Differentiation of Human Epidermal Cells Transformed by SV40

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ABSTRACT Human epidermal cells were transformed with DNA from wild-type SV40 virus or with DNA from a temperature-sensitive A mutant (*tsA209*). The SV40-transformed cells differed from nontransformed cells in their morphologic appearance, growth properties, and expression of certain characteristics associated with differentiation. The transformed cells were more variable in size and shape than their nontransformed counterparts and were less stratified and less keratinized. While the growth properties of the cells were similar under optimal growth conditions, the transformed cells could be propagated under stringent growth conditions that did not support the growth of nontransformed human epidermal cells. The transformants still required a 3T3 feeder layer for growth, remained anchorage dependent as assayed in soft agar, and were not tumorigenic in athymic nude mice. The expression of certain differentiated functions of the human epidermal cell, the presence of keratins and cross-linked envelopes, was decreased in the transformed cells, and these functions could be restored at the nonpermissive temperature in the *tsA209* transformed cells.

Human epidermal cells in culture undergo a defined program of differentiation reminiscent of that of the natural epithelium. Analogous to the in vivo situation, the cells of the cultured epithelium stratify (27). They consist of a basal layer of mitotically active cells giving rise to more differentiated cells in the upper cell layers (37). The cells contain abundant keratins (38) and in the final stages of differentiation assemble a cross-linked envelope beneath their plasma membrane (14, 29).

The establishment of transformed human epidermal cell lines offers numerous advantages in the study of cellular regulation and differentiation. First of all, normal human epidermal cells undergo senescence after a limited number of passages (15, 28). The establishment of cell lines would facilitate studies requiring prolonged passaging of human epithelial cells. Secondly, these studies would enable analyses of the transformed phenotype in human epithelial cells. Lastly, the use of a temperature-sensitive (ts) mutant allows one to modulate the expression of the transformed phenotype. At the permissive temperature (33°C), the transformed phenotype is expressed. At the nonpermissive temperature (40°C), the effects of the transforming virus are reversed and normal differentiated functions are reexpressed.

In our study, we have transformed human epidermal cells with DNA segments containing the transforming regions from both wild-type SV40 and from a ts mutant (*tsA209*). Steinberg and Defendi (35) have previously reported the transformation of human epidermal cells with wild-type SV40 virus. We used DNA fragments of SV40 that encompassed the entire transforming region, but were deleted of late sequences, to insure a nonpermissive virus-host cell interaction. These transformed human epidermal cells differ morphologically and biologically from their nontransformed counterparts. The expression of the two main properties of epidermal cell differentiation, keratins, and cross-linked envelopes, is dramatically altered in the transformed cells. Suppression of the transformed phenotype by growth at the restrictive temperature restores these functions.

MATERIALS AND METHODS

Tissue Preparation and Cell Culture of Human Epidermal Cells: Cultures of human epidermal cells were established from newborn human foreskin by a modification of the method of Hawley-Nelson et al. (18). Briefly, the tissue was placed onto filter paper with the dermis side down and floated overnight at 4°C on 0.25% trypsin dissolved in calcium-magnesium-free PBS (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY). The epithelium was lifted away from the dermis, minced, and placed in a trypsinizing flask containing 0.25% trypsin at 37°C for an additional 45 min. The cells were pelleted by centrifugation for 5 min at 800 *g* and resuspended in growth medium. When grown under optimal growth conditions, cells were inoculated into Medium 199 containing 10% fetal calf serum (FCS) and supplemented with 5 μg/ml insulin, 5 μg/ml transferrin, 20 ng/ml EGF, 5×10^{-7} M hydrocortisone, and 1×10^{-10} M cholera toxin (S. Banks-Schlegel and H. Green, unpublished data; 6).

For stringent growth conditions, cells were grown in Medium 199 containing only 2% FCS and 5×10^{-7} M hydrocortisone. Cells (10^4) were inoculated into 60-mm tissue culture dishes containing 5×10^5 irradiated 3T3 cells (27), and the medium was changed every 3 to 4 d.

Transformation of Human Epidermal Cells by DNA Transfection Using a Subgenomic Fragment of SV40 and a ts Mutant (tsA-209): When primary cultures of human epidermal cells were ~90% confluent, they were disaggregated into single cells with a solution containing 1% polyvinylpyrrolidine, 0.02% EGTA, and 0.1% trypsin dissolved in calcium-magnesium-free PBS (PET) (21). Following dissociation from the surface of the dish, the cells were pooled, pelleted by centrifugation, and resuspended in medium. Human epidermal cells (10^6/ml) were transfected in suspension with the cloned large *HhaI/Eco*RI fragment either of wild-type SV40, of a conditional mutant for the A gene function *tsA209* (22), or a conditional mutant for the A gene function which does not encode a functional small t antigen *tsA288* (33). This fragment contains an intact "early" or transforming region and is deleted of a segment in the late region essential for the expression of late genes and, therefore, for the full permissive expression of the virus. The DNA (5 μg/ml) was transfected onto the cells by calcium co-precipitation (13) with calf thymus DNA (25 μg/ml) as a carrier. Following exposure of the cells in suspension to DNA for 2 h, the cells were plated onto an irradiated 3T3 feeder layer and incubated at 37°C (wild-type DNA) or at 33°C (*tsA209* and *tsA288* DNAs). At each passage, the cells were monitored for the presence of SV40 T antigen.

