and 100 ng of cholera toxin per ml (milk epithelial cells). For labeling, the cultures on 50-mm plastic dishes were transferred to Dulbecco's modified Eagle's medium without phosphate plus 1% dialyzed calf serum and were labeled with 0.5 mCi of inorganic [^{32}P]phosphate each (1 Ci = 3.7×10^{10} becquerels) for 3 hr at 37°C.

Sera. The SV40 large T-specific antiserum, D2, was prepared in rabbits by D. P. Lane (17). The antigen, D2 protein, was purified from HeLa cells infected with the adeno-SV40 hybrid virus Ad2$^+$D2. In extracts of SV40-transformed or -infected cells, this serum reacts only with SV40 large T antigen and not with small T antigen, and it has no detectable activity against pp53 in the direct-binding radioimmunoassay described by Lane and Robbins (18). The monoclonal antibody from clone 122, directed against pp53, was isolated by E. G. Gurney and has been characterized (4). It reacts with both human and mouse pp53 and has no detectable activity against SV40 large T

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: pp53, 53,000-dalton host phosphoprotein; SV40, simian virus 40; T antigen, tumor antigen; SAC, *Staphylococcus aureus* Cowan 1 immunoadsorbent.

antigen in the direct-binding radioimmunoassay or against free large T antigen in immunoprecipitation. Our results would indicate that it is probably directed against a polypeptide rather than a carbohydrate determinant, but activity against other possible modifications of the protein has not been completely excluded.

Other methods for the preparation of cell extracts, immunoprecipitation, and gel electrophoresis have been described in detail (19, 20). Briefly, cultures were labeled with [^{35}S]methionine or inorganic [^{32}P]phosphate and lysed with 1% Nonidet P-40 at pH 8. Immunoprecipitation with specific antisera or monoclonal antibody was followed by collection of immune complexes on *Staphylococcus aureus* Cowan 1 immunoadsorbent (SAC) prepared as described (21). Proteins were separated by $NaDodSO_4$/polyacrylamide gel electrophoresis or by isoelectric focusing followed by gel electrophoresis (22).

RESULTS

Characterization of pp53 in SV80 Cells. Human cell line SV80, transformed by SV40, has been well characterized and widely used as a source of SV40 T antigens (23, 24). In this line, because SV40 large T antigen is present, we can ask, first, whether pp53 is present by the criterion of reaction with clone 122 antibody and, second, whether it has the expected property of forming a complex with large T antigen. Complex formation would show that it was functionally analogous in at least one respect to mouse pp53, and crossreaction with clone 122 antibody would show that it shared one particular determinant with mouse pp53. There was already good evidence for the presence of a protein with these properties in SV80 cells (4, 17). As shown in Fig. 1, clone 122 antibody precipitated a pp53 from extracts of SV80 cells and at the same time precipitated some of the SV40 large T antigen present in the extract. Only a fraction of the large T antigen was associated with the pp53, as was the case in the transformed mouse cells examined previously (25). This was clearly shown by using a specific serum directed against purified large T antigen protein. This precipitated all the available large T antigen, free or complexed, and this total is much more than that precipitated by clone 122 antibody (compare Fig. 1 track 3 with track 7). Both the anti-T serum and the clone 122 antibody were used at saturating levels to ensure that the serum input did not limit the amount of antigen precipitated. Fig. 1 also shows that treatment of the cells with tunicamycin during synthesis had no detectable effect on the mobility of either large T antigen or pp53 (compare track 3 with track 4). The same tunicamycin treatment did affect the synthesis and mobility of the heavy chain of HLA antigen, which is well characterized as a glycoprotein (26). This is shown by the effect of tunicamycin on the precipitation of the 44,000- to 40,000-dalton bands from the same extracts by the monoclonal antibody W6/32 (27) directed against HLA antigen (compare track 11 with track 12). This is by no means conclusive evidence, but it gives us no reason to think that pp53 has a substantial carbohydrate component. There is also no reason to think that clone 122 antibody is directed against a carbohydrate determinant; this antibody precipitated the pp53 from tunicamycin-treated cells as well as it did from untreated cells. In both cases it is still possible to argue that carbohydrate is present and involved but that tunicamycin fails to inhibit its synthesis, and we cannot rule this out. Tunicamycin inhibits only the dolichol sugar pathway, and glycosylation might occur by another pathway.

