MOLECULAR AND CELLULAR BIOLOGY, July 1982, p. 763–771
0270-7306/82/070763-09$02.00/0

Vol. 2, No. 7

Quantitation of a 55K Cellular Protein: Similar Amount and Instability in Normal and Malignant Mouse Cells

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Received 9 November 1981/Accepted 31 March 1982

Quantitative expression of a specific 55,000 (55K)-molecular-weight cellular protein was studied in two groups of mouse embryo fibroblast (clonal) cells originating from two parent clones, one of which possessed high tumorigenicity and the other of which possessed very low tumorigenicity. From the clone with low tumorigenicity, tumor lines and clones were obtained by selecting rare spontaneously transformed highly tumorigenic (mutant) cells. Cells were labeled during exponential growth for 3 h at 37°C, with [^{35}S]methionine, and the cellular 55K protein was immunoprecipitated with a monoclonal antibody and quantitated. There were low and approximately equal amounts of 55K protein in cells (clones) with both low and high tumorigenicity from both groups of cells, and there was no correlation at all between quantitative expression of 55K protein and of cellular tumorigenicity. There was ~10- to 20-fold more 55K protein in all simian virus 40-transformed T antigen-positive derivative clones, as shown previously. The T antigen-negative revertant tumor lines and clones obtained by an immunological in vivo selection method had low amounts of 55K protein, similar to the parent cell before simian virus 40 transformation. In all of the T antigen-negative cells, including the highly tumorigenic cells, degradation (turnover?) of the 55K protein was rapid, and a half-life of 15 to 60 min was estimated from pulse-chase experiments. In all of the T antigen-positive cells the 55K protein was stable (half-life > 10 h). In primary cells established from the tumors induced by highly tumorigenic cells there was a very low or no detectable amount of the 55K protein. This is in contrast to the primary cells obtained from early murine embryos in which we have reported high amounts of (stable) 55K proteins.

In cells transformed by the simian virus 40 (SV40) two proteins encoded by the early half of the SV40 DNA are synthesized: the SV40 large T antigen and the SV40 small t antigen. These antigens are detectable by immunoprecipitation with antisera to SV40-transformed cells. By the same method a protein of ~55,000 (55K) molecular weight was also detected in all of the SV40-transformed cells. This protein, however, turned out to be of cellular origin and was not encoded by the SV40 DNA (4, 10, 15, 21, 22, 26–28). It is intriguing that similar 53 to 56K cellular proteins were also found in mouse cells transformed in diverse other ways, such as in chemically induced sarcomas, in RNA virus-induced sarcomas and leukemias, and in a spontaneously transformed fibroblast (6). Also, similar 53 to 55K proteins were found in the human B-cell lymphomas and in many other established human tumor cell lines (5, 17). These observations led to an impression that the 53 to 55K protein is a "tumor antigen" and that it may be a correlate of the tumorigenic transformation of the cells (6, 9). However, the 55K protein was also observed in small amounts in normal mouse embryo fibroblast cells, such as 3T3 cells not transformed by SV40 (14, 15, 25). In the 3T3 cells, the 55K protein was found to be rapidly degraded once it was synthesized, but it was stable in the SV40-transformed 3T3 cells (25).

In the last few years we have developed and partially characterized closely related mouse cells with or without SV40 transformation and have determined quantitatively their cellular tumorigenicity in the syngeneic mouse. Among the cell lines we have developed are two groups of cells originating from two clones, both from the same AL/N mouse embryo mass cell line. One clone demonstrated very high tumorigenicity (median tumorigenic dose [TD_{50}] = 10^2 cells per mouse), apparently as a consequence of spontaneous transformation of the cells in tissue culture (23). The other clone demonstrated very low tumorigenicity (TD_{50} = $10^{6.4}$) (29). From

these two parent clones, various derivative cell lines and clones were obtained, with or without SV40 transformation (23, 29, 30). From some of the SV40-transformed T antigen-positive clones, SV40 T antigen-negative revertant tumor lines and clones were obtained through immunological selection against the SV40-transformed cells by transplantation rejection in the immunologically competent syngeneic host (23). Some such T antigen-negative clones were then chosen for retransformation by SV40 and also for selection for T antigen-negative revertants. The tumorigenicity of all of these cells was determined. These closely related mouse cells (clones) were then suitable for use in studying the correlation between the amount and stability of the 55K cellular protein and tumorigenicity (and also of the T antigen expression). Such a study is the subject of this communication.