Immunofluorescence Detection of SV40-T Antigen in Transformed Human Epidermal Cells: Cells on coverslips were fixed in acetone:methanol (1:1), stained with pooled serum from SV40 tumor-bearing hamsters for T antigen, and assayed by indirect immunofluorescence using fluorescein isothiocyanate-conjugated rabbit anti-hamster serum.

Immunoprecipitation of SV40-T Antigen from Transformed Human Epidermal Cells: Subconfluent cultures of transformed and control human epidermal cells were incubated for 4 h at 33° or 37°C with S^{35}-methionine (250 μCi/ml). Anti-SV40 T serum was incubated with the labeled NP-40 cell extracts, and the immunoprecipitated proteins were analyzed on 10% SDS polyacrylamide gels (20).

Analysis of Keratin Proteins Extracted from Normal and Transformed Human Epidermal Cells: Normal and transformed human epidermal cells were homogenized thoroughly in 20 mM Tris-HCl, pH 7.4, to remove most nonkeratin proteins (5, 38). The proteins were pelleted by centrifugation in a Beckman J2-21 centrifuge at 12,000 rpm for 15 min. This procedure was then repeated twice. The final pellet, containing mostly keratin proteins, was solubilized in 20 mM Tris-HCl, pH 7.4, 2% SDS, and 10 mM dithiothreitol (DTT). The protein from an equivalent number of cells was analyzed on an 8.5% SDS polyacrylamide slab gel (20).

Ionophore-induced Terminal Differentiation of Normal and Transformed Human Epidermal Cells: With the exception of HE-209, cultures of normal (HE) and transformed (HE-SV) human epidermal cells were grown at 33°C (permissive temperature for transformation in the ts mutant) and 40°C (restrictive temperature for transformation in the ts mutant). In the case of the ts mutant, HE-209, all cultures were grown at 33°C until the cultures were ~80–90% confluent. At this point, half of the cultures were switched to 40°C and left for 5 d before analysis. When the cultures were just confluent, the cells were detached from the dish with PET. After inactivation of the trypsin with serum, the cells were washed once with serum-free medium and then resuspended in 1.5 ml of serum-free medium containing 50 μg/ml of a solution of X-537A, a calcium ionophore (31), dissolved in dimethyl sulfoxide. After 3.5 h at 37°C, the cells were pelleted by low speed centrifugation and resuspended in PBS. A sample was removed to a hemocytometer chamber to obtain a total cell count. Small samples of concentrated solutions of SDS and reducing agent (DTT) were then added to obtain a final concentration of 2% and 10 mM, respectively. After a 5-min incubation at room temperature, a sample was again counted in a hemocytometer chamber to determine the number of cross-linked envelopes (2, 37).

Growth of Normal and Transformed Human Epidermal Cells in Soft Agar: Duplicate 60-mm dishes were seeded with 10^4 cells and 10^5 cells in 2 ml of 0.35% Noble-agar (Difco Laboratories, Detroit, MI) and growth medium over 4 ml of 0.6% agar base (23). Cultures were maintained at 33°, 37°, and 40°C and the cultures were overlaid twice weekly with 0.35% agar. Colony formation was assayed at 2 and at 3 wk.

Tumorigenicity Studies: Cells (5×10^6 to 10^7) derived from cultures of HE and HE-SV and HE-209 human epidermal cells were injected into athymic nude mice. In some cases, the animals were merely monitored weekly for tumor formation. In other cases, the animals were sacrificed after 8 d by cervical dislocation. The nodules which had formed were excised surgically and processed for histological examination.

RESULTS

Transformation of Human Epidermal Cells

Cells obtained from primary cultures of human epidermal cells (HE) were transfected with the large HhaI/Eco RI DNA fragment either of wild-type SV40 virus, of a tsA mutant of SV40 (*tsA209*) or of a ts double mutant of SV40 (*tsA288*). The cells were plated on irradiated 3T3 feeder cultures and were passaged just before confluence at 2–3 wk. While the untransfected cultures of human epidermal cells underwent senescence after four passages, the cultures transfected with DNA from wild-type SV40 and *tsA209* escaped senescence and were established into lines. Cultures transfected with DNA from the *tsA288* double mutant underwent senescence and could not be propagated beyond passage 4, similar to the control cultures. SV40 tumor (T) antigen was monitored by indirect immunofluorescence and, by the fifth passage, 60% of both the wild-type SV40 early region DNA transfected cells (HE-SV) and *tsA209* early region DNA transfected cells (HE-209) were found to be positive. By passage six, 100% of the cells were T-antigen positive. Immunoprecipitation of S^{35}-labeled proteins from the SV40-transformed human epidermal cells with SV40-tumor specific antiserum revealed a major band at 94 kdalton and a minor band at 17 kdalton, characteristic of SV40 large and small T antigens (Fig. 1). These proteins were not detected in nontransformed human epidermal cells and were not precipitated from the transformed cells by normal hamster serum.

