Establishment and Characterization of Human Renal Cancer and Normal Kidney Cell Lines

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ABSTRACT

We have reviewed our laboratory's efforts to establish continuous human renal cancer cell lines. During the 16-year period of 1972 through 1987, 498 successive attempts resulted in establishment of 63 renal cancer cell lines. Of these lines, 46 were derived from primary kidney tumors and 17 from metastatic sites (lung, brain, bone, and lymph node). Forty-three of these lines have been characterized with regard to morphology, growth kinetics, anchorage-independent growth, tumorigenicity in athymic nude mice, and expression of kidney cell surface antigens. These results were compared with data from primary short term cultures of normal kidney epithelium. The overall success rate of establishing continuous renal cancer cell lines was 12.7%. In general, no significant difference in success was noted based on whether the specimen was derived from a primary or a metastatic lesion. However, all successfully established lines were derived from tumors exhibiting clinically "aggressive" behavior. All cell lines expressed proximal tubular cell differentiation antigens. Significant morphological heterogeneity was observed among normal kidney as well as kidney cancer cell lines in vitro. No significant difference in doubling time was found between cell lines of renal cancer and passage 1 cultures of normal kidney epithelium. Twenty-one of 30 (70%) lines assayed formed clones on soft agar and 26 of 33 (79%) lines grew in athymic mice. Among the 25 lines which were assayed continuous renal cancer cell lines was 12.7%. In general, no significant difference in success was noted based on whether the specimen was derived from a primary or a metastatic lesion. These results were compared with data from primary short term cultures of normal kidney epithelium. The overall success rate of establishing continuous renal cancer cell lines was 12.7%. In general, no significant difference in success was noted based on whether the specimen was derived from a primary or a metastatic lesion. However, all successfully established lines were derived from tumors exhibiting clinically "aggressive" behavior. All cell lines expressed proximal tubular cell differentiation antigens. Significant morphological heterogeneity was observed among normal kidney as well as kidney cancer cell lines in vitro. No significant difference in doubling time was found between cell lines of renal cancer and passage 1 cultures of normal kidney epithelium. Twenty-one of 30 (70%) lines assayed formed clones on soft agar and 26 of 33 (79%) lines grew in athymic mice. Among the 25 lines which were assayed continuous renal cancer cell lines was 12.7%. In general, no significant difference in success was noted based on whether the specimen was derived from a primary or a metastatic lesion. However, all successfully established lines were derived from tumors exhibiting clinically "aggressive" behavior. All cell lines expressed proximal tubular cell differentiation antigens. Significant morphological heterogeneity was observed among normal kidney as well as kidney cancer cell lines in vitro. No significant difference in doubling time was found between cell lines of renal cancer and passage 1 cultures of normal kidney epithelium. Twenty-one of 30 (70%) lines assayed formed clones on soft agar and 26 of 33 (79%) lines grew in athymic mice. Among the 25 lines which were assayed continuous renal cancer cell lines was 12.7%. In general, no significant difference in success was noted based on whether the specimen was derived from a primary or a metastatic lesion. However, all successfully established lines were derived from tumors exhibiting clinically "aggressive" behavior. All cell lines expressed proximal tubular cell differentiation antigens. Significant morphological heterogeneity was observed among normal kidney as well as kidney cancer cell lines in vitro. No significant difference in doubling time was found between cell lines of renal cancer and passage 1 cultures of normal kidney epithelium. Twenty-one of 30 (70%) lines assayed formed clones on soft agar and 26 of 33 (79%) lines grew in athymic mice. Among the 25 lines which were assayed continuous renal cancer cell lines was 12.7%. In general, no significant difference in success was noted based on whether the specimen was derived from a primary or a metastatic lesion. However, all successfully established lines were derived from tumors exhibiting clinically "aggressive" behavior. All cell lines expressed proximal tubular cell differentiation antigens. Significant morphological heterogeneity was observed among normal kidney as well as kidney cancer cell lines in vitro. No significant difference in doubling time was found between cell lines of renal cancer and passage 1 cultures of normal kidney epithelium.

INTRODUCTION

The study of human tumors adapted to in vitro growth offers many advantages: (a) availability of unlimited numbers of cells, (b) ability to perform multiple and repeated experiments over long time intervals, (c) ability to study metabolic events in viable cells, (d) ability to manipulate and control cells in vitro in ways not possible in vivo, and (e) ability to exchange cell lines among several laboratories, thereby allowing study of identical material.

