Characterization of Human Uroepithelial Cells Immortalized in Vitro by Simian Virus 401

Brian J. Christian, 2 Linda J. Loretz, Terry D. Oberley, and Catherine A. Reznikoff3

Departments of Human Oncology [B. J. C., L. J. L., C. A. R.] and Pathology [T. D. O.], University of Wisconsin Medical School and Veterans Administration Hospital, Madison, Wisconsin 53792

ABSTRACT

Normal human uroepithelial cells (HUC) were transformed with simian virus 40 (SV40) in vitro. SV40-transformed HUC (SV-HUC) were selected by their ability to survive senescence which normally occurs in HUC between passages 4 and 6. At passage 6, 100% of SV-HUC stained positive for SV40 T-antigen. The epithelial nature of SV-HUC was confirmed by positive staining for human cytoplasmic keratins in all cells. SV-HUC have altered growth characteristics compared to HUC including the capacity to grow on plastic, independent of a collagen-gel substrate; loss of the dependence on medium supplements for optimal growth, loss of the dependence on feeder cells for growth at clonal density, and an apparently unlimited lifespan in culture (>2 years). Although SV-HUC have an increased percentage of viable cells and increased saturation density compared to HUC, the generation time of SV-HUC during log phase is similar to that of HUC. Cultures of SV-HUC are epithelial in appearance and show some morphological heterogeneity in cell size and shape. At the ultrastructural level, SV-HUC have numerous alterations such as, irregularly shaped nuclei and nucleoli, pleomorphic microvilli, and the lack of a glyocalyx on the cell surface. In addition, SV-HUC do not stratify in culture, suggesting an inability to differentiate. Unlike HUC, SV-HUC are capable of growth in soft agarose, a property which increased with serial passage. Yet, through at least P50, SV-HUC remained non-tumorigenic as determined by the inability to form tumors in athymic nude mice. This cell line of human epithelial origin may be suitable for studying the conversion of cells to tumorigenicity by subsequent treatment with another oncogenic agent.

INTRODUCTION

The transformation of cells in vitro, as defined by most criteria, has proven to be more difficult for human cells than for rodent cell types. The basis for this difference is unknown; however, several explanations have been postulated. For example, the technical aspects of cell culture may favor the growth and subsequent transformation of rodent cells over cells from human tissues, and furthermore the rodent karyotype may be apparently unlimited lifespan in culture (>2 years). Although SV-HUC have an increased percentage of viable cells and increased saturation density compared to HUC, the generation time of SV-HUC during log phase is similar to that of HUC. Cultures of SV-HUC are epithelial in appearance and show some morphological heterogeneity in cell size and shape. At the ultrastructural level, SV-HUC have numerous alterations such as, irregularly shaped nuclei and nucleoli, pleomorphic microvilli, and the lack of a glyocalyx on the cell surface. In addition, SV-HUC do not stratify in culture, suggesting an inability to differentiate. Unlike HUC, SV-HUC are capable of growth in soft agarose, a property which increased with serial passage. Yet, through at least P50, SV-HUC remained non-tumorigenic as determined by the inability to form tumors in athymic nude mice. This cell line of human epithelial origin may be suitable for studying the conversion of cells to tumorigenicity by subsequent treatment with another oncogenic agent.

INTRODUCTION

The transformation of cells in vitro, as defined by most criteria, has proven to be more difficult for human cells than for rodent cell types. The basis for this difference is unknown; however, several explanations have been postulated. For example, the technical aspects of cell culture may favor the growth and subsequent transformation of rodent cells over cells from human tissues, and furthermore the rodent karyotype may be inherently more unstable allowing rodent cells to be more easily transformed than human cells (1, 2). Interspecies differences notwithstanding, comparison of transformation between mesenchymal and epithelial cell types within a given species, as is illustrated by a comparison of SV40 immunization in human fibroblasts (3) and human keratinocytes (4), suggests that important differences intrinsic to the cell type also exist. Given these differences in the nature of transformation for various cell types and the fact that the majority of adult human cancers are derived from epithelial tissues (5), the study of transformation in a human epithelial cell type has particular relevance toward understanding mechanisms of carcinogenesis in humans.