Morphology of SV40 Transformed Human Epidermal Cells

Cultures of normal and transformed human epidermal cells

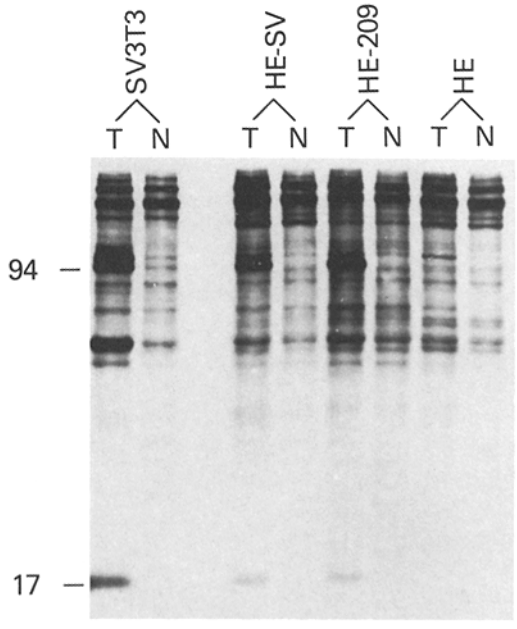

FIGURE 1 Immunoprecipitation of radio-labeled proteins extracted from normal and SV40-transformed human epidermal cells with SV40-tumor specific antiserum. Subconfluent cultures of HE, HE-SV, and HE-209 cells were labeled with S^{35}-methionine (250 μCi/ml) for 4 h at room temperature. The proteins were extracted from the cells, immunoprecipitated with either SV40-tumor specific antiserum (*T*) or normal hamster serum (*N*), and analyzed by SDS PAGE and autoradiography. Note the presence of a major band at 94 kdalton and a minor band at 17 kdalton in the transformed cells, characteristic of SV40 large and small T antigens.

(seventh passage) can be morphologically distinguished (Fig. 2). Nontransformed human epidermal cells are uniform in appearance, forming a cobblestone monolayer which undergoes an olderly and organized stratification when grown at either 33°C (Fig. 2*A*) or at 40°C (Fig. 2*B*). HE-SV cells grown at 33°C (Fig. 2*C*) or 40°C (Fig. 2*D*) were pleomorphic, varying in size and shape, tended to pile on top of one another in an unorganized fashion, and in some areas appeared more spindle-shaped than the nontransformed counterpart. A small proportion of the cells were multinucleate. At the permissive temperature for transformation (33°C), the human epidermal cells transformed by *tsA209* DNA (HE-209) resembled the cells transformed by wild-type SV40 DNA as shown in Fig. 2*E*. At the nonpermissive temperature (40°C), however, the cells were more uniformly cobblestone in appearance, did not pile up, and generally had the appearance of nontransformed human epidermal cells (Fig. 2*F*). Thus, the altered morphologic appearance of the SV40-transformed human epidermal cells ap-