MATERIALS AND METHODS

Cells. AL/N strain mouse embryo was used to establish a cell line, and this was the origin of all of the clones. Cells were cloned by a technique which assures single cell clones (29). Two clones, 104C and 210C, were the parents of two groups of cells. The relationships of the cells are outlined in Fig. 1. The SV40 T antigen-positive cells are indicated by asterisks; transformation was by the SV40 776 strain. Tumor lines were established in the culture as described (20). The T antigen-negative tumor lines were obtained from the SV40-transformed T antigen (and tumor-specific transplantation antigen)-positive clones after passage through the immunologically competent syngeneic mouse. The immunological selection against the SV40 T antigen-positive cells resulted in the rare revertant T antigen-negative cells growing out as tumors; the origin and the phenotypic properties of these T antigen-negative tumor lines and clones were described (23). The loss of the early viral gene sequences, but the retention of late SV40 sequences, in some of these lines and clones was also described (11). The following designations were used in earlier publications (11, 23): the 104C clone was referred to as T AL/N CL3; 106CSC, subclone 1; 124CSCT and 127CSCT, tumor lines 124 and 127; and 130CSCTC, tumor clone 130. The clone 140CSCTCSC and its sister clone 138CSCTCSC were obtained from 130CSCTC after retransformation by SV40 and recloning; 153CSCTCSCT is a tumor line from 138CSCTCSC (to be published). The origin and certain biochemical and biological properties of 210C, 215CSC, and 219CT cells were described (29, 30). The 238CT tumor line was obtained by a method similar to that used for the 219CT tumor line (30). The 242CTT line was a primary culture obtained from a tumor induced by 238CT as described previously (20).

Tumorigenicity. The TD_{50} was determined by intramuscular inoculation of 10-fold cell dilutions (minimum of three dilutions each) into batches of 6- to 8-week-old AL/N mice (10 mice per batch). The TD_{50} is given as $\log_{10}$ of the number of cells that produced rapidly ($\leq$10 weeks) lethal sarcomas in 50% of the mice inoculated, observed and calculated as in reference 23. Generally the TD_{50} values were calculated from data in which tumor incidence varied between 0 to 10 tumors per 10 mice within two 10-fold cell dilutions.

Cell growth and labeling. To facilitate the comparison of results, all of the cell lines and clones studied were in early and comparable tissue culture passage. All of the cells were grown in Dulbecco modified minimal essential medium containing 10% heat-treated dialyzed fetal calf serum (FCS) as described (20). When the cells were in logarithmic growth phase in near confluency (covering ~80% of the substratum), the cell layers were washed with 15 ml of Tris-buffered saline (pH 7.4) and labeled for 3 h at 37°C with 50 μCi (1,000 Ci/mmol) of L-[^{35}S]methionine per ml in 10 ml of methionine-free Dulbecco modified minimal essential medium containing 5% dialyzed FCS (4), unless stated otherwise.

Immunoprecipitation, electrophoresis, and fluorography. After being labeled, the cells were washed and suspended in extraction buffer (1, 4, 16) containing 0.1% Aprotinin (Sigma Chemical Co.) for protease inhibition. Immunoprecipitation on aliquots (0.5 ml) of cell extracts was with either monoclonal antibody, a supernatant of clone 122 hybridoma cells containing the antibody directed against the 55K protein in SV40-transformed mouse cells (8), or with normal mouse serum (N). The amount of the hybridoma 122 supernatants used in these assays was determined by titration against a constant volume of extract of SV40-transformed 215CSC cells (not shown), and the optimal volume of 200 μl of supernatant was used, unless stated otherwise.

The immunoprecipitates were incubated for 3 h at 4°C with a protein A-Sepharose solution, washed thoroughly, solubilized, and solutions from reactions with the 122 monoclonal antibody or with N sera containing approximately equal radioactivity were analyzed by electrophoresis on 10% sodium dodecyl sulfate-polyacrylamide gels (2, 4, 16). Marker proteins and the fluorography of the gels were also as described (1, 4, 16).

Quantitation of proteins. Counts per minute incorporated into (total) proteins were estimated after precipitation with cold trichloroacetic acid (TCA). For quantitation of the 55K protein the appropriate gel slices (~0.3 cm wide) were solubilized and counted as described (2). When indicated, the fluorograms were scanned by using a Shimadzu CS-910 dual wavelength chromatogram scanner equipped with a C-RIA data processor, and the area of the 55K band absorbance was integrated (2).

Pulse-chase experiments. Labeling was as described above, but for 30 min. The medium was then removed, the cells were washed twice with Tris-buffered saline (pH 7.4), and fresh unlabeled complete Dulbecco modified minimal essential medium containing 5% FCS was added. The cultures were kept at 37°C for the indicated chase periods.

