Transformation of Rat Cerebral Endothelial Cells by Rous Sarcoma Virus

CLEMENT A. DIGLIO, DAVID E. WOLFE, and PAUL MEYERS
Department of Pathology, Wayne State University School of Medicine, Detroit, Michigan 48201; and Department of Microbiology, State University of New York, Upstate Medical College, Syracuse, New York 13210

ABSTRACT Rat cerebral microvascular endothelial cells were infected with Schmidt-Ruppin Rous sarcoma virus-strain D (SR-RSV-D), an avian retrovirus. A single focus of transformed cells was isolated and the resultant cell line designated RCE-T1. The specificity for SR-RSV-D transformation was determined by virus rescue assay and demonstration of virus-specific antigens. RCE-T1 cells are virogenic when fused with chicken embryo fibroblasts (CEF) and do not produce infectious virus as demonstrated by the absence of detectable virus in culture fluid from these cells alone. Studies using an enzyme-linked immunosorbent assay (ELISA) for avian retrovirus-coded internal proteins show that RSV-transformed endothelial cells contain mainly p27 and react to some extent to p19 and p15 viral antigens. These data demonstrate conclusively that the transformation event was indeed due to SR-RSV-D. In addition, chromosome analysis confirmed these cells to be of rat origin.

RSV-transformed endothelial cells express the typical array of transformation-related properties such as anchorage-independent cell growth in soft agar, decreased cell adhesiveness, ability to grow in low serum, and capability of producing tumors in newborn rats. Demonstration of differentiated endothelial characteristics included positive immunofluorescent staining for factor VIII antigen and angiotensin-converting enzyme and histochemical localization of γ-glutamyl transpeptidase activity. This cell line should provide a useful model to study not only specialized biochemical and other functional characteristics of cerebrovascular endothelium but also the cellular mechanisms that involve the transition from normal to neoplastic expression.

Attempts to transfer normal differentiated tissues from an in vivo environment to defined culture conditions in vitro are often frustrating. In vitro, normal differentiated cells often fail to proliferate or cease to express differentiated cell properties beyond one or a few passages. We recently reported the establishment and maintenance of long-term primary endothelial cell cultures derived from rat cerebral microvessels (1). Although important information can be obtained from such cells maintained in primary culture, the ability to form subcultures and to obtain large numbers of cells while maintaining the differentiated characteristics of interest would obviously facilitate biochemical studies, as well as studies pertaining to cell growth properties and requirements. One approach for circumventing these limitations in culture is viral-induced cell transformation with a well characterized oncogenic virus.

While in vitro transformation of cells by DNA- and RNA-containing tumor viruses has been widely documented, reports on the successful transformation of vascular endothelial cells have been limited. Gimbrone and Fareed (2) described the successful transformation of human umbilical cord endothelial cells with SV40 viral DNA, but these transformed cells failed to routinely express normal differentiated endothelial cell properties. Rous sarcoma virus (RSV), an avian retrovirus, is capable of transforming a wide variety of differentiated cells of various species (3–9) and may even stimulate growth of differentiated cells under normal culture conditions (10–12). These observations prompted us to use the Schmidt-Ruppin-D strain of RSV, which can transform mammalian cells (13–15), as a transforming agent for rat cerebral endothelial cells in culture.

We report a number of features of an RSV-transformed

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cell line, derived from a single focus of transformed primary rat cerebral endothelial cells, including the expression of differentiated characteristics.

MATERIALS AND METHODS

Virus

The Schmidt-Ruppin strain of Rous sarcoma virus (SR-RSV-D) was originally obtained from Dr. Peter K. Vogt and was recloned twice by single-focus isolation and passaged at a low multiplicity of infection in chick embryo fibroblast (CEF) cells. The focus-forming titer was determined by a standard focus assay for RSV (16) with 2 μg/ml of polybrene incorporated into the medium during infection (17).

Cell Cultures and Transformation Procedures

Primary CEF of the C/E genotype were grown from a single White leghorn embryo as described by Dougherty et al. (18). These cells were avian retrovirus group specific (gs) antigen negative and chick helper factor (chf) negative when tested according to published criteria (19). All cell cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air.

We prepared primary rat cerebral microvascular endothelial cells and characterized as reported previously (1). We used morphologically homogeneous populations of endothelial cells in these studies. Primary cultures were maintained in Dulbecco's modified Eagle's medium (DME) supplemented with 20% fetal calf serum (FCS) (Grand Island Biological Company, Grand Island, NY), 25 μg/ml gentamicin and 150 μg/ml endothelial cell growth supplement (ECGS) (Collaborative Research Inc., Waltham, MA).