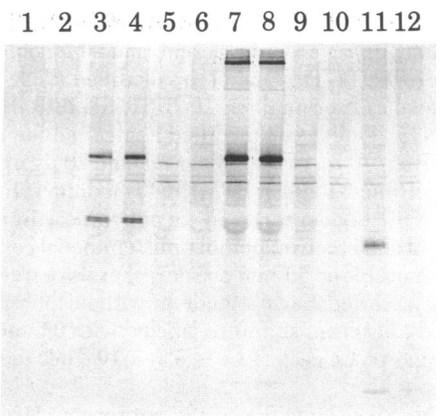

FIG. 1. Immunoprecipitation of large T antigen and pp53 from extracts of SV80 cells labeled in the presence and absence of tunicamycin. Parallel cultures of SV80 cells were labeled with [^{35}S]methionine for 2 hr at 37°C. One plate was first treated with tunicamycin (2 μg/ml) for 30 min, labeled in the same concentration of antibiotic, and then treated for 30 min with antibiotic in medium containing nonradioactive methionine. For the other plate, tunicamycin was omitted. Material from both plates was extracted with 1% Nonidet P-40 at pH 8.0; the proteins were immunoprecipitated and separated on a 7–20% (wt/vol) polyacrylamide gel. Tracks 1, 3, 5, 7, 9, and 11, culture without tunicamycin; tracks 2, 4, 6, 8, 10, and 12, culture with tunicamycin. Sera used: tracks 1 and 2, control hybridoma antibody; tracks 3 and 4, clone 122 hybridoma antibody; tracks 5 and 6, normal rabbit serum; tracks 7 and 8, D2 anti-T serum; tracks 9 and 10, normal mouse serum; tracks 11 and 12, W6/32 anti-HLA ascitic fluid.

Comparison of cell extracts labeled with [^{35}S]methionine or inorganic [^{32}P]phosphate showed that human pp53 is a phosphoprotein, as had already been shown for mouse pp53 (2). Human cells transformed by SV40 contain substantial amounts of free pp53 in contrast to the transformed mouse cells in which we could detect little or no pp53 apart from that complexed with large T antigen (4, 17, 28). The presence of free pp53 in human cells was demonstrated by a cascade experiment in which complexed pp53 was first precipitated by treatment with an excess of specific anti-T serum. This was followed by treatment with clone 122 antibody to precipitate the residual free pp53 (Fig. 2A, track 4). An extract of transformed mouse cells treated in exactly the same way showed no free pp53 (Fig. 2B, track 4). The fractions of pp53 found complexed and free in extracts of human cells depended on the conditions used. For example, slow freezing of extracts to −70°C before thawing and addition of antiserum halved the amount of complexed pp53 with a cor-

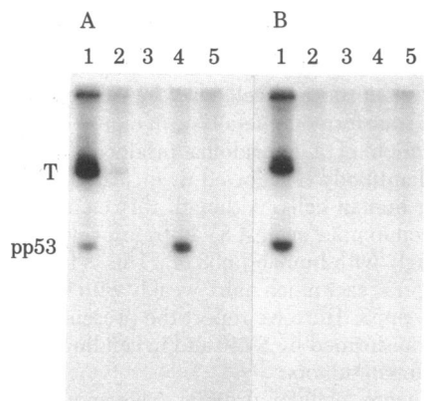

FIG. 2. Cascade immunoprecipitation of large T antigen and pp53 from SV40-transformed cells. Extracts of ^{32}P-labeled human (*A*) and mouse (*B*) cells were treated sequentially with antisera as follows: track 1, anti-T serum D2 and SAC; track 2, same as track 1; track 3, SAC alone followed by either 4 or 5; track 4, clone 122 antibody and SAC; track 5, same as track 1. After immunoprecipitation, the proteins were separated on a 12% polyacrylamide gel.