RESULTS

Selection and biological properties of the cells. The AL/N mouse embryo fibroblast clone 104C had very high tumorigenicity; the TD_{50} was 10^2 cells per mouse, and all of the tumors were

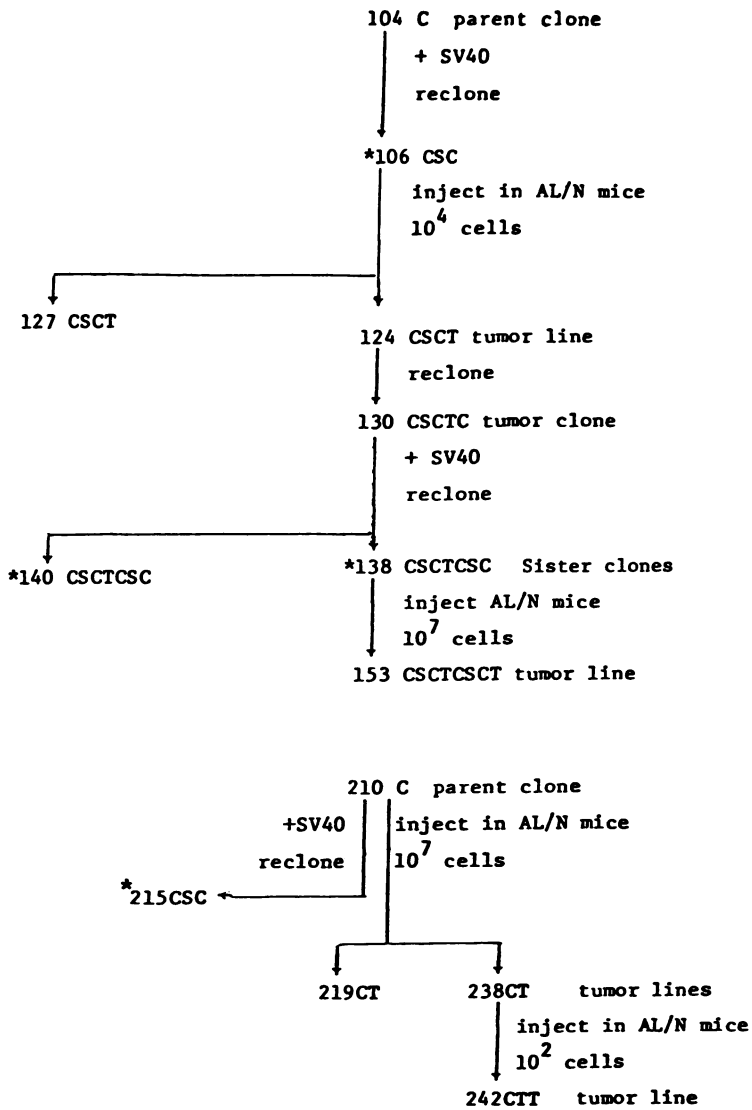

FIG. 1. Relationships of two groups of cells originating from two mouse embryo clonal cells. The numbers designate clones or cell lines. The relation and generation of the cells are indicated, with the postfix letters, in sequence (C, clone [or recloned]; S, SV40 transformed; T, tumor line) and described in the text. An asterisk before the number denotes that the cell is SV40 T antigen positive.

rapidly (≤10 weeks) lethal sarcomas. The tumorigenicity appears spontaneously in culture and remains in the reclones (23). After SV40 transformation, the SV40 T antigen-positive derivative clones, such as 106CSC, had TD_{50} values of 10^4 cells per mouse, apparently because the SV40 tumor (virus) specific transplantation antigen (TSTA) causes recognition and rejection of the cells in the immunologically competent syngeneic mouse (23). When 10^4 or 10^5 106CSC cells were injected into immunologically competent syngeneic mice and the resulting tumors were put back into culture, the cells of the resulting tumor lines (e.g., 124CSCT and 127CSCT) were T antigen and TSTA negative (23). The 124CSCT and 127CSCT cells are also negative for the early half of the SV40 DNA (11). The early half of the SV40 DNA encodes the T antigen. The T antigen is known to have high TSTA activity (3). Apparently, in a few 106CSC cells, reorganization of the DNA led to the loss of the early half of the SV40 DNA. The remain-

ing SV40 T antigen- and TSTA-positive cells are rejected (11). Thus, these rare revertant T antigen- and TSTA-negative cells are selected out and grow as tumors (11, 23). Cell lines and clones from such tumors showed the original high tumorigenicity ($TD_{50} = 10^2$) of the parent spontaneously transformed 104C clone. The relationships of these cells and their origin are outlined in Fig. 1, and their tumorigenicity and the presence of T antigen are summarized in Table 1.