Multiwell dishes containing seven to 10-d-old subconfluent cultures of primary rat cerebral endothelial cells ($\sim 5 \times 10^4 - 1 \times 10^5$ cells/2.1-cm^2 well) were infected with SR-RSV-D at a multiplicity of infection of 10–15. After 1 h of absorption at 37°C, the cultures were re-fed with DME plus 20% FCS and 150 μg/ml ECGS. After 5–7 d the culture medium was replaced with medium free of ECGS, and at 3–5-d intervals thereafter. 2 wk after infection, a focus of tightly packed, refractile, polygonal transformed cells was readily detected. These transformed rat cerebral microvascular endothelial cells, designated here as RCE-T1, were subsequently propagated in DME supplemented with 10% FCS and 25 μg/ml gentamicin. The cells were routinely transferred at a split ratio of 1:10 every 3–4 d, after dispersing the monolayer with 0.25% trypsin–0.06% EDTA. Cultures were frozen at various passage levels (4–70) with 10% dimethylsulfoxide (DMSO) and stored in liquid nitrogen.

Characterization Studies

GROWTH AND PLATING EFFICIENCY: Saturation densities and doubling times of RCE-T1 cells were measured at varying passage levels. Cells were seeded in 25-cm^2 flasks at 1×10^5 cells/flask. The medium was changed at days 3 and 7 of the experiment. On alternating days over a 9-d period, duplicate sets of cultures were trypsinized and the cells were counted in a hemocytometer. The doubling time of the cells was calculated from the exponential part of the growth curve and the saturation density, expressed as number of cells per 25-cm^2 flask, was calculated from the plateau level reached in each case.

To measure the effects of FCS concentration, the growth of two representative passage levels of RCE-T1 cells was tested in medium containing 0%, 0.5%, 5%, and 10% FCS. RCE-T1 cells were grown from an initial density of 1×10^5 cells/35-mm plastic tissue culture dish (Falcon Plastics, Cockeysville, MD). Cell counts were made at days 1, 2, 3, and 5 by treating duplicate cultures with trypsin-EDTA and counting the dispersed cells in a hemocytometer.

Plating efficiency of RCE-T1 cells (P_{42}) in soft agar was determined by adding various numbers of viable cells (as determined by trypan blue dye exclusion) to 2-ml portions of Eagle's MEM containing 10% tryptose phosphate broth, 10% FCS, 200 mM L-glutamine and 0.35% agar. The mixture was then plated in 60-mm tissue culture dishes containing a previously prepared and hardened base layer of 8 ml of the same medium and 0.87% agar. Macroscopic colonies of cells were counted after 14 d of incubation at 37°C in a humidified atmosphere of 5% CO_2 in air. The mean of quadruplicate plates from two experiments was used to determine the plating efficiency.

Cloning efficiency in liquid medium with RCE-T1 cells was also determined by seeding plates with 1 and 2×10^2 viable cells in DME with 10% FCS. After 14 d, with periodic medium changes, triplicate cultures were fixed with methanol, stained with May-Grunwald Giemsa, and the number of colonies was determined.

CHROMOSOME ANALYSIS: Control rat cerebral endothelial cells and various passages of RCE-T1 cells were harvested for chromosome analysis. Metaphase and prometaphase spreads were examined using differential ASG and GTG banding techniques by the method described by Sumner et al. (20).

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR AVIAN RETROVIRUS gs ANTIGEN: Antisera were obtained from hamsters with tumors induced by the Schmidt-Ruppin B strain of Rous sarcoma virus (21). IgG and peroxidase conjugate were prepared as described by Wilson and Nakane (22).

Standard antigen was an ether extract of purified avian myeloblastosis virus (AMV). Cell extracts fron normal rat cerebral endothelial cells and transformed RCE-T1 cells were made by twice freezing and thawing a 50% suspension in phosphate-buffered saline (PBS)–0.05% Tween-80, followed by 20 strokes with a Dounce homogenizer. The preparation was then clarified at 2,000 *g* for 20 min and the supernatant tested for gs antigen. The double antibody sandwich ELISA described by Clark and Dougherty (23) was used with the following modifications: flat-bottom Linbro microtiter plates (type 76-301-05) were sensitized with 0.15 μg of hamster anti-gs in 100 μl per well of 20 mM PBS (pH 6.8) containing 0.05% Tween-80 (PBS-Tween). Peroxidase-labeled hamster anti-gs IgG (0.75 μg/well) in 25 μl of PBS-Tween was then added. The Trinder reagent (25 mM Phenol, 2 mM 4-amino antipyrine, 0.8 mM H_2O_2) (24) in PBS-Tween at 100 μl/well was used as substrate for the enzyme reaction. The reaction was stopped after 30 min by the addition of 100 μl of 20 mM NaOH, and then the absorbance of each well was read at 492 nm in a Flow Titetek Multiskan plate reader.