Table 1. Human tumor cell lines and normal cell cultures

Cells	Type of tumor	pp53	Origin of cell line (ref.)
Lines			
Daudi	Burkitt lymphoma	+	5
Bristol 7	Lymphocyte transformed by EBV* *in vitro*	+	6
NALM 1	Chronic myelogenous leukemia	+	7
BT 20	Primary mammary carcinoma	+	8
SK BR3	Metastatic mammary carcinoma	+	9
T47D	Metastatic mammary carcinoma	+	10
Hs 578T	Primary mammary carcinosarcoma	+	11
Tera 1	Teratocarcinoma	+	9
Tera 2	Teratocarcinoma	+	9
MG	Teratocarcinoma	+	
BeWo	Choriocarcinoma	+	12
Jar	Choriocarcinoma	+	13
HeLa D98	Cervical carcinoma	−	14
Strains			
FS	Foreskin fibroblast culture	−	
LN 75	Skin fibroblast (Lesch–Nyhan)	−	15
Milk secondary culture	Mammary epithelium	−	16

* Epstein–Barr virus.

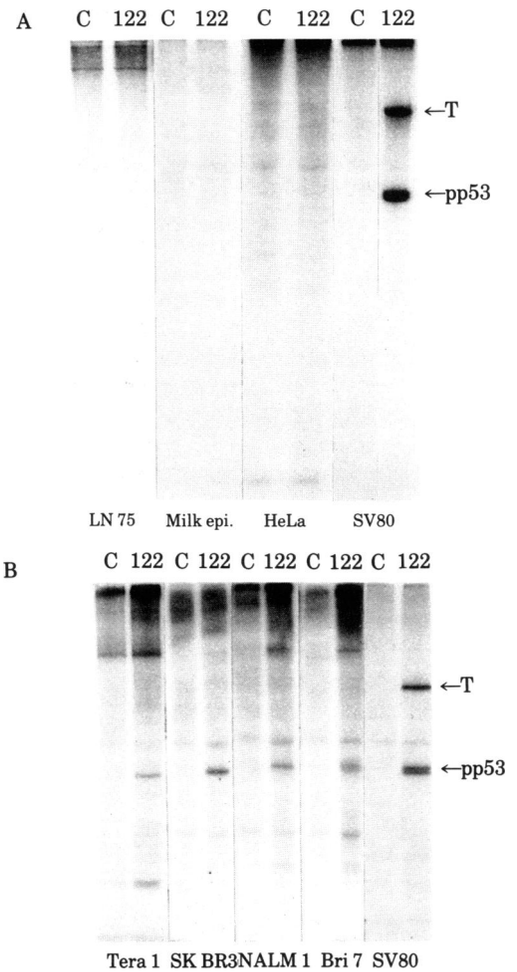

FIG. 3. Immunoprecipitation of pp53 from human tumor cell lines. Cultures of human tumor cells and control cells were labeled with inorganic [^{32}P]phosphate and immunoprecipitated with clone 122 antibody (122) or with control hybridoma supernatant (C). The cell types are indicated below each track: epi., epithelium; Bri 7, Bristol 7. After immunoprecipitation, the proteins were separated on 12% (*A*) or 10% (*B*) polyacrylamide gels.

responding increase in the amount of free pp53. The free pp53 may therefore result from the lower stability of the human pp53–large T antigen complex, rather than being an indication of the existence of two distinct classes of pp53.

Tumor Cell Lines. Cell lines derived from four widely different types of human tumor have been examined. These were from cervical and mammary carcinomas (primary tumor and pleural effusion), choriocarcinomas, teratocarcinomas, and a Burkitt lymphoma. All 12 lines of cells of these five types were positive for pp53 (Table 1). The appearance of the pp53 band varied in the different cell lines; for example, Daudi, Bristol 7, and Jar cells showed the same pp53 doublet as SV80 cells. The teratocarcinoma and mammary carcinoma cells gave only a single pp53 band, corresponding in mobility to the more rapidly migrating component of the SV80 doublet (Fig. 3). The only tumor cell line tested in which pp53 could not be detected was HeLa D98, initially derived from a cervical carcinoma (14). By using extracts of cells labeled with ^{32}P or with [^{35}S]methionine we were unable to detect specific precipitation of any band in the region of 50,000–60,000 daltons.

All the normal human cells that were tested proved to be negative for pp53 (Table 1). We were unable to detect specific precipitation of a band in the 53,000-dalton region with either ^{32}P- or [^{35}S]methionine-labeled extracts. If pp53 is present in these cells, it must be at a much reduced level such that it is below the background of nonspecific precipitation.