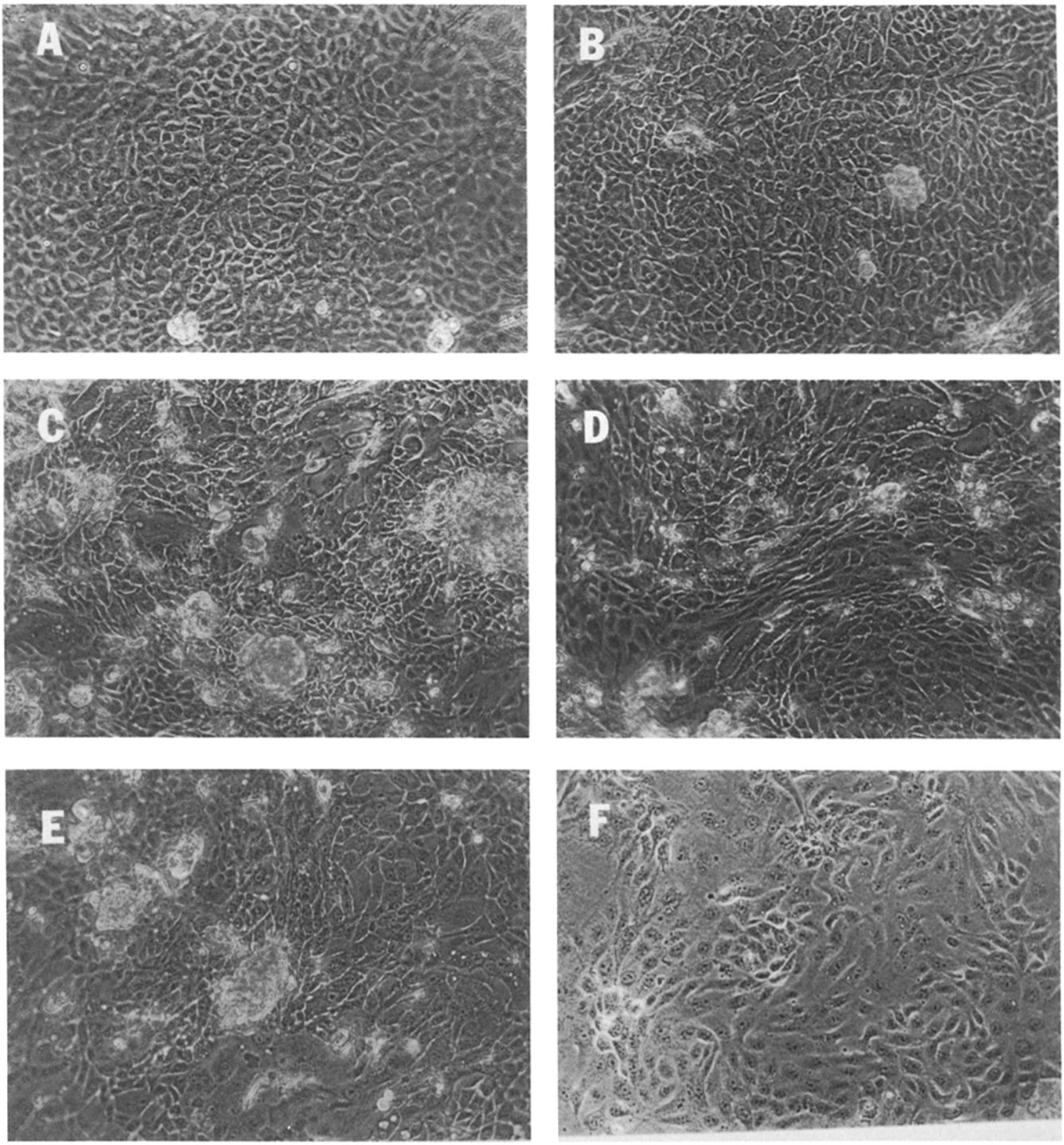

FIGURE 2 Morphology of cell cultures of normal and transformed human epidermal cells examined by phase-contrast microscopy. Cells were grown to confluence as described in Materials and Methods. Nontransformed human epidermal cells at 33°C (*A*) and 40°C (*B*); HE-SV cells at 33°C (*C*) and 40°C (*D*); HE-209 cells at 33°C (*E*) and 40°C (*F*). × 320.

pears to require a functional SV40 A gene product.

Histologic examination of the cultured epithelium derived from nontransformed (Fig. 3 *A*) or transformed human epidermal cells (Fig. 3 *B*) also indicates that the nontransformed and SV40-transformed epithelial cells differ morphologically. The cultured epithelium from the transformed cells does not undergo the orderly stratification of the nontransformed cells. Less keratinization of the most superficial layers of the epithelium of the transformed cells is demonstrated by the absence of keratohyalin granules that can be seen in the nontransformed counterparts (Fig. 3).

Growth Characteristics of SV40-transformed Human Epidermal Cells

The growth characteristics of HE-SV cells, HE-209 cells, and their nontransformed counterparts were next examined at both the permissive (33°C) and the restrictive (40°C) temperatures. Under optimal growth conditions, HE, HE-SV, and HE-209 cells grew to similar saturation densities (approximately 3.0×10^6 cells) with similar doubling times (~26 h) at 33°C (Fig. 4 *A*). At 40°C, however, HE-209 cells grew more slowly than the other cells with a doubling time of 57.6 h and stopped growing at a density of 0.57×10^6 cells (Fig. 4 *B*).

Under more stringent growth conditions, in which the cells were grown in medium supplemented only with 2% FCS and hydrocortisone, a difference between SV40-transformed human epidermal cells and their nontransformed counterparts was observed. At 33°C, the HE-SV and HE-209 cell lines attained a higher saturation density (3.0×10^6 cells and 1.7×10^6 cells, respectively) than control cultures of nontransformed HE cells (0.4×10^6 cells), Fig. 4 *C*. In addition, the doubling times for the transformed cell lines, HE-SV and HE-209, were ~38.4 and 43.2 h, respectively, while the nontransformed cells underwent only three to four doublings. At 40°C, both the nontransformed cells and the HE-209 cells grew poorly, reaching densities of only 0.25×10^6 cells and 0.12×10^6 cells, respectively (Fig. 4 *D*), indicating that the ability to propagate under stringent growth conditions requires a functional SV40 A gene product. In contrast, the HE-SV cells grew well, attaining a saturation density of 2×10^6 cells (Fig. 4 *D*).

Human epidermal cells, unless grown under special conditions (26), require a layer of irradiated 3T3 cells for clonal growth (27). We, therefore, examined the requirement of the SV40-transformed human epidermal cells at their sixth and