Received 10/30/86; revised 8/17/87; accepted 8/24/87.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported by NIH Grants CA-29525 and by a grant from the Pardee Foundation. Portions of this work have been presented at the American Association for Cancer Research Meeting, Los Angeles, CA, May 1986 (38).
2 Supported by NIH Training Grant 5-T32-CA 09471-03.
3 To whom requests for reprints should be addressed at Department of Human Oncology, Wisconsin Clinical Cancer Center, K4/550, 600 Highland Avenue, Madison, WI 53792.
4 The abbreviations used are: HUC, human uroepithelial cells; SV40, simian virus 40; SV-HUC, SV40-transformed human uroepithelial cells; FBS, fetal bovine serum; P, passage.

Neoplastic transformation of cultured cells, as is the case in the whole organism, is widely accepted to result from the accumulation of multiple cellular changes (2). Recent evidence in support of this statement comes from reports which compare the transformation of nonestablished early passage cells and immortalized cell lines by transfection with viral and cellular oncogenes (6–9). The consensus of these studies seems to be that transfection of an oncogene which imparts an extended culture lifespan to nonestablished cells cannot fully convert these cells to tumorigenicity, but can cooperate with a second class of oncogenes to achieve this endpoint. Cell lines which already exhibit the property of increased culture longevity require only the second oncogene to complete the neoplastic transformation (9). Thus, it can be hypothesized that a transforming event which imparts an extended lifespan to cultured cells may be an early step in the transformation pathway, enabling cells to be converted to tumorigenicity by a second agent (7, 9). The in vitro transformation of human cells by SV40 routinely imparts many of the altered phenotypic traits associated with transformed cells including morphological alterations, karyotypic changes, extended culture lifespan, altered growth requirements, and anchorage-independent growth (10). Yet, with few exceptions, transformation of human mesenchymal cells by SV40 does not result in demonstrable tumorigenicity (11–14) and neoplastic transformation of human epithelial cells by SV40 has not been reported. Therefore, an SV40-transformed human epithelial cell type with an extended culture lifespan may serve as a useful target cell type to study the mechanisms of tumorigenic transformation by treatment with a second oncogenic agent.

In this study we describe the partial transformation of HUC to an immortalized but nontumorigenic phenotype by SV40. We also characterize the SV40-immortalized cells with respect to phenotypic properties commonly associated with transformation in vitro.

MATERIALS AND METHODS

Cell Culture and SV40 Infection. Cultures of HUC were initiated from ureteral tissue explants obtained as a byproduct of kidney transplantation surgery, and cultured as previously described (15–17). Donor kidneys for transplantation surgery were prescreened and determined to be free of past or present urinary tract disease or malignancy (16). Briefly, the mucosal layer of tissue from individual human ureter segments was separated from the underlying tissue with forceps, minced into approximately 1-mm2 explants and plated (approximately 30 explants/100-mm dish) onto type I rat tail collagen-gel-coated dishes in approximately 1 ml of culture media. Ammonia-reconstituted collagen gels were prepared according to the method of Ehrmann and Gey (18) and pre-equilibrated in unsupplemented Ham's F12 medium for at least 48 h prior to use. The culture medium used for both HUC and SV40 was Ham's F12 (GIBCO, Grand Island, NY), supplemented with 5% FBS (Hyclone, Logan, UT), insulin (250 munits/ml; GIBCO), hydrocortisone (1 Mg/ml; Merck Sharp and Dohme, West Point, PA), transferrin (5 μg/ml; Sigma Chemical Co., St. Louis, MO), a mixture of nonessential amino acids (each amino acid at 0.1 mm; Microbiological Associates, Walkersville, MD), L-glutamine (2.0 mm; GIBCO), and dextrose (15 mm; Amend Drug and Chemical Co., Irvington, NJ).
This supplemented medium, referred to as 1% FBS-F12+ also contained penicillin (100 units/ml; Pfizer, Inc., New York, NY) and streptomycin (100 μg/ml; Pfizer). Cells were grown in humidified incubators at 37°C in an atmosphere of 5% CO2 and 95% air and received media changes three to four times per week. Routine dispersion and passage of both HUC and SV-HUC were performed using 0.1% EDTA (Sigma Chemical Co.) in Hank’s balanced salt solution (GIBCO) as previously described (15, 16). Cultures of SV-HUC through approximately P20 were split at a ratio of 1:3 when they became confluent. SV-HUC after approximately 20 passages in culture were split at a ratio of 1:10.