VIRUS RESCUE ASSAY: CEF and RCE-T1 cells were fused at 38°C by modifications of previously published methods (25–27). Briefly, 8 g of polyethylene glycol (PEG)-6000 (Baker Chemical Co., Sanford, ME) was autoclaved for 15 min. After 3–5 min of cooling, 10 ml of serum-free DME containing 15% DMSO was added, with stirring. Final concentrations of PEG and DMSO were 44.4% and 8.3%, respectively. CEF and RCE-T1, 1.0×10^4 cells/plate and 5×10^6 cells/plate, respectively, were plated together in 60-mm tissue culture dishes in DME containing 10% FCS and grown for 24 h. The medium was then removed, the cells were washed twice with serum-free DME, and 1 ml of the PEG-DMSO mixture was added to each plate. After 1 min, the PEG-DMSO was removed and the cell sheet was washed four times with serum-free DME, followed by two washes with DME containing 10% FCS. Finally, after the addition of growth medium (DME + 5% FCS) the plates were incubated. Controls consisted of plates with CEF alone and RCE-T1 cells alone. At various intervals the supernatant fluids were removed, filtered through 0.45-μm Millipore filters and stored at −75°C. The supernatants were later assayed on CEF for the presence of infectious virus by the standard focus assay for RSV (16).

HISTOCHEMICAL AND IMMUNOFLUORESCENT STAINING: Coverslip preparations of RCE-T1 cells were tested for the presence of factor VIII antigen and angiotensin-converting enzyme (ACE) using the indirect immunofluorescence technique as described previously (1). The histochemical localization of γ-glutamyl-transpeptidase was performed on acetone-fixed coverslip preparations of RCE-T1 cells using γ-glutamyl-4-methoxy-2-naphthylamide as a substrate, according to the method of Debault and Cancilla (28).

TUMORIGENICITY IN NEWBORN RATS: Confluent cultures of RCE-T1 cells (P_{12}) were trypsinized, suspended in medium with 1% FCS,

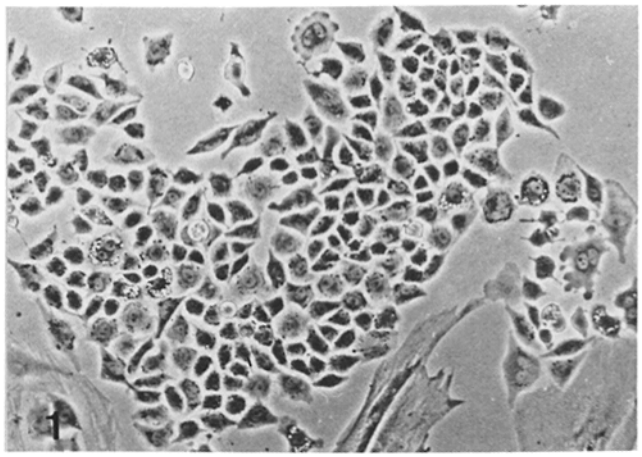


FIGURE 1 Phase-contrast micrograph showing a focus of RSV-transformed rat cerebral endothelial cells 12-d postinfection. The transformed cells have a polygonal shape and are closely apposed to each other. Most cells exhibit dense cytoplasm and contain a large nucleus with multiple nucleoli. × 125.

and counted. The cells were then pelleted and resuspended in Hanks' balanced salt solution (HBSS) at a concentration of 5×10^6–1×10^7 cells/ml, and 0.1 ml of the cell suspension was inoculated intraperitoneally in 2–5-d-old Fischer 344 rats to determine cell tumorigenicity. Tumor tissue was fixed in formalin and embedded in paraffin. Sections for light microscopic examination were stained with hematoxylin and eosin.