In 1962 the first human renal cancer cell line was established in continuous culture (1). Since then, 13 different laboratories have published (2-14) characterizations of 22 additional cell lines. While these reports have provided adequate details regarding the methods used, few have indicated the number of specimens or attempts which were made in order to establish these cell lines. Therefore, the relative ease of adapting human renal cancer to tissue culture has not been apparent.

The purpose of this report is to provide information about our experience with the establishment of 63 continuous RC cell lines and numerous short term NK cultures as well as to begin to characterize these cell types. Our first renal cancer cell line (SK-RC-1) was established in 1973. This paper reviews the previously unpublished experience of the 16 years 1972-1987, inclusive.

MATERIALS AND METHODS

Tissues. An estimated 1000 nephrectomies were performed at Memorial Sloan-Kettering Cancer Center during the years of this study. Fresh operative specimens were obtained from the Tumor Procurement Service (Department of Surgical Pathology) of Memorial Hospital and processed in our laboratory between January 1, 1972, and December 31, 1987. Specimens were handled stereilly by the pathologist, who provided at least 1 g of tissue from the tumor and, in the case of a nephrectomy, from an area of grossly normal-appearing kidney. These tissue samples were brought to the tissue culture laboratory in separate sterile specimen containers, placed in cold (4°C) medium (see below), and refrigerated until they could be processed (within 18 h).

Tissues, whether normal or neoplastic, primary or metastatic, were processed in an identical manner. Any surrounding fat, vessels, connective tissue, and capsule were excised. The specimen was minced finely with scissors and/or scalpel in a Petri dish containing 3–5 ml of 0.01 M sodium phosphate, 0.15 M NaCl (PBS), pH 7.2. This mixture was then transferred to a sterile 100-ml bottle containing 25 ml of 0.25% trypsin and 0.02% collagenase in PBS. The mixture was stirred at 37°C for 30 min with a magnetic stirring bar. In approximately one third of cases, the enzyme treatment was omitted, without an apparent change in success rate. An equal volume of complete medium (Eagles minimal essential medium, 1% nonessential amino acids, 2 mM glutamine, 100 µl/ml penicillin, 100 µg/ml streptomycin, 7.5% fetal bovine serum) was added and the cells were washed twice with fresh complete medium by pelleting at 1000 rpm for 10 min. Cell viability was not ascertained and seeding density was not tightly controlled. Typically, a 25–50-µl cell pellet was seeded per 25-cm² flask area, using either T25 or T75 tissue culture flasks (Falcon Labware, Lincoln Park, NJ), in complete medium. Flasks were placed in a 37°C incubator and maintained in a 5% CO₂ atmosphere.

Maintenance of Cell Cultures. Cultures were fed twice weekly and passed shortly after becoming confluent. Cells were removed from the flask by pouring off the old medium, rinsing the flask with sterile PBS, and adding 0.25% trypsin/0.1% EDTA at 37°C until the cells floated off the plastic (usually 2–5 min). Complete medium was added and the cells were washed twice with fresh complete medium by pelleting at 1000 rpm for 10 min before resuspension in complete medium and seeding to new flasks. In general, cultures were split 1:2 to 1:8.

Cultures are routinely tested every 4 to 6 weeks for Mycoplasma by the Microbiology Laboratory at our institution. Any contaminated flasks were discarded. In addition, aliquots of cells were cryopreserved in complete medium/10% dimethyl sulfoxide and stored in liquid nitrogen after every 5–10 passages.

Growth Kinetics. Five to 10 × 10⁶ cells/well were plated in Falcon 3047 multiwell tissue culture plates (Falcon Labware, Lincoln Park, NJ). The number of cells in each of triplicate wells was counted after 24, 48, 72, 96, and 120 h, using a Coulter counter (Coulter Electronics, Inc., Hialeah, FL). Cells were fed every other day. The doubling time of cell populations was determined during the logarithmic growth phase.