For SV40 infection, primary 7- to 10-day-old growing cultures of HUC were dispersed and reseded at 1 x 105 cells/collagen-gel-coated 100-mm dish in 3 ml of serum-free F12+ which contained wild type SV40 (provided by Dr. Janet E. Mertz, McArdle Laboratory, University of Wisconsin) at a multiplicity of 220 plaque-forming units/cell. After a 4-h incubation period, at which time the cells had attached to the substrate, the virus-containing medium was replaced with 1% FBS-F12+. The SV40-infected and mock-infected control cultures were maintained in the log phase of growth by subculturing prior to confluence. At P4, control and SV40-infected cultures containing senescing cells were cocultured with preirradiated (6000 rads, cesium source) Swiss 3T3 fibroblasts as feeder cells. SV-HUC were selected by their ability to survive senescence which inevitably occurred in mock-infected HUC cultures between P4 and P6, after 1 to 2 months in culture. Two lines of SV-HUC, designated SV-HUC-1 and SV-HUC-2, were independently derived from separate HUC cultures in different experiments.

Immunofluorescent Staining for SV40 T-Antigen and Keratin. For indirect immunofluorescent staining, cells grown on coverglasses were washed with PBS (10 mM phosphate buffer, 133 mM NaCl, pH 7.2) and fixed with ice-cold acetone. Hamster antiserum prepared against Green Monkey Kidney cells (SV40 permissive) were inoculated with preirradiated (6000 rads, cesium source) Swiss 3T3 fibroblasts as feeder cells. SV-HUC were selected by their ability to survive senescence which inevitably occurred in mock-infected HUC cultures between P4 and P6, after 1 to 2 months in culture. Two lines of SV-HUC, designated SV-HUC-1 and SV-HUC-2, were independently derived from separate HUC cultures in different experiments.

Immunofluorescent staining was performed as described (9). Briefly, cultures were fixed with 1% paraformaldehyde in PBS for 15 minutes, washed with PBS, and permeabilized with 0.1% Triton X-100. Cells were then incubated with primary antibody for 1 hour at room temperature, washed with PBS, and subsequently incubated under the same conditions with FITC-conjugated secondary antibody. Nonimmune rabbit serum (Jackson Immunoresearch) was used as a negative control. Coverslips were mounted with glycerol and examined with a Nikon inverted microscope using UV epifluorescence.

RESULTS

In Vitro Establishment of HUC by SV40. Primary cultures of HUC initiated from tissue explants grow exponentially for 3 to 4 passages, approximately 35 population doublings. Beyond this point, the proliferative capacity of the culture declines as the cells senesce. The appearance of normal HUC at P1 is characterized by a uniform population of cells with an oval or polygonal shape. Between P1 and P3, SV40-infected and mock-infected control cultures were morphologically indistinguishable. At P3, the control cultures contained several small areas of rounded cells, suggestive of an increased viability and mitotic activity. These areas were not observed in the HUC control cultures. At P4, the SV40-infected cultures contained several small areas of rounded cells, suggestive of an increased viability and mitotic activity. These areas were not observed in the HUC control cultures. At P4, the SV40-infected cultures contained several small areas of rounded cells, suggestive of an increased viability and mitotic activity. These areas were not observed in the HUC control cultures.
Fig. 1. Phase-contrast photomicrographs showing morphology of (A) normal HUC at P1, (B) area of apparent increased mitotic activity among senescing cells in an SV40-infected HUC culture at P5, (C) SV-HUC-1 at P10 showing cytoplasmic vacuolization, and (D) SV-HUC-1 at P20 showing epithelial morphology and loss of cytopathology. Bars, 100 μm.

control cultures eventually senesced, the cultures of SV40-infected cells treated in the same manner contained some cells which continued to divide. When the SV40-infected cultures approached confluent density, they were serially passaged without feeder cells and continued to divide. Although SV-HUC were successfully isolated from some culture dishes which were not initially passaged with feeder cells, the SV40-infected cultures which were passaged with feeder cells yielded an increased number of transformants (data not shown).