RESULTS

Transformation of Primary Rat Cerebral Endothelial Cells

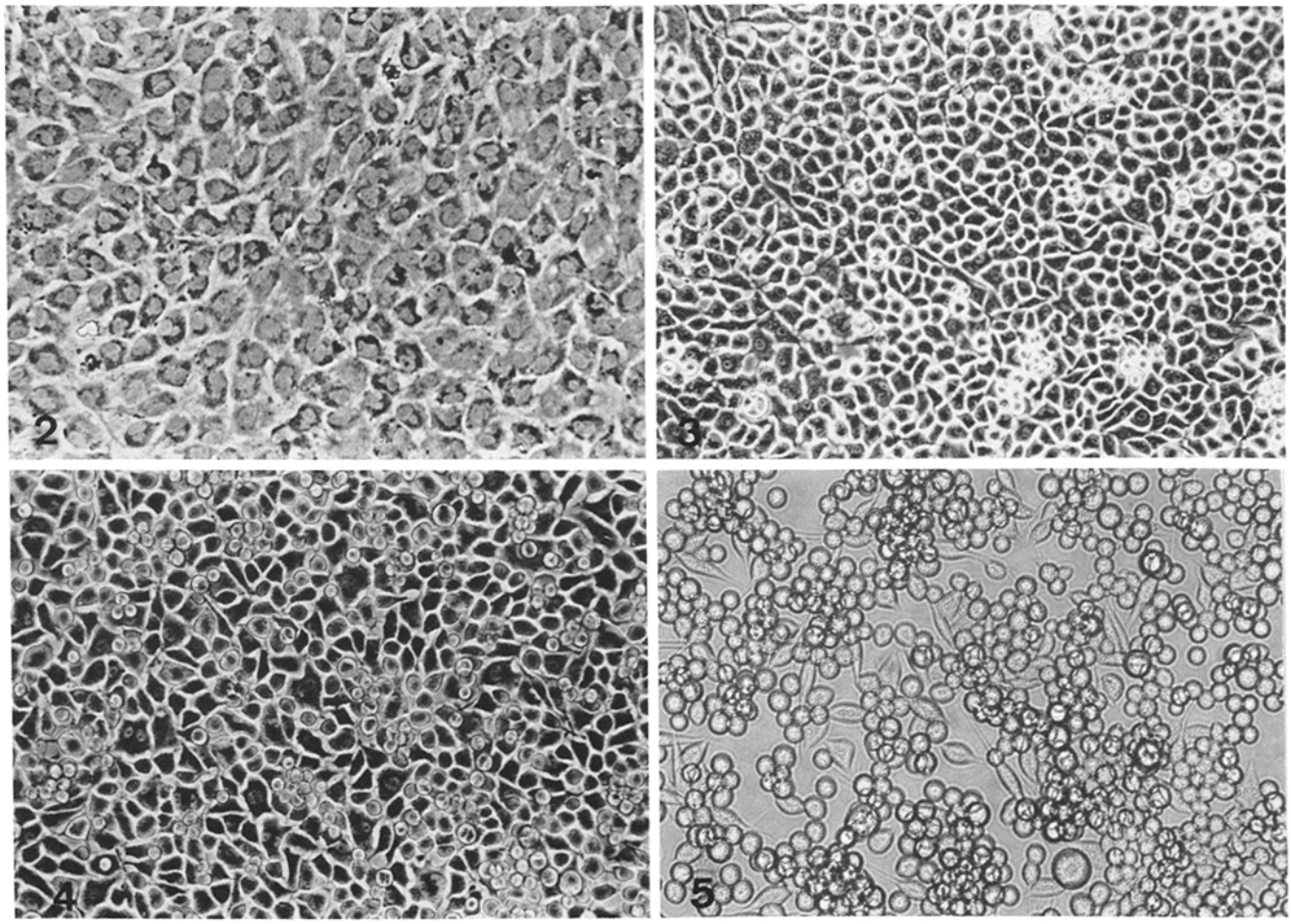
12 d after subconfluent homogeneous primary endothelial cells derived from rat cerebral microvessels were infected with SR-RSV-D, a single focus of tightly packed, refractile, small epithelioid-like cells was noted (Fig. 1). 7 d later, this population of transformed cells was isolated using a cloning penicylinder and then serially cultured. During this period, both infected and mock-infected control cerebral endothelial cell cultures were maintained in complete medium without ECGS. Foci of transformed cells were never observed in the control culture. Control primary cerebral endothelial cells, without the presence of ECGS, gradually deteriorated and cell death ensued (1). In addition, no contaminating smooth muscle or pericyte outgrowth was observed in control and transformed cultures during the period of observation.

Morphology of the RSV-transformed Cerebral Endothelial Cell Line

Normal rat cerebral endothelial cells in primary culture exhibit density-dependent inhibition of growth and form a single cell thick monolayer with a characteristic "cobblestone" appearance (Fig. 2). The transformed RCE-T1 cells in culture also form a flat, rather homogeneous sheet of polygonal cells closely apposed to one another (Fig. 3). These transformed cells do not form multilayers in the flattened state but, upon reaching confluence, they gradually round up and form grape-like clusters that eventually detach from the culture surface (Figs. 4 and 5).