Comparison of pp53 from Tumor Cell Line and SV80 Cells. Further evidence that the pp53 from tumor cells was similar to that from SV40-transformed cells was obtained from two-dimensional gel electrophoresis (22). Previous studies with mouse pp53 (25) had shown that it gave several spots on two-dimensional gels. As shown in Fig. 4, several species with different isoelectric points made up the fast and slow components of the human pp53 doublet, and both components streaked in the isoelectric focusing dimension. There were always a major species with a pI $\approx$ 6.7 and a minor species with a pI $\approx$ 7.2. This property of streaking in the isoelectric focusing dimension is shared with SV40 large T antigen (19). The patterns of the pp53 species from the tumor cells were similar to the pattern from SV80 cells, except that Hs 578T, SK BR3, NALM 1, and teratocarcinoma pp53 species showed only the faster migrating species, as already seen on one-dimensional gels. The distribution of radioactivity from ^{32}P and [^{35}S]methionine also varied to some extent with the different cells. For example, in Daudi extracts there was less ^{32}P in the more acidic species of pp53 relative to ^{35}S.

All the tumor cell lines were checked for the presence of any SV40 T antigen. No specific precipitation of material corresponding to the 94,000-dalton large T antigen or of smaller proteins could be detected by using a potent antiserum specific for SV40 large T antigen. There is, therefore, no evidence for involvement of SV40 in any of these tumor cell lines or of contamination of the lines with SV40. As shown below, some of the cell lines were susceptible to infection by SV40 and produced substantial amounts of large T antigen after infection.

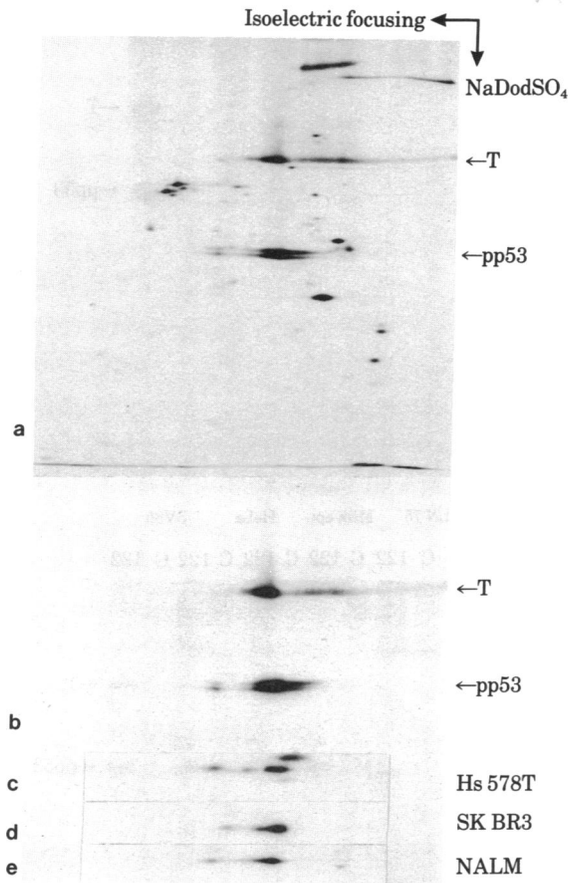

FIG. 4. Isoelectric focusing of pp53 from human tumor and SV40-transformed cells. Cell extracts were immunoprecipitated with clone 122 antibody and the proteins were separated by the method of O'Farrell *et al.* (22). (*a*) [^{35}S]Methionine-labeled SV80 cell extract. (*b*) [^{32}P]Phosphate-labeled SV80 cell extract. (*c*, *d*, and *e*) [^{32}P]Phosphate-labeled extracts from human tumor cell lines; only the pp53 region is shown, aligned so that regions of the same pI are vertically below each other and correspond to *b*.

The pp53 from the various tumor cell lines would be expected to have the property of associating with SV40 large T antigen if it were analogous to that of SV80 cells. Infection of Hs 578T cells with SV40 caused an increase in the incorporation of ^{32}P into pp53, and provided large T antigen with which pp53 could associate. As shown in Fig. 5, tracks 6 and 10, clone 122 antibody precipitated the pp53 from the infected cells and with it a fraction of the SV40 large T antigen present in the extract. Only a small amount of the large T antigen was complexed with pp53 (compare the complexed large T antigen in track 6 with the total large T antigen in track 8) as compared with SV80 cells.