The next 5 to 10 passages of SV-HUC (P5-15) were characterized by a heterogeneous morphological appearance. This was accompanied by a background of chronic cell lysis and detachment of dead cells from the monolayers, which was most prevalent as the cultures approached confluence. Complete lysis of SV-HUC cultures did not occur. The morphological appearance of a representative area of SV-HUC at P10 is shown in Fig. 1C. The majority of cells exhibited a characteristic epithelial morphology with single uniformly shaped nuclei. However, numerous multinucleated giant cells were also present, as were cells containing cytoplasmic vacuolization. As SV-HUC cultures were serially passaged, the degree of cell shedding into the medium and the numbers of giant cells gradually decreased. In contrast to cultures at earlier passages (P5-15) in which cells grew in patches with many open areas, SV-HUC cultures by P20 (Fig. 1D) were capable of growing in confluent sheets. With continued propagation, cultures of SV-HUC became more morphologically uniform, however, always retained a proportion of cells with a heterogeneous appearance.

The establishment of postsenescent SV-HUC cultures followed a similar progression to apparently immortal lines (>2 years in culture) from two separate SV40 infections, designated SV-HUC-1 and SV-HUC-2. The SV-HUC with an extended life did not enter a "crisis" phase as defined by a quiescent or greatly reduced period of growth, which has been described for SV40-transformed fibroblasts (3). This is in contrast to SV40-transformed endothelial cell strains derived from a human ureter (23), which exhibited an approximate 2-fold increase in lifespan, but eventually underwent senescence. At earlier passages (P5-15) of SV-HUC, the growth and viability of the cultures appeared to be very sensitive to cell density. Cultures maintained subconfluent remained viable, whereas cultures which were allowed to become confluent did not survive. This dependence on low cell density for growth diminished with serial passage, after which SV-HUC grew with increasing vigor.

The production of infectious virus, as detected by lysis of Green Monkey Kidney cells, was different for the two cell lines tested. Conditioned medium and cell lysates tested negative for infectious SV40 at P15 and P30 from SV-HUC-1, while SV-HUC-2 tested positive for infectious SV40 at P10 and P25.

Immunofluorescent staining for SV40 T-antigen at P6 was detected within the nuclei of 100% of the cells of both SV-HUC-1 (Fig. 2A) and SV-HUC-2, but not in normal HUC. The SV40 T-antigen staining persisted with time in culture, through at least P45 of SV-HUC-1, the last passage tested. SV-HUC (Fig. 2B) and normal HUC (photo not shown) stain positively for cytoplasmic keratin, a marker used to confirm the epithelial nature of the cells. As controls, human fibroblast cells tested negative for keratin staining (photo not shown).

Growth of SV-HUC on Collagen and Plastic Substrata. The
growth of HUC, SV-HUC-1, and SV-HUC-2 was compared on collagen-gel and plastic substrata (Table 1). The 24-h postseeding cell attachment for all cell types was greater on the collagen-gel substrate than on plastic. Optimal growth of normal HUC was achieved only when the cells were cultured on collagen gels. The generation time of HUC grown on the plastic substrate was 3- to 4-fold longer than when grown on the collagen gels. Early passage (P10) SV-HUC-1 also required a collagen-gel substratum for significant growth, as shown by an increased generation time when these cells were cultured on plastic. The increased cell death and detachment from the dishes which was apparent in early passage (P5-15) SV-HUC cultures as they approached confluence (vide supra) was evidenced by the low cell densities at 14-day postseeding compared to HUC, particularly on the collagen substrate. When the early passage SV-HUC-1 were in the exponential phase of growth, however, their generation time was similar to that of HUC. As SV-HUC were serially passaged, the requirement of a collagen-gel substrate for optimal growth was lost as is demonstrated by comparable generation times for SV-HUC (P25) cultured on either plastic or collagen-gel substrates. In addition, the growth rates of these cells grown on either substrate were similar; and not different from that of normal HUC grown on collagen gels. SV-HUC grown on either substrate attained a higher saturation density than that of HUC grown on collagen gels. This was primarily due to a tighter packing of SV-HUC within the monolayer, as these cells were not found to stratify (vide infra). The viability of SV-HUC, assessed during the log phase of growth or at confluence, was higher than that of HUC. This difference in viability was most marked when cells were grown on collagen gels and assessed at confluence (Table 1; compare HUC, 64%, and late passage SV-HUC-1, 82%). Comparison of SV-HUC-1 and SV-HUC-2 indicates that transformants generated independently, had similar growth properties.