Growth Behavior of RCE-T1 Cell Line

Fig. 6 represents the growth curve of RCE-T1 cells at various passage levels (10, 40, and 70) maintained in medium with 10% FCS. Population doubling times were 20.4 h (passage 10), 16.2 h (passage 40), and 16.8 h (passage 70) indicat-



FIGURES 2–5 Fig. 2: Phase-contrast micrograph of normal rat cerebral endothelial cells in primary culture. The cells form a packed monolayer with the typical "cobblestone" appearance. × 150. Fig. 3: Phase-contrast micrograph of a monolayer of RCE-T1 cells 3 d in culture. The culture consists of a confluent, flattened sheet of rather homogeneous epithelioid-appearing cells. A few small clusters of rounded cells can be seen at this stage. × 125. Fig. 4: Same culture as Fig. 3 after 4 d in culture. Note the marked increase in number of rounded cells that are detaching from the culture dish. × 125. Fig. 5: Same culture as described in Figs. 3 and 4 after 5 d in culture. Most of the transformed cells are rounded and form large clusters. Note the loss of adhesiveness of these cells to the culture surface. × 125.

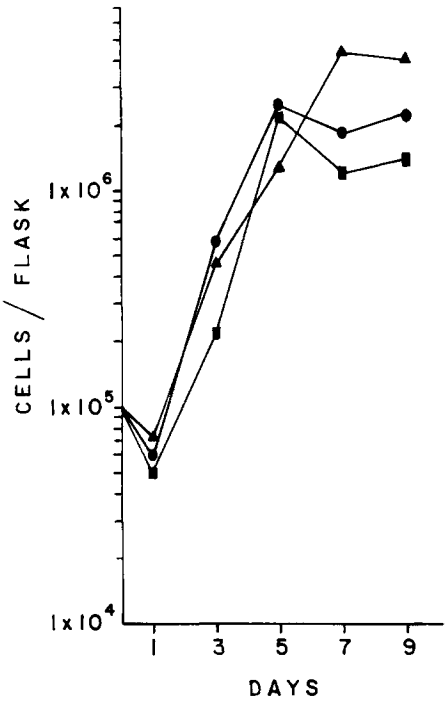


FIGURE 6 Cell growth of RCE-T1 cells at different passage levels. Cells from each passage were plated in duplicate at 1 × 10^5 cells/25 cm^2 flask in DME containing 10% FCS and incubated. Cells from each passage level were harvested by trypsin-EDTA treatment at 1, 3, 5, 7, and 9 d and counted in a hemocytometer. Each point represents the average number of viable cells obtained. ■, passage 10. ●, passage 40. ▲, passage 70.

ing an increase in growth rate at higher passage levels. Saturation density levels were similar for passages 10 and 40 with ~1 × 10^5 cells/cm^2, while a noticeably higher cell density was found at passage 70 (1.7 × 10^5 cells/cm^2). A progressive increase in relative plating efficiency was also noted with increasing number of passages (data not shown).

Growth curve studies of transformed RCE-T1 cells (passages 27 and 57) cultured in various serum concentrations are shown in Fig. 7. Cells from both passages grew comparably in medium supplemented with either 10% or 5% FCS and exhibited significant growth potential when maintained in medium with as little as 0.5% FCS. Although cultures maintained without FCS did achieve relative plating efficiency levels of >40% they failed to grow, which is in marked contrast to normal rat cerebral endothelial cells that require both high concentrations of serum and the presence of ECGS for optimum growth (1). Additional evidence of transformation was obtained by assaying the growth of RCE-T1 cells in semisolid media. This type of culture exhibited a plating efficiency of 24% in soft agar, as compared with a plating efficiency of 28% in liquid medium.

Chromosome Studies

Comparison of chromosomal patterns in both RCE-T1 cell preparations at various passage levels (4–12) and control primary rat cerebral endothelial cells exhibited a normal male rat karyotype of 42, xy that is in agreement with published standards and conclusively demonstrated that the transformed cells were of rat origin.

Persistence of the SR-RSV-D Genome in RCE-T1 Cells

Both RCE-T1 cells and rat control endothelial cells were tested for the presence of RSV gs antigens by ELISA. The RCE-T1 cell line yielded a positive reaction at a dilution of 1/100 of the 50% cell extract, indicating that the viral gs protein content was ~10 ng/mg of wet weight cells. No reaction was detected with extracts of normal control rat cerebral endothelial cells. It should be noted that the antisera used react predominantly with avian retrovirus p27, and to a lesser extent with p19 and p15 viral proteins. Since avian retrovirus-transformed mammalian cells may contain gs antigens (29–31) without the entire genome being present in a rescueable form, fusion experiments were conducted with permissive host cells (i.e., CEF). Focus-forming virus was detectable in fused CEF-RCE-T1 cultures 5 d after PEG-DMSO treatment (Table I). Virus titer increased progressively up to day 8 and declined thereafter, due to aging and overcrowding of the cultures. The absence of detectable virus in supernatant fluids from RCE-T1 cell cultures indicates that these cells are not producing infectious virus. Although serotyping was not performed, the morphology of the foci induced on CEF by the rescued virus released from CEF-RCE-T1 heterokaryons was identical to that of the original SR-RSV-D employed to transform the rat endothelial cells. It is apparent from these results that RCE-T1 cells are virogenic, in that they contain the intact genome SR-RSV-D and that infectious virus is rescueable by fusion with permissive CEF.