Comparison of pp53 Species by Partial Proteolysis. The similarity between pp53 from tumor cell lines and that from SV80 cells was also borne out by comparison of the products of partial proteolytic cleavage of these proteins. As shown by Cleveland *et al.* (29), proteins isolated by gel electrophoresis give characteristic patterns of smaller polypeptides as relatively stable intermediates during digestion with proteases such as *S. aureus* V8 protease. This technique showed that the two components of the SV80-derived 53,000-dalton band were closely related, giving similar patterns of polypeptides (Fig. 6 *b* and *c*). The first three intermediate polypeptides retained the small differences of mobility shown by the intact polypeptides. With the BT 20 pp53 (Fig. 6*a*), the pattern corresponded to that of the faster migrating component of the SV80 doublet (Fig. 6*b*), in

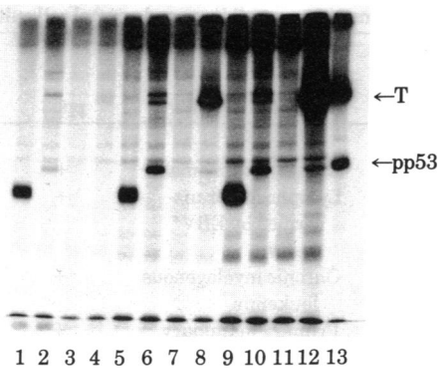

FIG. 5. Infection of mammary carcinoma cells with SV40. Cultures of uninfected Hs 578T cells (tracks 1–4) and the same cells 24 hr (tracks 5–8) and 48 hr (tracks 9–12) after infection with SV40 (20 plaque-forming units/cell) were labeled with ^{32}P. Each extract was immunoprecipitated with anti-HLA monoclonal antibody as control (tracks 1, 5, and 9), clone 122 antibody to precipitate pp53 and associated SV40 large T antigen (tracks 2, 6, and 10), control normal rabbit serum (tracks 3, 7, and 11), and D2 anti-T serum to precipitate large T antigen and any associated pp53 (tracks 4, 8, and 12). After immunoprecipitation, the proteins were separated on a 10% polyacrylamide gel with SV80 large T antigen and 53,000-dalton markers (track 13).

agreement with the mobility of the intact 53,000-dalton polypeptide. The pp53 doublet from Daudi cells (Fig. 6*d*) gave a pattern similar to that of the SV80 pp53 doublet. At least 10 polypeptides with molecular weights from 10,000 to 50,000 were seen to correspond in mobility in the digests of the different pp53 species from SV80, Daudi, and BT 20. Some differences were detectable between the patterns, however, indicating that the proteins were similar but not identical. This was already clear from the slight differences of mobility already mentioned. Other proteins, such as the 45,000-dalton band cut from the same gels, showed a pattern clearly different from that of pp53 (Fig. 6*e*).

DISCUSSION

Human cells transformed by SV40 contain a protein corresponding to the 53,000-dalton protein previously found in association with the virus-coded large T antigen in SV40-transformed mouse cells. The proteins from mouse and human cells are similar in size, are phosphoproteins, react with clone 122 an-

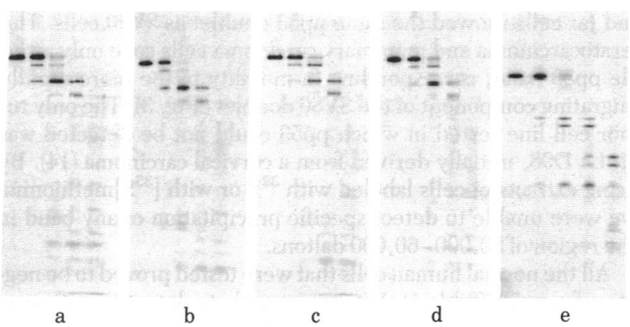

FIG. 6. Partial proteolysis comparison of pp53 species. Extracts of [^{35}S]methionine-labeled cells were immunoprecipitated with clone 122 antibody and the pp53 were separated by gel electrophoresis. The gel bands were excised, and the polypeptides were digested with increasing amounts of *S. aureus* V8 protease for 1 hr at 37°C. The protease concentrations in each panel were, from left to right, 0, 0.2, 2, 20, and 200 μg/ml. The pp53 species were derived from BT 20 (*a*), SV80 lower band (*b*), SV80 upper band (*c*), and Daudi (*d*). The 45,000-dalton band (*e*) was cut from the BT 20 gel and serves as a control.

tibody, and associate with SV40 large T antigen. In addition to the large T antigen-associated pp53, SV40-transformed human cells also contain free pp53 (4, 17).