Effect of Serum and Medium Supplements on the Growth of SV-HUC. The dependence of HUC and SV-HUC-1 on the presence of FBS and medium supplements for optimal growth on collagen gels was determined (Table 2). Ham's F12 based medium containing 1% FBS and a combination of medium supplements (listed in "Materials and Methods") has been previously established to significantly improve the growth of HUC (15, 16). Deletion of either FBS or the combination of medium supplements from the medium diminishes the growth of HUC. Culture of HUC in Ham's F12 medium without both of these components does not support growth. The relative importance of FBS and the medium supplements for growth of early passage SV-HUC-1 (P15) was similar to that of HUC; with the addition of the combination of 1% FBS and the medium supplements to the basal medium producing the best growth; either medium component alone producing about half maximal growth; and Ham's F12 without FBS and the medium supplements not supporting growth. At P25, SV-HUC-1 had a diminished requirement for the medium supplements for optimal growth. Growth of SV-HUC-1 in the presence of serum, both with and without the medium supplements, (Table 2; compare 1% FBS-F12 and 1% FBS-F12+) resulted in similar growth. In the absence of serum (Table 2; compare F12 and F12+), the addition of medium supplements to the medium only slightly enhanced the growth of SV-HUC-1 at this passage. The growth promoting effects of FBS towards SV-HUC-1 were maintained with serial passage.

Clonal Growth of SV-HUC. The conditions for the growth of HUC and SV-HUC-1 at clonal density were compared (Table 3). Clonal growth of HUC in 1% FBS-F12+ was achieved only when the cells were cocultured with Swiss 3T3 feeder cells. Even at a relatively early passage (P15), SV-HUC-1 exhibited some clonal growth in the absence of feeder cells. SV-HUC-1 at P30 had completely lost the requirement of feeder cells for clonal growth, growing similarly in the presence or absence of the feeder cells. In the absence of 3T3 feeder cells, the importance of FBS and medium supplements for the clonal growth of SV-HUC-1 was determined. Under these conditions, the minimal amount of clonal growth in SV-HUC-1 (P15) was abolished by the deletion of either serum or the combination of supplements from the medium. At P30, the medium supplements were not essential for the clonal growth of SV-HUC-1 (P30), although their presence stimulated growth approximately 3-fold. The presence of 1% FBS in the culture medium, however, continued to be necessary for the clonal growth of SV-HUC-1.

Ultrastructural Alterations of SV-HUC. Transmission electron microscopy was performed on postconfluent cultures of normal HUC at P1 (Fig. 3, A-C) and SV-HUC-1 at P20 (Fig. 4, A-C). The ultrastructural features of HUC have been described in detail (15, 17). In contrast to HUC which have

---

Table 1  Comparison of growth of SV-HUC on collagen and plastic substrates

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Substrate</th>
<th>24-h Attachment (% of seeded cells)</th>
<th>Generation time (h)</th>
<th>Viability at log phase (%)</th>
<th>Saturation density (total cells × 10⁶)</th>
<th>Viability at confluence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HUC</td>
<td>Collagen</td>
<td>67 ± 6</td>
<td>39</td>
<td>76 ± 2</td>
<td>2.2 ± 0.1</td>
<td>64 ± 5</td>
</tr>
<tr>
<td>(P1)</td>
<td>Plastic</td>
<td>51 ± 6</td>
<td>131</td>
<td>58 ± 5</td>
<td>0.9 ± 0.1</td>
<td>73 ± 6</td>
</tr>
<tr>
<td>SV-HUC-1</td>
<td>Collagen</td>
<td>57 ± 15</td>
<td>43</td>
<td>85 ± 1</td>
<td>0.2 ± 0.1</td>
<td>80 ± 1</td>
</tr>
<tr>
<td>(P10)</td>
<td>Plastic</td>
<td>37 ± 15</td>
<td>115</td>
<td>82 ± 2</td>
<td>0.1 ± 0.1</td>
<td>75 ± 3</td>
</tr>
<tr>
<td>SV-HUC-1</td>
<td>Collagen</td>
<td>56 ± 1</td>
<td>40</td>
<td>87 ± 3</td>
<td>5.8 ± 0.5</td>
<td>82 ± 2</td>
</tr>
<tr>
<td>(P25)</td>
<td>Plastic</td>
<td>32 ± 5</td>
<td>40</td>
<td>87 ± 3</td>
<td>4.6 ± 0.3</td>
<td>79 ± 6</td>
</tr>
<tr>
<td>SV-HUC-2</td>
<td>Collagen</td>
<td>51 ± 5</td>
<td>40</td>
<td>77 ± 3</td>
<td>4.4 ± 0.5</td>
<td>76 ± 2</td>
</tr>
<tr>
<td>(P20)</td>
<td>Plastic</td>
<td>35 ± 5</td>
<td>40</td>
<td>69 ± 2</td>
<td>3.0 ± 0.1</td>
<td>51 ± 3</td>
</tr>
</tbody>
</table>