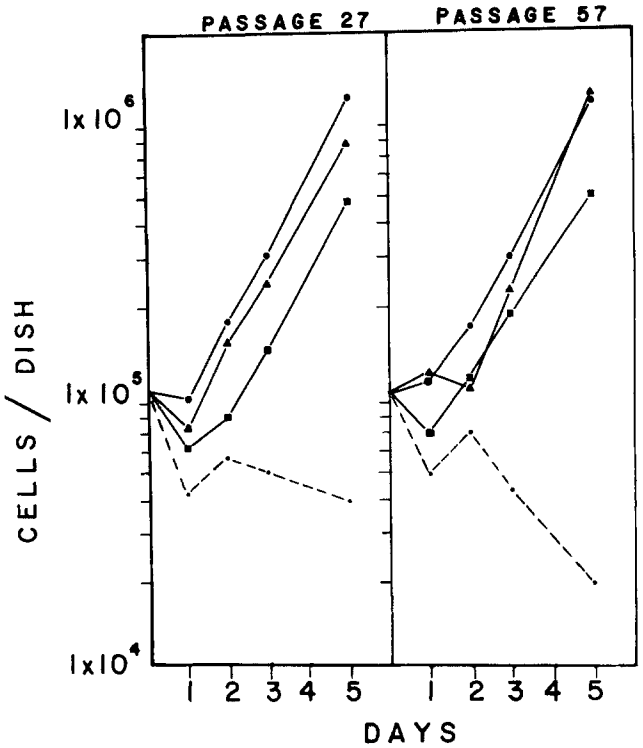


FIGURE 7 Effect of various serum concentrations on the growth of RCE-T1 cells. Passage 27 and 57 RCE-T1 cells were plated in duplicate at 1 × 10^5 cells/35 mm tissue culture dish in DME supplemented with various concentrations of FCS (●--●, 0%; ■, 0.5%; ▲, 5%; ●, 10%). Duplicate dishes from each series were harvested by trypsin-EDTA treatment at 1, 3, and 5 d and counted in a hemocytometer. Each point represents the average number of viable cells obtained.

Differentiated Endothelial Properties of RCE-T1 Cells

As previously reported, rat cerebrovascular endothelial cells are capable of retaining morphologic and antigenic characteristics of vascular endothelium in long-term primary cell culture (1). Because a single transformed colony was isolated upon infection of a homogeneous population of cerebral endothelial cells, it was of interest to determine whether or not RSV-transformed endothelial cells were still capable of retaining differentiated endothelial related markers. Fig. 8 represents positive fluorescent staining for the presence of factor VIII antigen in the transformed cell line. Positive fluorescence was also demonstrated using rabbit antibody to rat lung angiotensin converting enzyme (ACE) (Fig. 9). The fluorescence observed had a perinuclear localization. Strong positive immunofluorescent activity for factor VIII antigen and ACE was seen in the RCE-T1 cell line at passage levels 4 through 50, after which fluorescence reactivity gradually decreased and eventually was not detected after passage 70. The RCE-T1 cell line also exhibited the presence of γ-glutamyl transpeptidase enzyme (γGTP) activity (Fig. 10) by histochemical staining, which is in contrast with the loss of this enzyme activity during in vitro cultivation of normal cerebrovascular endothelial cells (28). Although γGTP activity was demonstrated, variation in the degree of expression of this enzyme was noted when biochemically quantitated (Diglio, C. A., and M. L. Caspers, manuscript in preparation).

TABLE 1

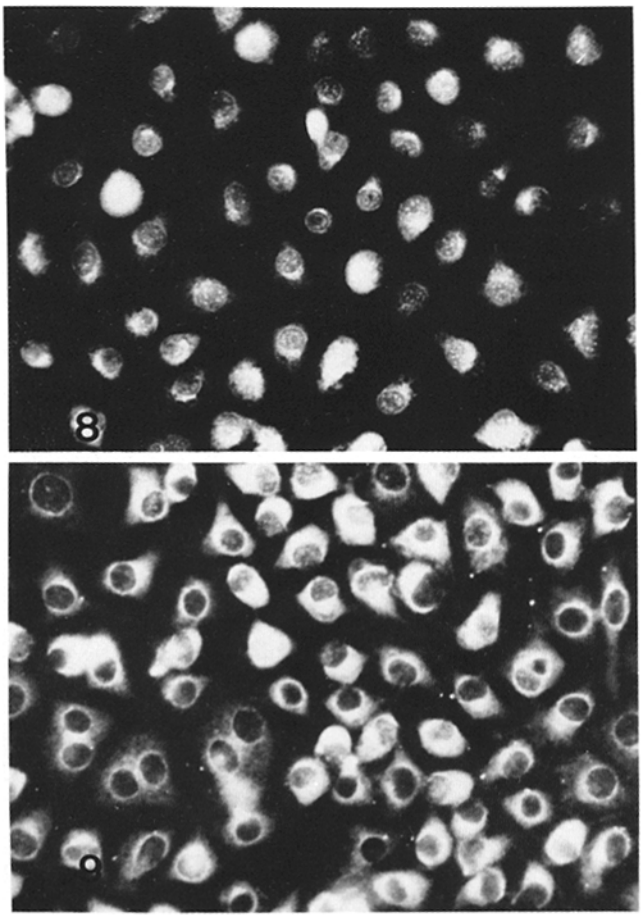
Virus Rescue in Transformed RCE-T1 Cells