Another mouse embryo fibroblast, clone 210C, was chosen as a "normal" parent clone, as this clone and its reclones have very low tumorigenicity ($TD_{50} = 10^{6.4}$) (29). Transformation of the 210C clone by SV40 does not result in any change in the low tumorigenicity or change in cell growth property in tissue culture (29). However, the derivative (215CSC) cells synthesize the SV40 T antigen (4, 29). When the parent non-SV40-transformed normal 210C cells were injected in large numbers (10^7) into the syngeneic mice, tumors were formed. When the resulting tumors (from separate mice) were reestablished in culture, the resulting tumor lines, such as 219CT and 238CT, had very high tumorigenicity ($TD_{50} \leq 10^2$). Apparently, rare variant (mutant) tumorigenic cell(s) are selected for tumorigenicity during the transplantation procedure (30). When the resulting tumorigenic cells were recloned, all of the subclones tested possessed the high tumorigenicity (recloning of 238CT gave 16 subclones, all with TD_{50} values $\cong 10^2$ [data not shown]).

Detection of the 55K protein. We used the cell lines described above to study the relationship between the amount of the 55K cellular protein and other cell properties. For standard quantitation the incorporation of [^{35}S]methionine in a 3-h labeling period was employed in preconfluent, exponentially growing cells, as described above. The fluorogram in Fig. 2 illustrates the separation of the labeled immunoprecipitated proteins. In the SV40-transformed 215CSC cells, the SV40 T antigen and substantial amounts of the ~55K protein precipitated with the specific monoclonal antibody 122, but not with the normal serum. The 55K cellular protein is known to interact with the SV40 T antigen; thus, both proteins coprecipitate with the 55K specific monoclonal antibody (8). A smaller amount of 55K protein also precipitated with the 122 antibody from the normal 210C cells and from the spontaneously transformed 219CT cells (see below). There is no substantial difference in the doubling times of 210C and 215CSC cells (29). Note that in none of these cells is there a sign of any other protein which specifically precipitates with the 55K monoclonal antibody.

The amount of the 55K protein was estimated in the various cells by using the gel separation shown in Fig. 2. The results are given in Table 1. The quantitation of the 55K protein was repro-

TABLE 1. Properties of mouse cells

Cell no.	Derivation[a]	Tumorigenicity[b] (TD_{50})	SV40 T-antigen[c]	Radioactivity in 55K-protein[d] (% cpm × 10^{-4})	
				N	122
104	C	2	−	0.09	0.8
106	CSC	4	+	0.10	17.7
127	CSCT	<2	−	0.09	1.9
124	CSCT	2	−	0.10	1.9
130	CSCTC	2	−	0.09	1.9
140	CSCTCSC	>7	+	0.12	19.7
153	CSCTCSCT	3.5	−	0.09	2.0
210	C	6.4	−	0.10 (0.08)	0.9 (0.8)
215	CSC	6.4	+	0.12 (0.09)	18.2 (19.0)
219	CT	<2	−	0.11 (0.11)	1.9 (1.8)
238	CT	2	−	0.10	1.8
242	CTT		−	0.10	1.9

[a] A cell line established from an AL/N mouse embryo fibroblast was cloned (23, 29) and two clones, 104C and 210C, were used to establish the derivative cells. The relation and history of the cells are indicated with the postfix letter (see legend to Fig. 1). All clones and cell lines were (direct) linear descendants of each other in order of presentation, representing subpopulations of cells after the given treatment (transformation by SV40, S; cloning, C; establishment of tumor lines, T, etc.), as shown in the legend to Fig. 1. Some of the cells underwent selection procedures in vivo, as described in the text, and possess different phenotypic properties.

[b] The TD_{50} denotes tumorigenicity (defined in the text). $TD_{50} = \log_{10}$ of the number of cells producing rapidly lethal sarcomas in 50% of the mice inoculated.

[c] Positive or negative by nuclear immunofluorescence and also by immunoprecipitation of the cellular extract, using SV40 T serum (cf. Fig. 2).