Cell lines derived from spontaneous human tumors contain proteins very similar to the pp53 from SV80 cells. The criteria of similarity were extensive. In addition to being phosphoproteins with similar mobilities, both pp53 species displayed the determinant recognized by clone 122 antibody and had similar behavior in isoelectric focusing. The association of pp53 with SV40 large T antigen is a very specific interaction, as shown by the lack of any other major proteins in immunoprecipitates of cell extracts with either specific anti T or pp53 antibodies. After infection of human tumor cell lines with SV40, the amount of incorporation of ^{32}P into pp53 increased substantially. Some of this newly synthesized pp53 became associated with SV40 large T antigen, as shown by the coprecipitation of the two proteins by specific antibodies to either of them. The amount of participation of pp53 in the complex with SV40 large T antigen in the SV40-infected human tumor cells was somewhat less than that seen in SV80 cells, but otherwise the situation was similar. The last criterion of similarity in patterns of partial proteolysis is related to the underlying similarity in the overall structure of the proteins and of their amino acid sequences. Minor differences of amino acid sequence would probably not be detected. The conclusion drawn from these partial proteolysis comparisons between the various pp53 species was that they were very similar, although probably not identical. There is no evidence of SV40 involvement with any of the tumors, and it seems likely that the situation is analogous to that found in transformed mouse cells in which pp53 (or p53) appeared in cells transformed by a great variety of agents (3). Because normal human cells, especially epithelial cells, cannot be maintained in tissue culture except for relatively short periods, it is difficult to compare transformed cell lines with their normal counterparts. However, actively growing cultures of several types of normal human cell, skin fibroblasts, or milk epithelial cells showed no sign of specific precipitation of pp53, either phosphate- or methionine-labeled. We can, therefore, say that the rate of synthesis of pp53 that we find in transformed cells is greatly increased over that in normal cells because we cannot be sure whether normal cells contain levels of pp53 below our limits of detection or no pp53 at all. Several cell lines, either from Burkitt lymphomas or transformed by Epstein–Barr virus *in vitro*, have been examined by Klein *et al.* (30). Some, but not all, of these showed a 53,000-dalton protein or proteins associated with the nuclear antigen of Epstein–Barr virus. It is likely that these are similar to the pp53 described here, and this gives support to the suggestion that the presence of the pp53 may be associated with at least some types of transformation. Because pp53 is a nuclear protein both in SV40-transformed mouse cells and in virus-free 3T12 mouse cells (4, 28), it may be involved in control of DNA replication and, by being synthesized constitutively, result in uncontrolled replication. However, there is insufficient data to allow us to choose among the many hypotheses. The striking result is that a variety of human tumor cell lines share with the virus-transformed cell SV80 a distinctive feature that is not shown by untransformed cells. This is the presence of a group of 53,000-dalton proteins.

After this paper was written, we tested another line of tumor cells 33I, derived from a cervical carcinoma (31), and found that it was clearly positive when examined for the presence of pp53. The apparent absence of pp53 in HeLa cells is therefore not a general characteristic of all cell lines derived from cervical carcinomas.

We are indebted to the following for supplying us with cell cultures: Dr. N. Auersperg (33I), Dr. J. Fogh (Tera 1, Tera 2, BT 20, and SK BR3), Dr. M. Greaves (NALM 1), Dr. Y. Ito (SV A31 E7), Dr. G. Klein (Daudi), Dr. D. Livingston (SV80), Dr. B. Migeon (LN 75), Dr. W. A. Nelson-Rees (Hs 578T), Dr. R. Weiss (BeWo and Jar), E. G. and G. Mason Research Institute (T47D), and Searle Research Laboratories (Bristol 7). We thank Dr. M. Crumpton for W6/32 monoclonal antibody, Dr. D. P. Lane for the D2 antibody, and Dr. M. Owen for tunicamycin and advice on its use.