Anchorage-independent Growth. A bottom layer of 0.6% agar was prepared with complete medium and Bacto-Agar (Difco Laboratories,
Table 1 Characterization of mouse monoclonal antibodies detecting human renal epithelial cell antigens

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<tr>
<th>Designation of antibody (Ig subclass)</th>
<th>Designation of antigen</th>
<th>Biochemical characterization of antigen</th>
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<tr>
<td>mAb S4 (γ2a)</td>
<td>URO-2</td>
<td>M, 160,000 glycoprotein</td>
<td>Glomerular visceral and parietal epithelium</td>
<td>Tumor cell epithelium</td>
<td>15-18</td>
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<tr>
<td>mAb F23 (γ2a)</td>
<td>URO-3</td>
<td>M, 140,000 glycoprotein (aminopeptidase N)</td>
<td>Proximal tubules and fibroblasts</td>
<td>Tumor cell epithelium and fibroblasts</td>
<td>16-18, 21</td>
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<tr>
<td>mAb S27 (γ1)</td>
<td>URO-4</td>
<td>M, 120,000 glycoprotein (adenosine deaminase binding protein)</td>
<td>Proximal tubules and thin loop of Henle</td>
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<td>mAb F31 ( μ)</td>
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<td>Proximal tubules (pars recta)</td>
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* Monoclonal antibodies identifying the URO antigens are available from Signet Laboratories (Dedham, MA).

RESULTS

Establishment of Cell Lines

Renal Cancer. A total of 498 renal cancer specimens were seeded in culture during the period of this study. A total of 363 (73%) specimens were derived from male patients; 135 (27%) specimens were from female patients. This yields a male to female ratio of 2.69:1, consistent with that generally reported for renal cancer (23). In all, 378 of the 498 (76%) were derived from nephrectomy specimens; 120 (24%) specimens were from metastatic lesions (Table 2).

Approximately 50% of specimens survive one passage, with gradual attrition down to 15% surviving six passages. In general, cultures which have survived greater than six passages...
appear to have the ability to survive indefinitely in vitro (24). We have arbitrarily defined "established continuous cell lines" as those cultures which survive 10 passages and 1 year in continuous culture. These lines are then given an SK-RC designation. Sixty-three continuous RC lines have been established in 498 attempts, for an overall success rate of 12.7%. Overall, no significant difference was observed in establishing lines from primary [46 of 378 (12.2%)] or from metastatic [17 of 120 (14.2%)] lesions.

The 63 lines were derived from 60 patients. In 3 cases, 2 separate lines were developed from different sites. Medical records were reviewed for 40 of the 60 patients whose tumor specimens became established in culture. It was found that, in all cases, these tumor specimens exhibited clinically "aggressive" behavior. Of the 40 patients, 35 either had at the time of surgery or developed soon thereafter metastatic disease. The remaining 5 patients (SK-RC-3, -7, -11, -34, and -40) all had main renal vein involvement at the time of surgery but did not develop metastatic disease during follow-up of at least 4–8 years. These findings indicate that a tumor must be biologically aggressive if it is to adapt to tissue culture. The site from which the specimen is derived (i.e., primary versus metastatic) appears unimportant.

The sex of the donors was reviewed to determine any impact on the likelihood of adapting tumors to culture. Fifty-one of 363 (14%) male and 12 of 135 (8.9%) female-derived tumors adapted to culture. This difference was not statistically significant.

Morphologically, these renal cancer cell lines take several forms (Fig. 1). Some are epithelioid with distinct cell borders, others have irregular or poorly defined borders, and others consist of spindle cells. Some lines contain cytoplasmic vacuoles or granules. None of the renal cancer lines exhibits contact inhibition.

Normal Kidney. Normal kidney epithelium can be adapted to in vitro growth on virtually every attempt. Unlike the cancer lines, however, these cultures survive only an average of three passages. These passages can be spread over a period of 2–3 months. Alternatively, these cells can be cryopreserved at early passage (passage 1 or 2), thawed, and reseeded for later use if desired. Normal kidney cells adhere and begin dividing rapidly in culture, in some cases earlier than their neoplastic counterparts. Morphologically, several different cell types are seen in a typical normal kidney culture (Fig. 2). One may note polygonal, round, and ellipsoidal cells. These cell types organize and grow among each other in different patterns. Contact inhibition is seen and the cells appear to cease dividing when a confluent monolayer has formed. Renal epithelial cells can easily be distinguished from fibroblasts. Fibroblast overgrowth has not been a significant problem in our experience.