[d] The preconfluent exponentially growing cells were labeled in media reinforced with 5% FCS and then subjected to extraction, immunoprecipitation, and electrophoresis as described in the text and shown in Fig. 2. The total TCA-precipitable radioactivity in the cell extracts (1 ml) was on the average 2×10^8 cpm. One-half of the extract (0.5 ml) was precipitated with the normal serum (N), the other half was precipitated with 200 μl of 122 monoclonal antibody. Total immunoprecipitable radioactivity with the antibody corresponded to 0.01 ± 0.003% of the total TCA-precipitable radioactivity. After gel electrophoresis (as in Fig. 2) of approximately equal amounts of immunoprecipitated radioactivity from each cell, the amount of the 55K protein was determined by elution and counting of the gel segments as described in the text. The percent counts per minute (% cpm) of the 55K protein was then calculated as the percent radioactivity of TCA-precipitable radioactivity and was given for each cell type. Values in parentheses were obtained by densitometer scanning of the fluorograms (see text).

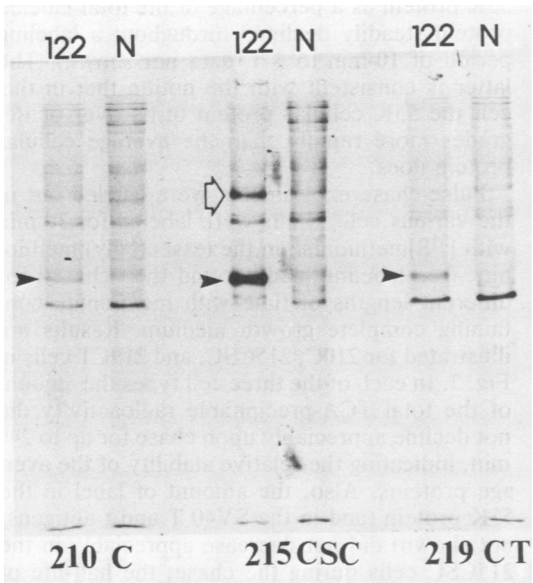

FIG. 2. Detection of the 55K protein in various cells. Labeling was under standard conditions using 50 μCi of [^{35}S]methionine per ml in subconfluent cultures for 3 h at 37°C. After extraction, immunoprecipitation with 200 μl of monoclonal 122 antibody or with normal mouse serum (N), and electrophoresis (see text), the fluorogram shows the presence of a large amount of specifically precipitating 55K protein in the 215CSC cells and small amounts in the 219CT and 210C cells (dark arrows). The SV40 T antigen-positive 215CSC cells also contain the 94K SV40 T antigen (open arrow). Overexposed fluorograms are presented, using equal input radioactivities, to detect low levels of any other specific coprecipitating protein.

ducible (data not shown; for 215CSC, see reference 2). Results were consistent for certain cell types. In all of the T antigen-negative cells, the amount of the 55K protein precipitating with the monoclonal antibody was 10- to 20-fold above the control values obtained from the same cell by immunoprecipitation with control normal sera. All of the T antigen-negative cells possessed low amounts of such specifically immunoprecipitable 55K protein ($\sim 1 \times 10^{-4}$% of the total TCA-precipitable protein in the clones and $\sim 2 \times 10^{-4}$% in the derivative "tumor" lines and clones obtained after the in vivo passage. The significance of this relatively small difference is not known).

All of the T antigen-positive cells possessed significantly more of the immunoprecipitable 55K protein ($\sim 20 \times 10^{-4}$% of the total TCA-precipitable protein as compared with T antigen-negative parent cells or as compared with the derivative revertant cells and clones). This correlation was without exception in both groups of cells, and it held through repeated cycles of SV40 transformation and selection of the T antigen-negative revertants. When the 130CSCTC T antigen-negative clone was retransformed by SV40, the resulting T antigen-positive 140CSCTCSC clone had about the same amount of ($\sim 2 \times 10^{-3}$%) 55K protein as the other T antigen-positive cells. The T antigen-negative revertant derivative line 153CSCTCSCT had $\sim 2 \times 10^{-4}$% 55K protein, similar to that of the T antigen-negative 130CSCTC clone. The above results are consistent with previous observations in which the SV40 T antigen interacts with and stabilizes the cellular 55K protein (8, 12, 19, 25); further, they extend such observations by presenting data on SV40 T antigen-negative revertant cells and on retransformations of such revertants by the SV40 viruses (see below).

In these groups of mouse fibroblast cells there was no correlation at all between the cellular tumorigenicity and the amount of the 55K protein synthesized and detected under the standard conditions employed. The normal clone, 210C, which possessed very low tumorigenicity, the derivative rare "mutant" 219CT cells and the 238CT cells, both of which possessed high tumorigenicity ($TD_{50} \leq 10^2$), and all of the cells of the first groups which also possessed similar high tumorigenicity (104C, 124CSCT, 127CSCT, and 130CSCTC) possessed low and near equal amounts of detectable 55K protein (1×10^{-4}% to 2×10^{-4}% of the total protein). Thus the amount of detectable 55K cellular protein under the standard conditions is not a correlate of cellular tumorigenicity. The above data document and significantly extend preliminary statements made concerning the presence or absence of the 55K protein in some of these cells (24).