1. Lane, D. P. & Crawford, L. V. (1979) *Nature (London)* **278**, 261–263.
2. Linzer, D. I. H. & Levine, A. J. (1979) *Cell* **17**, 43–52.
3. 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.
4. Gurney, E. G., Harrison, R. O. & Fenno, J. (1980) *J. Virol.* **34**, 752–763.
5. Klein, E., Klein, G., Nadkami, J. S., Wigzel, H. & Clifford, P. (1967) *Lancet* **ii**, 1068–1070.
6. Goodfellow, P., Barnstable, C., Jones, E., Bodmer, W. F., Crumpton, M. J. & Snary, D. (1976) *Tissue Antigens* **7**, 105–117.
7. Minowada, J., Tsubota, T., Greaves, M. F. & Walters, T. R. (1977) *J. Natl. Cancer Inst.* **59**, 83–87.
8. Lasfargues, E. Y. & Ozzello, L. (1958) *J. Natl. Cancer Inst.* **21**, 1131–1140.
9. Fogh, J. & Trempe, G. (1975) in *Human Tumor Cells in Vitro*, ed. Fogh, J. (Plenum, New York), pp. 115–159.
10. Keydar, I., Chen, L., Karby, S., Weiss, F. R., Delarea, J., Radu, M., Chaitcik, S. & Brenner, H. J. (1979) *Eur. J. Cancer* **15**, 659–670.
11. Hackett, A. J., Smith, H. S., Springer, E. L., Owens, R. B., Nelson-Rees, W. A., Riggs, J. L. & Gardner, M. B. (1977) *J. Natl. Cancer Inst.* **58**, 1795–1800.
12. Patillo, R. A. & Gey, G. O. (1969) *Cancer Res.* **28**, 1231–1236.
13. Patillo, R. A., Ruckert, A., Hussa, R., Bernstein, R. & Delfs, E. (1971) *In Vitro* **6**, 398.
14. Gey, G. O., Coffman, W. D. & Kubicek, M. T. (1952) *Cancer Res.* **12**, 264–265.
15. Migeon, B. R., Norum, R. A. & Corsaro, C. M. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 937–941.
16. Taylor-Papadimitriou, J., Shearer, M. & Stoker, M. G. P. (1977) *Int. J. Cancer* **20**, 903–908.
17. Lane, D. P. & Crawford, L. V. (1980) *Proc. R. Soc. London Ser. B* **210**, 451–463.
18. Lane, D. P. & Robbins, A. K. (1978) *Virology* **87**, 182–193.
19. Crawford, L. V. & O'Farrell, P. Z. (1979) *J. Virol.* **29**, 587–596.
20. Crawford, L. V., Pim, D. C. & Lane, D. P. (1980) *Virology* **100**, 314–325.
21. Kessler, S. W. (1975) *J. Immunol.* **115**, 1617–1624.
22. O'Farrell, P. Z., Goodman, H. M. & O'Farrell, P. H. (1977) *Cell* **12**, 1133–1142.
23. Todaro, G., Green, H. & Swift, M. R. (1966) *Science* **153**, 1252–1254.
24. Livingston, D. M., Henderson, I. C. & Hudson, J. (1974) *Cold Spring Harbor Symp. Quant. Biol.* **39**, 283–289.
25. Crawford, L. V., Lane, D. P., Denhardt, D. T., Harlow, E. E., Nicklin, P. M., Osborn, K. & Pim, D. C. (1980) *Cold Spring Harbor Symp. Quant. Biol.* **44**, 179–187.
26. Snary, D., Goodfellow, P., Hayman, M. J., Bodmer, W. F. & Crumpton, M. J. (1974) *Nature (London)* **247**, 457–461.
27. Barnstable, C. J., Bodmer, W. F., Brown, G., Galfre, G., Milstein, C., Williams, A. F. & Ziegler, A. (1978) *Cell* **14**, 9–20.
28. McCormick, F. & Harlow, E. (1980) *J. Virol.* **34**, 213–224.
29. Cleveland, D. W., Fischer, S. G., Kirschner, M. W. & Laemmli, U. K. (1977) *J. Biol. Chem.* **252**, 1102–1106.
30. Klein, G., Luka, J. & Zeuthen, J. (1980) *Cold Spring Harbor Symp. Quant. Biol.* **44**, 253–261.
31. Auersperg, N. (1964) *J. Natl. Cancer Inst.* **32**, 135–163.