6069
Table 2 Effects of serum and medium supplements on the growth of HUC and SV-HUC

<table>
<thead>
<tr>
<th>Test media</th>
<th>FBS (%)</th>
<th>Attached viable cells (× 10^6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HUC (P1)</td>
</tr>
<tr>
<td>F12</td>
<td>0.1 ± 0.1</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>F12+</td>
<td>2.1 ± 0.3</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>F12+</td>
<td>2.5 ± 0.8</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>F12+</td>
<td>5.1 ± 0.3</td>
<td>3.2 ± 0.1</td>
</tr>
</tbody>
</table>

Table 3 Growth requirements of SV-HUC and HUC at clonal density

<table>
<thead>
<tr>
<th>Test medium</th>
<th>FBS</th>
<th>Feeder cells</th>
<th>Colony forming efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HUC (P1)</td>
<td>SV-HUC-1 (P15)</td>
</tr>
<tr>
<td>F12+</td>
<td>1%</td>
<td>±</td>
<td>18 ± 5</td>
</tr>
<tr>
<td>F12+</td>
<td>1%</td>
<td>- 0</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>F12+</td>
<td>1%</td>
<td>+ 0</td>
<td>0</td>
</tr>
<tr>
<td>F12*</td>
<td>1%</td>
<td>- 0</td>
<td>0</td>
</tr>
</tbody>
</table>

regularly shaped nuclei, SV-HUC-1 had very irregular nuclei which were sometimes larger in size, and often showed prominent clefiting. Multiple nucleoli of variable size and shape were also observed in SV-HUC-1 (compare Figs. 3B and 4B). Although cell processes were observed to overlap in cultures of SV-HUC-1, these cells did not form multilayers of cells with overlapping nuclei as was shown in cultures of HUC (compare Figs. 3A and 4A). In addition, the microvilli on the cell surface of SV-HUC-1 were pleomorphic, often elongated, and branched and lacked a glycoalyx. This is in contrast to the uniformly shaped microvilli and the presence of a well-developed glycoalyx on the surface of HUC (compare Figs. 3C and 4C).

Among the ultrastructural characteristics in common between HUC and SV-HUC-1 were numerous junctional complexes between cells, similar amounts of intracellular intermediate filaments, rough endoplasmic reticulum, and mitochondria. The altered ultrastructural features of SV-HUC-1 and SV-HUC-2 were found to be similar. Additionally, the ultrastructural alterations were similar regardless of whether the cells were grown on collagen-gel or plastic substrates.

Anchorage-independent Growth and Tumorigenicity of SV-HUC. Normal HUC were not capable of growth in semisolid media, nor did they produce tumors when inoculated into athymic nude mice (17). In contrast, SV-HUC were capable of forming colonies in 0.3% agarose. The frequency of colony formation of SV-HUC-1 in soft agarose progressively increased as cells were serially passaged in vitro, from approximately 3 × 10^(-2) at P10 to approximately 3 × 10^(-2) at P35. SV-HUC-2 also tested positive for growth in semisolid medium (approximately 4 × 10^(-4) at P15).

Both SV-HUC-1 and SV-HUC-2 continued to be nontumorigenic when 3–5 × 10^6 cells were inoculated s.c. into nude mice at all passages tested (SV-HUC-1, 0 tumors/32 inoculations, P10-50; SV-HUC-2, 0 tumors/11 inoculations, P10-25). Thus, the increase in anchorage-independent growth of SV-HUC-1 after serial passage was not associated with the spontaneous acquisition of tumorigenicity.