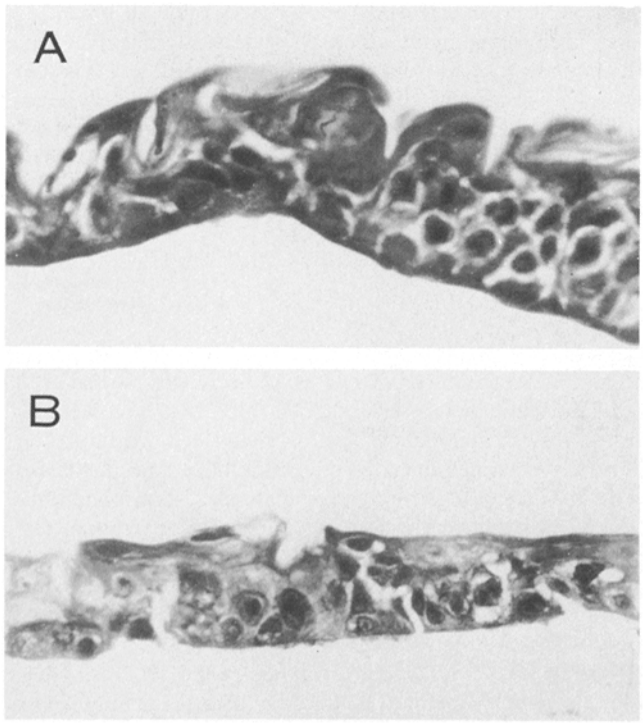

FIGURE 3 Cultured epithelium derived from normal and transformed human epidermal cells. Normal and transformed human epidermal cells were grown to confluence at 33°C as described in Materials and Methods. After incubation for 1 h at 37°C with the enzyme Dispase II (3, 17), the epithelium detached as an intact sheet of cells. The epithelium was attached to filter paper, fixed, embedded, sectioned, and stained for histological examination. (*A*) Cultured epithelium from HE cells. (*B*) Cultured epithelium from HE-SV cells. × 160. Note the more limited stratification and absence of keratohyalin granules in the epithelium from the transformed cells.

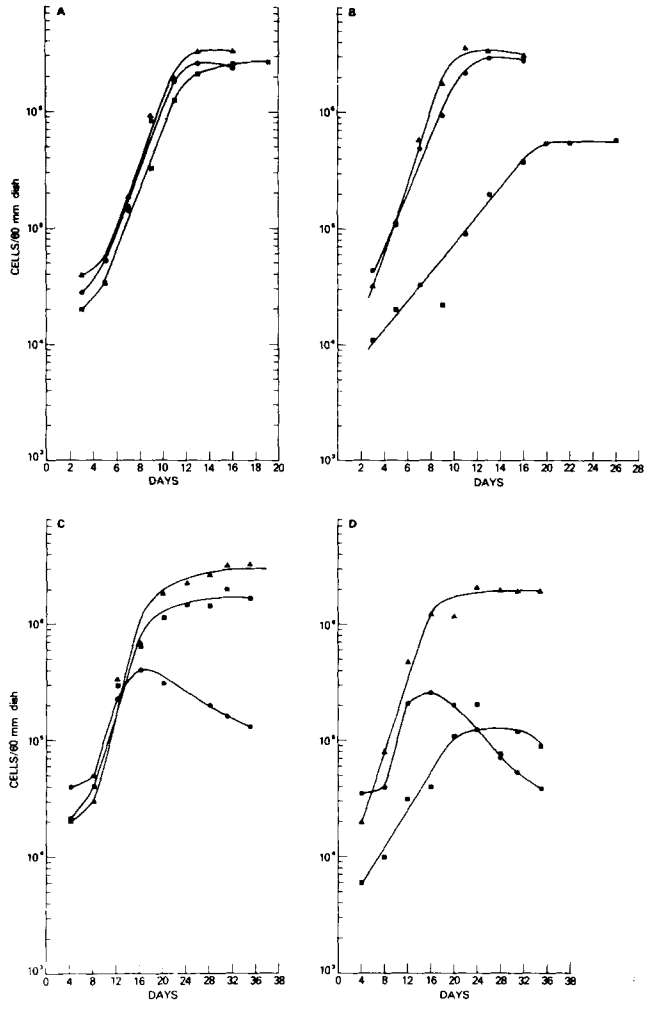

FIGURE 4 Growth of normal and transformed human epidermal cells under optimal and stringent conditions. HE, HE-SV, and HE-209 cells were grown under either optimal conditions (Medium 199 containing 10% FCS supplemented with 5 μg/ml insulin, 5 μg/ml transferrin, 20 ng/ml EGF, 5×10^{-7} M hydrocortisone, and 10^{-10} M cholera toxin) or stringent conditions (Medium 199 containing only 2% FCS and 5×10^{-7} M hydrocortisone). HE (●), HE-SV (▲), and HE-209 (■) cells were grown under optimal growth conditions at 33°C (*A*) or 40°C (*B*) or under stringent conditions at 33°C (*C*) or 40°C (*D*).