Synthesis and half-life of the 55K protein in various cells. For further study, three closely related cell lines, the parent 210C clonal cells, the SV40-transformed derivative 215CSC cells, and the tumorigenic variant derivative 219CT cells, were selected. Using different serum concentrations (2, 5, and 10% [vol/vol] FCS), but otherwise employing the standard labeling conditions as in Table 1, there was only a slight (~10%) serum concentration (or by implication of cell growth rate)-dependent increase in the relative amounts (percent of total TCA-precipitable protein) of the 55K protein in all three cells (data not shown). By using 30-min labeling periods instead of the 3-h periods, there was a significant (~threefold) increase in the relative amount of the labeled 55K protein over that of the average TCA-precipitable cellular proteins in the two non-SV40-transformed cells (from 1×10^{-4} and 2×10^{-4} to 7×10^{-4}). This is consis-

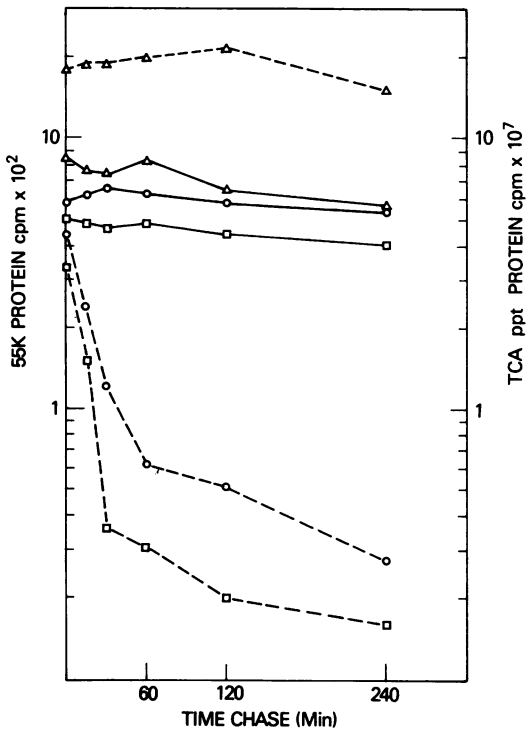

FIG. 3. Pulse-chase experiments in three related cells. Equal amounts of the parent nontumorigenic 210C cells (○), the derivative tumorigenic 219CT cells (□), and the derivative SV40-transformed 215CSC cells (△) were seeded, grown, and labeled during exponential growth phase in 150-cm² Falcon flasks with 50 μCi of [³⁵S]methionine per ml at 37°C in methionine-free medium containing 5% FCS for 30 min. For chase, the cells were washed well, and the medium was replaced with complete nonradioactive medium containing 5% FCS for the lengths of time indicated. Immunoprecipitation was with 122 hybridoma supernatant. After analysis (described in Fig. 2 and Table 1) the radioactive counts in the total TCA-precipitable protein (solid lines) and in the 55K protein (broken lines) were determined.

tent with a (labile) 55K protein being synthesized more rapidly in the non-SV40-transformed cells than the average TCA-precipitable protein. (The relative amounts of label in the 55K protein in the SV40 T antigen-positive 215CSC cells remained about the same [$\sim 2 \times 10^{-3}$%] after either 30-min or 3-h labeling periods. This latter result implies that complexing with the T antigen and stabilization occur rapidly [i.e., ≤30 min] after the synthesis of the 55K protein in SV40-transformed cells.)

Detailed kinetic analysis was carried out on 210C cells. The uptake of label into the 55K protein was faster than the uptake into the average cell proteins using up to a 20-min labeling period; however, the relative amount of the 55K protein as a percentage of the total labeled protein steadily declined throughout a labeling period of 10-min to 3-h (data not shown). The latter is consistent with the notion that in this cell the 55K cellular protein turns over or degrades more rapidly than the average cellular protein does.