Antigenic Phenotype

Selection of mAbs. The mAbs utilized in this study, and the antigens they define, have been well characterized with respect to immunochemistry and tissue distribution in vivo and in vitro (Table 1). We and others (22, 25, 26) have shown that antigenic expression can change as cells adapt to a tissue culture environment. There are four patterns which characterize this shift: antigens which are expressed in vivo can remain expressed in vitro (on—on) or may be turned off in vitro (on—off) or antigens not expressed in vivo can be turned on (off—on) or remain unexpressed (off—off). Before antigen expression can be used as a basis for extrapolating between in vivo and in vitro settings, one must determine that the particular antigens are expressed or not expressed in a consistent fashion (i.e., on—on; off—off). The mAbs selected for this study have the characteristic of consistent patterns of reactivity or expression both in vivo and in vitro. The specificity of these mAbs for renal epithelial antigens in vivo, the derivation of the specimens from normal kidney or renal cancers, and the demonstration of a consistent pattern of expression of these antigens in vivo and in vitro offer assurance that the cells growing in vitro are indeed derived from renal epithelia.

Normal Kidney Cells. Antigen expression of more than 50 normal kidney cell lines, each derived from a different donor, was studied in detail. Each line displayed an identical pattern of antigen expression when assayed at passage 1. That is, three of four (URO-2, -3, and -4) PT cell markers reacted with at
least 90% of the cells of each line. A subpopulation of 10–20% of the cells in all NK cultures reacted with the fourth PT antigen (URO-8), which is expressed only by cells of the straight portion of the proximal tubule (19).

Renal Cancer Cells. Forty-three long term renal cancer cell lines have been phenotyped using the same panel of mAbs (Fig. 3). All 43 cell lines expressed at least one of the four PT differentiation antigens (URO-2, -3, -4, and -8) assayed. Forty-one of 43 (95%) lines expressed at least two distinct PT-specific antigens.

In 28 lines, antigen expression has been tested at both early (passage 3 to 20) and late (50 to 150+) passage. Loss of expression of these PT antigens over time in tissue culture was a relatively rare event. Antigen loss was, however, observed with URO-3 (mAb F23). Six of 28 cell lines (SK-RC-6, -17, -35, -45, -47, and -62) demonstrated partial or complete loss of this antigen when assayed at late passage. Loss of URO-2 (mAb S4) was noted in one line, SK-RC-18, and decreased reactivity for URO-8 (mAb F31) was noted in two lines, SK-RC-21 and SK-RC-37. All other cancer lines which were assayed at early and late passage maintained the same antigenic phenotype.

No consistent correlation was noted between antigenic phenotype and histological subtype (e.g., clear cell or granular cell) of renal cancer.

Growth Characteristics

Doubling Time. The DT of RC lines ranged from 30 to 96 h, with a mean of 46 h (Fig. 3). No significant difference was observed between cell lines derived from a nephrectomy specimen (mean, 48 h) and lines obtained from metastatic sites (mean, 44 h). Four different normal kidney lines in passage 1 were found to double in a similar time interval (40–42 h). However, growth slowed down in passage 2 (DT = 96 h) and typically stopped in passage 3. Normal kidney cultures only rarely survive five passages.

Anchorage-independent Growth. Twenty-one of 30 (70%) lines formed clones in soft agar with cells plated at a concentration of 15,000 cells/ml (Fig. 3). The site of origin of the cell line (i.e., primary versus metastatic) did not appear to have an impact on its ability to grow on soft agar. Normal kidney cells did not grow on soft agar.

Tumorigenicity. Twenty-six of 33 (79%) RC lines formed tumors when injected s.c. into male nude mice (Fig. 3). Seventeen of 23 (74%) lines derived from primary lesions grew in nude mice, while 9 of 10 (90%) metastatic lines formed tumors. This difference was not statistically significant.

DISCUSSION

Analysis of 498 consecutive attempts to establish continuous renal cancer cell lines from primary and metastatic kidney tumor lesions demonstrates that kidney cancer can be adapted to growth in vitro in approximately 13% of all attempts. Since we are aware of only one other report (2) which indicated how many attempts were required to establish their line(s), the success rate of establishing renal cancer cell lines has not been previously appreciated. In the report by Giard et al. (2), 2 lines were established in 21 attempts (9.5%), consistent with the results reported here. This success rate is paralleled only by that achieved with the in vitro growth of human melanomas. In other human cancers the yield has been in the range of 1% or less (2).