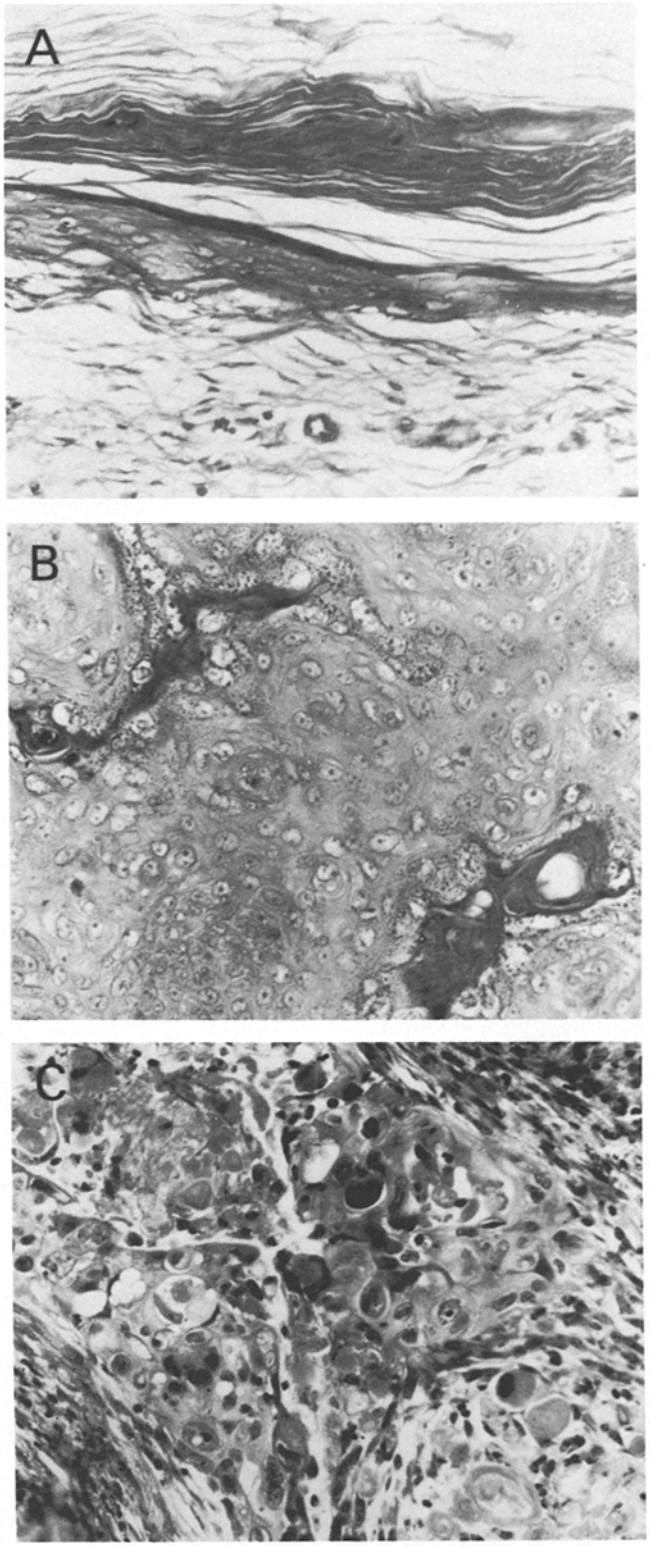

FIGURE 5 Morphology of nodules of normal and transformed human epidermal cells injected into athymic nude mice. Cells (approximately 10^7) obtained from just confluent cultures of HE, HE-SV, and HE-209 cells were injected subcutaneously into athymic nude mice. 8 d after inoculation, the nodules were removed, fixed, embedded, sectioned, and stained for histological examination. (*A*) Epithelial cyst formed by normal human epidermal cells. Note the arrangement of the cells into a fully organized, stratified squamous epithelium resembling epidermis. (*B*) Cellular wall of cyst formed by HE-SV transformed human epidermal cells. Note the unorganized arrangement of the cells with features characteristic of a keratinizing, squamous cell carcinoma. (*C*) Cellular mass formed by HE-209 transformed human epidermal cells. Note the unorganized arrangement of the cells, similar to the HE-SV cells. × 100.

eighth passage for a 3T3 feeder cell layer. The HE-SV, HE-209 cells, and nontransformed HE cells were plated at clonal densities (5×10^3 cells) onto 60-mm dishes in the presence or absence of irradiated 3T3 cells. In the case of HE-SV cells, one to three colonies (colony-forming efficiency = 0.02–0.06%) formed on the dish without feeders, while for HE-209 cells and nontransformed HE cells no colonies formed. When these HE-SV transformed cells which had arisen in the absence of feeder support were subcultured at clonal densities again with or without feeder support, however, they grew well only in the presence of feeder support. These results indicate that the SV40-transformed HE cells, like normal HE cells, require a 3T3 feeder layer for growth.

To test for anchorage-independent growth, we plated HE-SV and HE-209 transformed human epidermal cells from sixth and eighth passages at 10^4 and 10^5 cells per dish in 0.35% agar. Control cultures of nontransformed human epidermal cells were treated identically. The cultures were maintained at 33°, 37°, and 40°C and assayed at 2 and 3 wk for colony formation. In each case, no colonies were detected.

To assess the tumorigenicity of the SV40 transformed human epidermal cells, we injected subcutaneously into athymic nude mice approximately 10^7 cells obtained from cultures of HE-SV cells, HE-209 cells, and nontransformed HE cells. In each case, nodules formed within 24 h and increased in size for ~2 wk before regressing. Examination of the nodules formed by these cells at 2 wk, however, indicated some remarkable morphologic differences. In agreement with a previous report (11), human epidermal cells injected into nude mice formed an organized keratinized epithelium resembling normal epidermis (Fig. 5*A*). The nodules derived from the transformed cells, however, did not resemble normal epidermis but had much less differentiated characteristics. HE-SV cells formed a structure resembling a keratinizing, squamous cell carcinoma (Fig. 5*B*). The nodules formed by HE-209 were also very undifferentiated in appearance with bizarre cells and prominent individual cell keratinization (Fig. 5*C*).