Supernatant from cells	Virus titer*			
	3	5	8	11
			(day)	
CEF + RCE-T1	<0.5	2×10^2	8.1×10^3	8.2×10^1
CEF alone‡	<0.5	*NT*§	*NT*	<0.5
RCE-T1 alone‡	<0.5	*NT*	*NT*	<0.5

* Titer on CEF in focus-forming units/milliliter at times shown after cell fusion.

‡ Mock fusion.

§ *NT*, not tested.



FIGURES 8 and 9 Fig. 8: RCE-T1 cells (passage 32) exhibiting cytoplasmic localization of factor VIII antigen by indirect immunofluorescence. × 260. Fig. 9: Immunofluorescent localization of angiotensin-converting enzyme activity in RCE-T1 cells (passage 32). Note the prevalent perinuclear fluorescence in some of these cells. × 260.

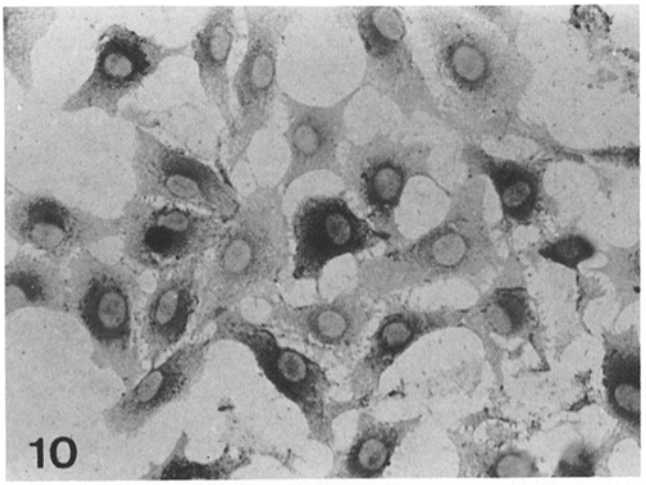
Tumor Formation

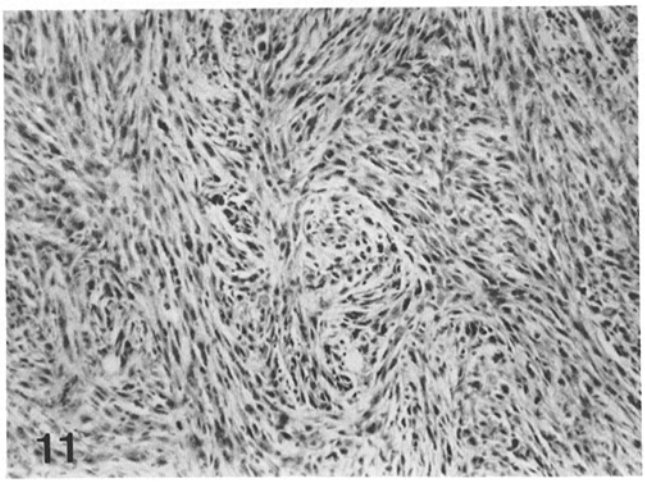
All seven newborn rats injected with RCE-T1 cells developed numerous intraperitoneal neoplastic nodules within 15–20 d postinjection and were most numerous and largest over the peritoneal surface of the diaphragm and the adjacent peritoneum. Most of the tumor tissue grew as well-circumscribed nonencapsulated nodules, with only some of them showing local invasion of adjacent fibrovascular adipose tissue, hepatic and pancreatic parenchyma. Extensive bilateral pulmonary metastases were also noted in two of the rats. The morphology of tumors induced by the RCE-T1 cell is shown in Fig. 11. The predominant histologic pattern of these tumors was consistent with that of a poorly differentiated spindle cell sarcoma frequently displaying well-developed storiform areas. The highly elongated cells were arranged in semiparallel or disorderly arrays. Mitotic figures were common. Multifocal areas of necrosis were present in most nodules and markedly prominent in the pulmonary metastases.