Karyology of SV-HUC. A karyotypic analysis of SV-HUC-1 was performed at P15. The cell line showed a bimodal chromosomal distribution, with approximately 50% of the cells near diploid (modal number 44) and 50% of the cells in the tetraploid range (modal number 88). The chromosome pattern was heterogeneous, showing various random chromosome changes in individual cells. Both the diploid and tetraploid populations showed seven consistent marker chromosomes, namely, 5p+, del(6)(p11), 9q+, 11p+, 15q−, 19p+, and an Xp+. All cells examined contained at least five of the seven markers. In addition, most of the cells in both the diploid and tetraploid populations had no normal number 15 chromosomes due to translocations resulting in the 5p+ and Xp+ markers. The tetraploid population was not an exact doubling of the diploid as it consistently had a 14q/21q translocation affecting two number 14 and two number 21 chromosomes, while the diploid component had normal number 14 and 21 chromosomes but a 6q/14q translocation instead (Fig. 5). On extended serial cultivation (i.e., at P50), additional chromosomal changes were observed in the SV-HUC-1 line. A detailed analysis of these karyotypic changes will appear elsewhere.

DISCUSSION

In this study, we describe the in vitro transformation of human uroepithelial cells to apparently immortal, but nontumorigenic cell lines by SV40. In vitro transformation by SV40 has been previously described for a variety of other human cell types of both mesenchymal (3, 24) and epithelial origin (25–33). Qualitatively, the characteristics of the SV40-infected cultures leading to the establishment of SV-HUC with an extended lifespan were most similar to those described for SV40-transformed epithelial cells, in particular keratinocytes; and differed from those described for transformed human fibroblasts. The lack of noticeable morphological alterations or a piling-up of SV40-infected HUC is in contrast to the early formation of distinct foci composed of dense overlapping cells which has been described following SV40 infection of human fibroblasts (24) and has also been observed in our laboratory in SV40-infected human ureteral fibroblasts. The inability to select for SV-HUC on the basis of obvious morphological changes is most likely related to the epithelial nature of the cells and a similar lack of early marked morphological changes indicating transformation has been noted in the SV40 transformation of human keratinocytes (27). In addition, SV-HUC did not undergo a crisis phase as defined by cell death or a diminished rate in cell growth which is characteristically observed in SV40-transformed human fibroblasts after an initial period of accelerated growth (3, 24). At early passages after selection of cells with an extended lifespan, cultures of SV-HUC did exhibit extensive cell lysis and increased cell death particularly as the cultures became dense, which may be analogous to the crisis periods described by others. Yet, when these cultures were maintained in log phase, SV-HUC grew at a rate similar to that of HUC and have continued to grow for a period of more than 2 years without a decrease in growth rate. A crisis phase has been reported in SV40-transformed human epithelia derived from amnion (26) and breast (30) tissue as well as keratinocytes (4) and the frequency with which postcresis lines could be recovered ranged from rare (26, 30) to frequent (4). In agreement with the SV40 transformation of HUC, Taylor-Papadimitriou and coworkers have reported a complete absence of a crisis phase for SV40-transformed keratinocytes (29). Properties intrinsic to the original cell type may in part determine the
culture longevity of the SV40 transformants, however, factors such as the virus type, multiplicity of virus infection, age of donor tissue, and culture conditions can also influence SV40 transformation (24). Steinberg and Defendi (31) reported a large variability in the extent to which their different keratinocyte lines exhibited the crisis phase and indicated that the emergence of cell lines from cultures in crisis could be enhanced by maintaining the cultures in log phase. That culture conditions can influence the survival of the transformants was demonstrated in the present study, since coculture of SV40-infected HUC with feeder cells was observed to enhance the number of cells surviving senescence. This is consistent with the finding that early passage SV-HUC had culture requirements for growth at clonal density similar to that of HUC and did not acquire the properties of more autonomous growth until later passages. In that transformation by SV40 occurs with low frequency, culture conditions which are optimal for growth of cells at low density may be required to increase the probability of establishing transformants.

Previous studies have demonstrated that cells transformed by SV40 are capable of being propagated under culture conditions which do not support the growth of their normal counterparts (10). Similarly, later passages of SV-HUC were capable of growth on plastic, in semisolid medium and growth independent of both medium supplements and feeder cells at clonal density. Acquisition of the altered phenotypic properties in SV-HUC was a gradual process, with early passage cultures exhibiting traits more similar to HUC than later passage cells. Although the karyotype of SV-HUC-1 at early passage demonstrated a considerable degree of heterogeneity, five of seven consistent marker chromosomes were present in all of the cells examined. Since it is unlikely that these markers all arose independently in different cells, at some point prior to P15, SV-HUC-1 had most likely originated from a single cell. In agreement with these results, other studies have shown progressive independence of cloned lines of transformants from normal medium and substrate requirements with serial subcultivation (4, 29). Thus, SV40-transformed cells of clonal origin exhibit a genetic lability and a capacity to readily adapt to culture conditions.