Differentiated Properties of SV40-transformed Human Epidermal Cells

The differentiated characteristics of human epidermal cells in tissue culture were next examined for HE-SV and for HE-209 cells at the permissive and restrictive temperature. The properties examined included the quantitative and qualitative analysis of keratin proteins, the analysis of the ionophore-induced cross-linked envelopes as a measure of terminal differentiation, and pattern formation.

KERATIN PROTEINS: Following repeated extraction of the nontransformed and transformed human epidermal cells with dilute aqueous buffer, the remaining water-insoluble proteins were analyzed on SDS polyacrylamide gels (Fig. 6). In each case, the sample was derived from an equivalent number of cells. The proteins were found to be concentrated in the region of 40 to 58 kdalton, characteristic of keratin proteins. Immunoprecipitation of radio-labeled proteins from these cells with keratin antiserum confirmed that these proteins were keratins (data not shown). At both 33° and 40°C, HE-SV transformed human epidermal cells were found to contain reduced quantities of keratins when compared to the nontransformed normal

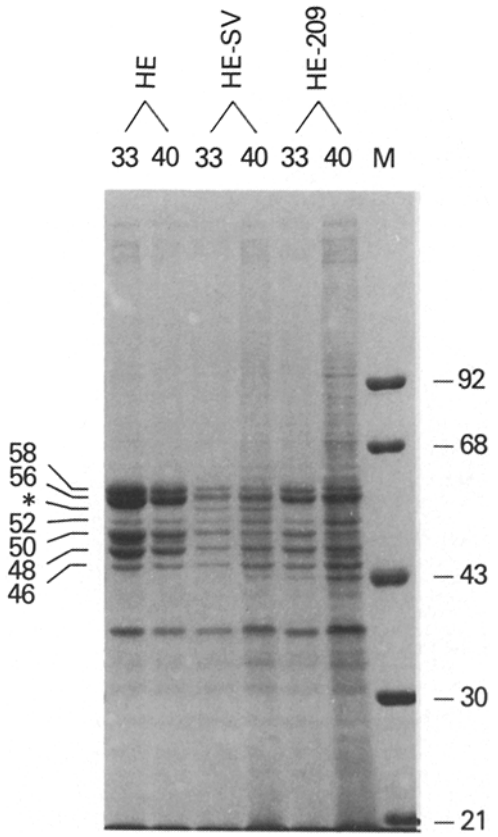

FIGURE 6 Keratin proteins extracted from normal and transformed human epidermal cells. Keratin proteins were extracted from confluent cultures of HE, HE-SV, and HE-209 cells grown at 33°C and 40°C. A protein sample derived from an equivalent number of cells was analyzed by SDS PAGE. The position of molecular weight markers (lane designated *M*) run concurrently on the gel is shown at the right-hand side of the gel. The molecular weights ($\times 10^3$) assigned to the keratin proteins from the nontransformed HE cells are shown at the left-hand side of the gel and were derived from the best fitting curve for the standards. The 54-kdalton keratin present in the HE-SV transformed cells is indicated by an asterisk.

counterparts. Qualitatively, the keratins present in nontransformed cells were all present in the HE-SV cells although an additional 54-kdalton species was detectable in the HE-SV cells. The decreased amount of keratins present in SV40-transformed HE cells appears to be functionally linked to the expression of the transformed phenotype. Whereas the HE-209 cells also made lower levels of keratins at the permissive temperature (33°C), keratin production was increased significantly at the restrictive temperature and approached levels found in normal human epidermal cells grown at 40°C. Qualitatively, the keratins present in the transformed HE-209 cells were similar to those of normal cells, and only the HE-SV cells contained the 54-kdalton keratin species.

CROSS-LINKED ENVELOPES: Terminal differentiation of normal and transformed human epidermal cells was induced by the calcium ionophore, X537A (31). The extent of terminal differentiation was assayed by determining the percentage of cells which contained cross-linked envelopes, structures which are resistant to SDS and reducing agent (2, 37) (Table I). At the permissive temperature (33°C), only 45.8% and 28.2% of HE-SV and HE-209 transformed cells, respectively, were able to form cross-linked envelopes compared to 89.6% of the

TABLE I

Cross-linked Envelopes in HE and HE-SV and HE-209 Cells Grown at 33°C and 40°C

Cells	Temperature	Cross-linked envelopes
	°C	% of total cells
HE	33	89.6 ± 1.2*
	40	67.2 ± 0.2
HE-SV	33	45.8 ± 2.9
	40	29.0 ± 5.8
HE-209	33	28.2 ± 4.0
	40	62.8 ± 0.5

Normal and transformed human epidermal cells were grown to confluence and then induced to undergo terminal differentiation by the calcium ionophore, X537A, as described in the Materials and Methods. Following estimation of the total number of cells, the cells were dissolved in SDS and DTT at a final concentration of 2% and 10 mM, respectively, leaving behind only the cross-linked envelopes. Their numbers were determined by counting in a hemocytometer chamber under phase optics. The percentage of total cells containing envelopes was calculated.