DISCUSSION

As reported previously, long-term primary cerebrovascular endothelial cell cultures have been maintained under restricted culture conditions that required high serum concentration and medium supplemented with ECGS. Confluent cultures exhibited contact inhibition of growth, expressed normal differentiated properties, but lacked the capacity for further proliferation and subculture (1). In another study, Gimbrone and Fareed (2) described the first successful transformation of human vascular endothelium with SV40 virus. However, the resultant cell lines exhibited altered morphology and loss of endothelial-related properties (2).

The present study describes the successful transformation of rat cerebral microvascular endothelial cells with an RNA tumor virus and, to our knowledge, the first establishment of a continuous cell line of RSV-transformed endothelial cells. Although this line, designated RCE-T1, expresses typical transformation-related properties (growth in soft agar, tumorigenicity), it still is capable of retaining normal differentiated endothelial cell characteristics. In vitro virus-induced transformation allowed us to circumvent the culture limitations of primary cultures. Moreover, the occurrence of a single, well-isolated focus of transformed cells was of great advantage, in that the resultant population can be considered to likely be a clonal isolate derived from infection of a single target cell.





FIGURES 10 and 11 Fig. 10: Histochemical localization of γ-glutamyl transpeptidase in RCE-T1 cells (passage 23). × 750. Fig. 11: Light micrograph showing the interweaving spindle-cell pattern characteristic of the intraperitoneal RCE-T1 tumor nodules. Hematoxylin and Eosin stain. × 100.

The first apparent indicators of transformation in cerebrovascular endothelial cells were the morphological appearance and the induction of sustained proliferation. RCE-T1 cells were highly refractile and mimicked to some degree normal endothelium as seen by phase-contrast microscopy (1, 32). In addition, the transformed cells in the flattened state did not form multilayers, except that upon reaching saturation density they gradually rounded up, detached, and formed grapelike clusters. The loss of adhesiveness in culture is a characteristic feature of RSV-transformed cells (33). The features described above are in contrast with the pattern of SV40-transformed human vascular endothelium that expressed multilayering overgrowth and altered morphology (2).

The maintenance of the transformed state by RSV requires the continuous expression of the viral transforming gene, *src* (34–37). RSV-infected mammalian cells frequently contain virus-specific antigens as well as all of the viral genetic information necessary for the production of complete virus, and infectious RSV can be rescued from these cells by fusion with susceptible avian fibroblasts in the absence of any exogenous or endogenous helper virus (29). The presence of RSV viral antigens in RCE-T1 cells and the ability of fused RCE-T1 and chicken embryo fibroblasts to release SR-RSV-D virus clearly indicate that the transformation event was virus-specific. In addition, cytogenetic analysis demonstrated that these transformed cells are of rat origin.

Virus-induced transformation results in a number of phenotypic changes that are collectively termed "the transformed phenotype." The alterations include changes in cell shape, decreased cell adhesiveness, lack of density-dependent inhibition of growth, ability to grow in vitro in low serum concentrations, and tumorigenicity (38, 39). RCE-T1 cells possess all of these stable transformation properties and also have the ability to produce colonies in soft agar, whereas normal cells fail to form colonies. The acquisition of this property of anchorage independence of growth has been shown to correlate well with tumorigenicity (40). RCE-T1 cells inoculated into newborn rats induced tumors that are consistent with the histopathological features of sarcomas.

RCE-T1 cells are an exception to the general finding that differentiated cellular functions often are not expressed following viral transformation. For example, cells of mesodermal origin transformed with RSV usually fail to express normal differentiated functions (8, 41). While the shutoff of these so-called "luxury" molecules can be circumvented when cells are transformed by temperature-sensitive (ts) mutants of RSV and then grown at the nonpermissive temperature (5–7), RCE-T1 cells that were transformed by wild-type RSV and are, within the passage levels studied (4–70), an exception to this rule. Both factor VIII antigen and angiotensin converting enzyme activity were demonstrated in these cells by indirect immunofluorescence. However, it should be mentioned that the expression of these endothelial-related markers in RCE-T1 cells could not be demonstrated at higher passage levels (>70) where marked morphologic alterations also occurred. We recently isolated several morphologic variants from the parent RCE-T1 cell line as well as possible revertants. Examination of these cloned populations for retention of endothelial-related functions and expression of transformation-related properties is currently underway.

In summary, the results show that it is possible to transform rat cerebral endothelial cells with RSV and that these cells retain some of the specific differentiated properties of the original cells in culture. This cell line should then be useful for studying specialized biochemical and functional characteristics of cerebrovascular endothelium. In addition, the RCE-T1 cell line may provide a model to study the mechanism of cell transformation from normal to neoplastic expression.

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