The relatively high success rate of adapting renal cancers to in vitro growth is further enhanced by the ability to grow the autologous normal cellular counterpart, i.e., renal proximal tubular cells, under identical conditions. The study of human renal cancer in vitro, therefore, provides a unique and unparalleled experimental system to study biochemical, immunological, metabolic, and genetic differences between normal and transformed cells. It is interesting that both renal cancers, which are generally slow growing in vivo, and adult proximal tubular cells, which do not divide in vivo, should adapt to grow in vitro so much better than other cell types.

Since our initial description (24) of short term cultures of normal human kidney epithelium, additional reports (6, 27–29) have been published. The experience of Matsuda et al. (7) was quite consistent with our own. They successfully derived epithelial monolayers in 10 of 10 cases. They, too, noted "considerable heterogeneity in cellular morphology." Contrary to the experience of Matsuda et al. and our own, Detrisac et al. (27) found a uniform morphology in their cultures, which, by histochemical and immunological profile, appeared to be pure cultures of proximal tubular cells. This difference may be related to the fact that Detrisac's cultures were derived from the outer cortex only, while our cultures utilized both the kidney cortex and medulla. In addition, Detrisac et al. grew their cultures on extracellular matrix, which could exert a selective pressure. We are currently attempting to correlate antigenic phenotypes with particular morphological characteristics.

The average DT of 46 h for our RC lines (range, 30 to 96 h) is in complete agreement with other reports (6–8). We did not find a significant difference in DT between primary, metastatic,
or normal kidney (passage 1) cell lines. However, as also reported by Matsuda et al., we observed a prolonged DT in passage 2 NK cells (96 h). These lines eventually stopped growing completely in passage 3 to 5. Detrisac et al. grew NK cells on an extracellular matrix and by doing so were able to extend the life span of their NK cultures from 3 to 16 passages.

Twenty-one of 30 (70%) RC lines form colonies on soft agar. Anchorage-independent growth is considered a typical feature of transformed cells (30) and has been observed in other RC lines as well (6, 7, 11). In a study by Stiles et al. (31), no strong association between anchorage-independent growth and tumorigenicity in athymic nude mice was revealed. This is consistent with our data for 25 RC lines tested for both growth in soft agar and tumorigenicity in nude mice. Results of these assays were concordant in 17 lines (60%); 4 lines (16%), despite forming clones on soft agar, did not grow in nude mice; the 4 remaining lines (16%) formed tumors in the animals but did not grow in soft agar. Cell lines which could grow both on agar and in nude mice did not appear to be clinically more aggressive than those which possessed either trait individually.

Derivation of these cell lines from renal cancer specimens and expression of restricted renal tubular antigens by these cell lines confirm that the lines are in fact renal cell carcinomas. The pattern of antigenic expression by these cell lines parallels our immunohistochemical findings with fresh frozen sections of renal cancers. That is, they express proximal tubular differentiation antigens, although not uncommonly they fail to express this full complement of these antigens.

It is interesting that all of the cell lines which could adapt to immortal growth in vitro were derived from tumors which exhibited clinically aggressive features (e.g., metastasis or main renal vein invasion). Conversely, the finding that only 14.2% of metastatic tumors could be established in vitro implies that biological aggressiveness is necessary but not always sufficient for establishment of a cell line. Further, if biological aggressiveness is a necessary characteristic, it is interesting that the success rate is not significantly different based on whether the specimen is derived from a metastatic lesion (the sine qua non of clinical aggressiveness) or a localized primary renal cancer. One explanation for this observation is that all renal cancer primaries contain biologically aggressive cells. This is, perhaps, not surprising in view of the fact that approximately 50% of patients with renal cancer have metastatic disease (25% overt, 25% occult) at the time of initial diagnosis. But the converse, that 50% of patients presenting with renal cancer never develop metastasis, even when in some cases the primary tumor is enormous, would have argued that a major proportion of renal cancers are clinically "benign" and do not possess metastatic potential. This latter position is not consistent with our findings here.

In summary, this study shows that renal cancer cells can successfully be adapted to in vitro growth. This has provided us with continuous cultures from primary as well as metastatic lesions from bone, brain, lung, adrenal, and other soft tissue sites. In addition, the study of renal cancer offers the unique opportunity to have readily available under the same conditions the normal autologous counterpart. This variety of material allows comparative analysis of genetic, biochemical, and/or immunological differences between the tumor cell and its normal counterpart, between primary and metastatic lesions, and between metastatic lesions of different sites.

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REFERENCES

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