* Mean ± range of two experiments.

nontransformed cells. Whereas only 29.0% of the HE-SV transformed cells grown at 40°C contained cross-linked envelopes, at this restrictive temperature 62.8% of the HE-209 cells were found to contain envelopes, approximating the value found for the nontransformed epidermal cells (67.2%) (Table I). Thus, the expression of the transformed phenotype leads to the loss of a defined characteristic of human epidermal cell differentiation.

PATTERN FORMATION: In the final stages of differentiation, human epidermal cells have been observed to undergo pattern formation resulting in structures resembling those of human dermatoglyphs (16). The expression of the transformed phenotype in the HE-SV and HE-209 cells was found to interfere with the process of pattern formation. These cells did not form the ridges and whorls exhibited by their nontransformed counterpart (data not shown).

DISCUSSION

In this paper, we report the transformation of human foreskin epidermal cells by subgenomic segments of DNA containing the intact transforming "early" regions of wild-type SV40 and a tsA mutant of SV40 (*tsA209*). The wild-type SV40-transformed line HE-SV was characterized by changes in morphology and growth and by a quantitative loss of epidermal cell differentiated properties. The HE-SV cells were heterogeneous in size and shape and did not undergo organized stratification. Under growth conditions optimized for nontransformed HE cells, no significant difference in the growth properties of normal and SV40-transformed cells were observed. However, the transformed cells had a reduced requirement for serum and growth factors and could be propagated under conditions that did not support the growth of nontransformed cells. The dependence of these altered morphologic and growth characteristics on a functioning SV40 A gene product was demonstrated with the HE-209 cells. Maintenance of these properties comprising the transformed phenotype in HE-209 cells is temperature sensitive; at 33°C the cells have a transformed phenotype, and at 40°C they have a normal, nontransformed phenotype. Interestingly, in parallel cultures we were unable to transform human epidermal cells with the equivalent *HhaI/Eco*RI DNA fragment of the SV40 double tsA/deletion mutant 288, which

has the same tsA mutation as *tsA209* but does not encode a functional small t antigen (33). This result suggests that the SV40 small t antigen may be required for the transformation of primary cell cultures.

Differentiation of epidermal cells has been shown to be accompanied by an increase in the amount of keratin and/or the appearance of new keratin species (1, 4, 5, 10, 12, 34, 36, 39). We demonstrate a quantitative reduction of differentiated epidermal cell functions in the SV40-transformed human epidermal cells. Steinberg and Defendi (35) have previously shown that SV40 transformation of human epidermal cells results in the decrease in keratins by histochemical staining. The HE-209 cells, however, quantitatively contain as much keratin as the nontransformed HE counterparts at the restrictive temperature. The loss of epithelial cell specific properties concomitant with a functional SV40 transforming protein (A gene product) and its reexpression at the restrictive temperature have been observed in human placental cells (7, 8) and in rat fetal hepatocytes (9, 32). Yoshimura et al. (40) have also recently reported transformation of chick epidermal cells by a temperature-sensitive mutant of Rous Sarcoma virus (RSV) in which the functional src gene abolished some differentiated cell functions. This correlation between expression of the transformed phenotype and the loss of differentiated function has been observed also with mesenchymal cells (19, 24, 25). In the final stages of differentiation, epidermal cells acquire a cross-linked envelope beneath their plasma membrane (14, 29). The cross-linked envelope forms in cells when cell biosynthetic processes have been inhibited (30, 31), and the calcium ionophore X537A is capable of inducing the formation of these envelopes. SV40-transformed human epidermal cells have a lower capacity to form envelopes (~50% of control values). The HE-209 cells, however, retain this property at the restrictive temperature and form envelopes at a level ~93% that of nontransformed epidermal cells at 40°C. In conclusion, these findings show the expression of the SV40-transformed phenotype results also in the loss of this property of epidermal cell differentiation.

The establishment of transformed human epidermal cell lines (especially with the use of ts mutants) may provide valuable models for enhancing our understanding of malignant transformation in human epithelial cells. Until recently, most attempts at understanding neoplastic transformation have derived from studies using fibroblasts as a model system.

Studies employing these established cell lines offer distinct advantages. Human epidermal cells have been demonstrated to exhibit a "finite" lifespan in which the cells become senescent after approximately three to five passages (depending on the age of the donor) (15, 28). The epithelial cell lines which we have established enable one to propagate the cells beyond normal senescence and to undertake studies which require multiple passages of these cells. Moreover, these cell lines provide the opportunity to examine different levels of expression of differentiated properties in these transformed human epidermal cells. The use of the ts mutant allows one to modulate this transformed phenotype. Since the HE-209 cells grown at the restrictive temperature approximate "normal" human epidermal cells, these cells may also be useful for in vitro studies involving the regulation of differentiation in human epidermal cells. At present, we are using these cell lines to try and propagate human papilloma viruses which do not appear to impart an "indefinite" lifespan to the cells they infect, as do the other oncogenic viruses. We will employ various agents to try and effect the expression of the virus in these cells. It is possible that SV40 may provide a complementation or "helper effect" for the papilloma virus.

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