Pulse-chase experiments were carried out in the various cells. Cells were labeled for 30 min with [³⁵S]methionine in the (essentially) methionine-free labeling medium and then chased for different lengths of time with methionine containing complete growth medium. Results are illustrated for 210C, 215CSC, and 219CT cells in Fig. 3. In each of the three cell types the amount of the total TCA-precipitable radioactivity did not decline appreciably upon chase for up to 240 min, indicating the relative stability of the average proteins. Also, the amount of label in the 55K protein (and in the SV40 T and t antigens, not shown) did not decrease appreciably in the 215CSC cells during the chase; the half-life of >10 h can be estimated. In contrast, in the two non-SV40-transformed cells, the amount of label in the 55K protein decreased rapidly, especially during the first 60 min of the chase. This occurred in both the tumorigenic 219CT cells and in the normal 210C, and a half-life of 15 to 20 min can be estimated for the 55K protein. Turnover of the 55K protein and of the SV40 T antigen in the other cell lines shown in Fig. 1 was measured by similar pulse-chase experiments, and results are summarized in Table 2.

TABLE 2. Half-life estimates[a] in the various cells

Cell[a]	Half-life	
	55K protein[b]	Large T antigen
104C	120 min	
*106CSC	>10 h	>10 h
127CSCT	60 min	
124CSCT	60 min	
130CSCTC	60 min	
*140CSCTCSC	>10 h	>10 h
*138CSCTCSC	>10 h	>10 h
153CSCTCSCT	60 min	
210C	60 min	
*215CSC	>10 h	>10 h
219CT	60 min	

[a] Asterisk before the cell number denotes the SV40 T antigen-positive clone.

[b] From pulse-chase experiments similar to those described in the legend to Fig. 3. These estimates on the T antigen-negative cell lines are only approximate, and accurate values are probably lower (see Fig. 3). In these experiments there was no 15-min chase, and for immunoprecipitation, a selected serum from hamsters bearing SV40-induced tumor was used (1, 24).

The results in Fig. 3 and Table 2 confirm earlier similar observations on the 3T3 nontumorigenic mass mouse cell line and on an SV40-transformed derivative cell (25). They also show, for the first time, the instability or high turnover rate of the 55K protein in several highly tumorigenic spontaneously transformed mouse cells.

Absence or low level of the 55K protein in primary cell cultures from a tumor. We have observed previously that primary embryo cell cultures from midgestation or earlier mouse embryos possess significant amounts (~50%) of a 55K protein which is similar to that detected in the SV40-transformed mouse cells. We also found that after tissue culture transfers the amount of the 55K protein rapidly declines to the low levels observed in established cell lines and clones (2, 24). A primary cell culture was prepared from a tumor (242CTT) and tested for stable 55K protein by the standard 3-h labeling technique. The amount of specifically immunoprecipitating 55K protein was low (Fig. 4) in quantity and was comparable to the other non-SV40-transformed established cell lines and clones reported in Table 1. Thus, in contrast to primary mouse embryo cells, the primary cells from this mouse tumor, originating from the highly malignant 238CT cells, do not contain significant amounts of (stable) 55K protein.

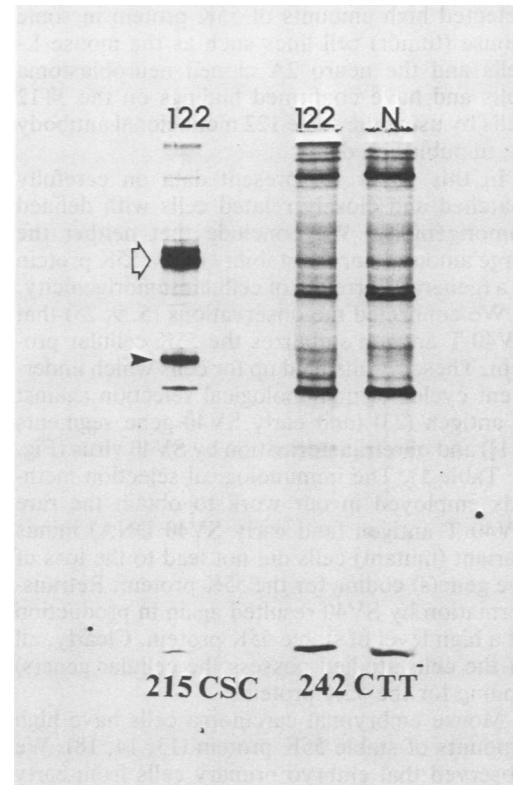

FIG. 4. The 55K protein is not detectable in primary culture of a tumor line. The 238CT tumor line cells were used to form a tumor in syngeneic mice as described in the text, and the primary culture (denoted 242CTT) was labeled under the standard conditions for 3 h and tested for 55K protein as described in the legend to Fig. 3. For positive control the 215CSC cells are also shown. Overexposed fluorograms were used to detect any other specific coprecipitating protein. No specific immunoprecipitable 55K protein is detectable in the 242CTT primary culture.