Postconfluent cultures of SV-HUC had a higher viability and attained higher saturation densities than normal HUC. The increased confluent density, however, was not associated with an increase in growth rate during the exponential phase of growth. Similar results have been obtained for SV40-transformed keratinocytes (32) and Kaighn et al. reported a decrease in the maximal growth rate for various lines of SV40-transformed prostate epithelial cells (28). Thus, SV-HUC do not exhibit growth inhibition which is operative in cultures of normal HUC and continue to divide at cell densities which would suppress growth of HUC. One possible explanation for the increased viability and saturation density of SV-HUC is an inability to differentiate. The loss of differentiated functions of SV40-transformed human epithelial cells has been clearly dem-

---

**Fig. 3.** Transmission electron micrographs of normal HUC showing (A) the formation of multilayers of cells, (B) the regular appearance of nuclei, and (C) the regular shape of microvilli and the presence of a surface glycocalyx. The cells were seeded on plastic dishes and were fixed at 14 days postconfluence. Bars, 1.0 μm, 10.0 μm and 100 nm for A, B, and C, respectively.
TRANFORMATION OF HUMAN UROEPITHELIAL CELLS BY SV40

Fig. 4. Transmission electron micrographs of SV-HUC-1 showing (A) lack of multiple cell layers, (B) irregularly shaped nuclei, and (C) the presence of pleomorphic microvilli lacking a glycocalyx. All conditions as in Fig. 3.

Fig. 5. Karyotype of a near-tetraploid cell of SV-HUC-1 at P15. Consistent markers include: 5p+, del(6)(p11), 9q+, 11p+, 15q−, 19p+, and Xp+.

Demonstrated in the keratinocyte system where the transformants lose the ability to stratify and do not progress to form terminally mature squamous cells (4, 29, 32). Postconfluent cultures of normal HUC stratify to form multilayers of cells in which the nuclei overlap. This stratification usually does not exceed three cell layers and the superficial layer of cells exhibits a well-developed glycocalyx. Although stratification is not indicative of complete terminal differentiation of HUC (34), it may rep-
resent an initial step in this process. In agreement with the characteristics of SV40-transformed keratinocytes, SV-HUC did not show stratification and thus, the increased saturation density was representative of a tighter packing of cells. In addition, the plasma membrane of SV-HUC completely lacked a glycocalyx.

The surface microvilli of SV-HUC were branched and irregular in appearance in comparison to the uniformly shaped microvilli of HUC. Pleomorphic microvilli have been observed on uroepithelium from human transitional cell carcinoma biopsies of the urinary bladder as well as carcinomas from other tissues (35). Also, the formation of altered microvilli on rodent uroepithelium can be induced both in vivo and in vitro by experimental bladder carcinogens and represents a very early change in the carcinogenic process (36, 37). Although the significance of pleomorphic microvilli as a marker of neoplasia is controversial, the observation that SV-HUC, a transformed but nontumorigenic cell line, possess these altered microvilli is consistent with their appearance in vivo at an early stage following carcinogen treatment.

In summary, a line of human uroepithelial cells has been developed and characterized, and early passage cells (P15) have been cryopreserved for future experimentation. Since these cells have an extended lifespan but remain nontumorigenic, characteristics associated with an early step in the neoplastic transformation of normal cells in vitro (6–9), this cell line is suitable for studies of subsequent steps of neoplastic transformation.

In a preliminary report, we have described tumorigenic conversion of SV-HUC after exposure to 3-methylcholanthrene (38). It will be particularly relevant to test the transforming capability of recognized human bladder carcinogens towards SV-HUC.

ACKNOWLEDGMENTS

The authors express their appreciation to Dr. Lorraine F. Meisner and Dr. Shi-qi Wu of the University of Wisconsin State Laboratory of Hygiene for the karyotypic analysis. We also wish to thank Melanie Ignjatovic for her expert technical assistance and Karen Blomstrom for her careful preparation of this manuscript.

REFERENCES

Characterization of Human Uroepithelial Cells Immortalized in Vitro by Simian Virus 40


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/47/22/6066

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.