DISCUSSION

We quantitated the amount of the stable 55K cellular protein, as detected after 3-h labeling of exponentially growing cells, by using optimal conditions for immunoprecipitation and a monoclonal antibody. We compared closely related clonal derivative mouse cells with or without SV40 transformation. Also, we determined carefully the tumorigenicity of the cells. In the mouse cells studied which were not transformed by SV40 or which were selected out by immunological means and were T antigen negative, there was no correlation at all between cellular tumorigenicity and the amount of the detectable 55K protein. A clone with low or negligible tumorigenicity had approximately the same low amount of 55K protein as that of the directly derived highly tumorigenic cell lines (see lower half of Table 1). A similar low amount of 55K protein was present in several other T antigen-negative and highly tumorigenic clones and cell lines (see upper half of Table 1; also reference 4). We also determined the stability of the 55K protein in the closely related (clonal) mouse cells. All of the highly tumorigenic cells tested possessed unstable 55K protein, with half-lives ($\leq$60 min) similar to that of a (parent) cell with low or negligible tumorigenicity (Table 2).

Others reported that a similar 53 to 55K protein was present in mouse cells transformed by diverse means, but not in normal fibroblast (6); they postulated that the protein is transformation related, but gave no tumorigenicity data (6, 9). Many (but not all) human tumor lines were found to possess the 55K protein (5, 7). In some normal cells, such as in thymocytes (6, 9) and in kidney epithelial cells (7), the 55K protein was also observed, presumably related to the growth of these cells in culture; however, no quantitative data were presented. In the normal mouse mass 3T3 cell line only a small amount of the 55K protein was detected (15, 25). In the tumorigenic 3T12 cells a higher amount was detected (18), and in some experiments the 122 monoclonal antibody was used (8). We have also

detected high amounts of 55K protein in some mouse (tumor) cell lines such as the mouse L-cells and the neuro 2A cloned neuroblastoma cells and have confirmed findings on the 3T12 cells by using the same 122 monoclonal antibody (8; unpublished data).

In this paper we present data on carefully matched and closely related cells with defined tumorigenicity. We conclude that neither the large amount, nor the stability of the 55K protein is a (general) correlate of cellular tumorigenicity.

We confirmed the observations (5, 9, 25) that SV40 T antigen stabilizes the 55K cellular protein. These results held up for cells which underwent cycles of immunological selection against T antigen (23) (and early SV40 gene segments [11]) and of retransformation by SV40 virus (Fig. 1, Table 1). The immunological selection methods employed in our work to obtain the rare SV40 T antigen (and early SV40 DNA) minus variant (mutant) cells did not lead to the loss of the gene(s) coding for the 55K protein. Retransformation by SV40 resulted again in production of a high level of stable 55K protein. Clearly, all of the cells studied possess the cellular gene(s) coding for the 55K protein.

Mouse embryonal carcinoma cells have high amounts of stable 55K protein (13, 14, 18). We observed that embryo primary cells from early mouse, rat, and hamster embryos produce a high amount or stable 55K protein or both (2, 24). In contrast, primary tumor cells did not produce (a significant amount of) stable 55K protein (Fig. 4). The reason for this is unknown; however, this finding is consistent with the major thesis of the present paper, namely, that the amount or the stability (or both) of the 55K protein is not a correlate of tumorigenicity.

The amount of 55K protein in mouse, rat, and hamster embryo primaries declines with embryo age (2). The two-dimensional fingerprints of the [^{35}S]methionine tryptic peptides of the 55K proteins from the early embryos of these species and from the SV40 transformed cells (for example, from 215CSC) of the same species are virtually identical (2). The two-dimensional fingerprints of these peptides (and also of monkey and human SV40-transformed cells) show evolutionary conservation of the 55K proteins (2, 26, 27). We presume that 210C or 219CT cells possess the "embryo" protein similar to that of the related 215CSC cells. However, because of the low amounts of the 55K protein present in the 210C and 219CT cells, we have no two-dimensional fingerprint data to support this proposition.

There was no reproducibly detectable coimmunoprecipitating (putative stabilizing) protein observed in the embryo cells (2) and in the mouse embryonal carcinoma cells (not shown) or in the non-SV40 transformed normal or tumorigenic cells studied in this paper. We are further analyzing these proteins and are also attempting to unravel control mechanisms for the expression of the 55K protein in various cells, mechanisms which, in part, may operate on other than posttranslational level.

ACKNOWLEDGMENT

We thank E. Gurney for the monoclonal antibody 122-producing hybridoma cell and for advice.

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