Establishment and Cell Cycle Kinetics of a Human Squamous Cell Carcinoma in Nude Mice and in Vitro

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ABSTRACT

A human squamous cell carcinoma of the vulva was xenografted to athymic, nude mice. A tissue culture cell line designated SqCaVu-1H was derived from a second-passage xenograft. The growth characteristics and cell cycle kinetics in the xenografts and in SqCaVu-1H cells were compared. Approximately 80% of the tumor implants produced growing xenografts which had a 2-week latent period followed by Gompertzian growth with a doubling time of 5 to 30 days at 35 days postimplantation. The cell cycle kinetics of the xenografts revealed a heterogeneity from region to region within the tumor. G2 phase and S phase in the xenografts are approximately 8 and 13 hr, respectively.

The SqCaVu-1H cells contain only human chromosomes. The modal chromosome number was 64. SqCaVu-1H cells produce plasminogen activator during logarithmic growth, and they produce tumors when injected s.c. into athymic, nude mice. Logarithmically growing SqCaVu-1H cells have a population-doubling time of 21.8 hr in tissue culture. In vitro, their cell cycle duration is approximately 16.9 hr, with G2 phase at 5.6 hr and S phase at 8.6 hr. Comparison of the growth of the same human tumor cells under in vivo and in vitro conditions serves to emphasize that tumor cell proliferation depends strongly on the microenvironment. The varied proliferation characteristics are correlated with their varied inhibition of deoxyuridine incorporation into DNA because of exposure to methotrexate. Logarithmically growing SqVaCu-1H cells had a 50% inhibitory dose of 2.2 × 10^-6 M. Plateau-phase cells in vitro had a 50% inhibitory dose of 9 × 10^-6 M, while the inhibition of cells from the xenografts was nearly dose independent.

INTRODUCTION

Previous reports have described the tumorigenicity of HeLa cells, a tissue culture line derived from a human epithelioid carcinoma of the cervix (12, 17), but the cell cycle kinetics of these xenografts has not been investigated (1, 10, 33). Previously published data on cell cycle kinetics of human tumor xenografts in athymic (hereafter called nude) mice are rare. The cell cycle kinetics of a human fibrosarcoma and a malignant melanoma xenografted to nude mice has been reported to compare favorably with previously published cell cycle parameters of similar tumors (28). Cell cycle kinetic data of xenografts from human carcinomas of the lung and tongue presented by Shimosato et al. (30) are also within the range of prior clinical data. No previous report of a primary xenograft of a human squamous cell carcinoma of the female genital tract to nude mice has come to our attention.

The purposes of the present paper are to report the establishment of a human squamous cell carcinoma of the female genital tract growing as xenografts in nude mice and in tissue culture, to partially characterize the biological properties of these cells, and to compare the cell cycle kinetics between xenografts in nude mice and an in vitro culture of the tumor cells derived from the same xenografts.

MATERIALS AND METHODS

Xenografts. Tumorous tissue which was diagnosed as a very poorly differentiated squamous cell carcinoma of the vulva by our consulting pathologist, Dr. Alan Ng, was taken from surgery and placed in McCoy’s 5A tissue culture medium with 20% fetal calf serum (Grand Island Biological Co., Grand Island, N. Y.). All sample preparations were made using sterile technique. Seventy-five min later, the medium was replaced with fresh medium containing polymyxin B sulfate, 500 units/ml (Burroughs Wellcome Co., Research Triangle Park, N. C.), bacitracin, 50 units/ml (The Upjohn Co., Kalamazoo, Mich.), and nystatin, 250 units/ml (E. R. Squibb & Sons, Inc., New York, N. Y.). The tissue was incubated for 30 min at 37º in this mixture and then minced into pieces approximately 3 mm in diameter. One tumor fragment was surgically implanted s.c. into each of 4 sites in 2 female RNC nude mice, 20 weeks of age (Dr. O. P. Miniats, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada) (9). The mice were maintained disease free in a specific-pathogen-free colony. Five to 13 weeks after implantation, serial transplantations were performed.

Starting with the third passage, all subsequent transplantations were performed in BALB/c nude mice of either sex who were 4 to 6 weeks of age (ARS/Sprague-Dawley, Madison, Wis.). Tumors from various passages were intermittently biopsied and processed for histological examination. The tumor size of xenografts was measured with calipers and calculated by dividing by 2 the product of the length, width, and height in mm. The growth of 12 tumor nodules in 6 mice was followed over the first 3 transplantations, including the primary implantation from the patient. Tumor size was plotted as a function of time after implantation. Best fits of tumor growth data to the Gompertzian growth equation and to an exponential growth
Squamous Cell Carcinoma in Nude Mice and in Vitro

Cell Cycle Kinetics. Mice bearing tumor nodules from the first, second, and fifth transplantation passages were given i.p. injections of \([^{3}H]dThd\), \(2 \muCi/g\), 1.9 Ci/mmol (New England Nuclear, Boston, Mass.), for PLM analysis. At various times after injection, biopsies were sequentially removed from different sites of the same or other tumors from the same mouse which was lightly anesthetized with ether. Care was taken to biopsy areas not previously traumatized by previous biopsies. Each mouse carried one or 2 tumors. Several mice bearing tumors were used whenever insufficient tumor tissue was borne by any single mouse. This method of biopsy was adopted for 2 reasons: (a) it parallels the method of biopsy used in clinical cell cycle kinetics studies in which sequential biopsies are required from the same tumor; (b) it spares the relatively great expense of using a single mouse bearing a single tumor for each time point assayed. Tumor nodules used in these studies with a single exception ranged from approximately 1.8 to 6.5 cm in diameter and were 34 to 50 days post-implantation at the time of the study. Biopsies were fixed and processed for histology, and 4-μm sections were mounted on microscope slides for autoradiography. Microscope slides were deparaffinized, hydrated, and dipped in NTB-3 emulsion (Eastman Kodak, Rochester, N. Y.). After 2 to 3 weeks of exposure, the slides were developed in D-19 (Eastman Kodak) developer and stained with hematoxylin and eosin. Grain counts of local backgrounds were determined over 10 areas, each 170 sq μm. Backgrounds were typically less than 1 grain per nuclear area, which was approximately 170 sq μm. Cells were counted as labeled when the number of grains over the nucleus was greater than the average local background in an area of 340 sq μm, i.e., more than twice background. All phases of mitosis, prophase through telophase, were counted for PLM assays. At least 100 mitoses were counted per biopsy. Labeling indices were determined from the first biopsy from the PLM assay. Over 500 cells were counted for each labeling index and each mitotic index.

The growth fraction was estimated by the equation

\[
GF = \frac{L_{obs}}{L_{theo}}
\]

where \(GF\) is the growth fraction, \(L_{obs}\) is the observed labeling index, and \(L_{theo}\) is the theoretical labeling index. The theoretical labeling index was calculated by the method of Cleaver (8). Independent estimates of the growth fractions of medium-sized and very large tumors were accomplished by injecting \([^{3}H]dThd\), 2 μCi/g, 1.9 Ci/mmol i.p. every 10.5 hr for 52.5 hr into 2 mice bearing sixth-passage tumors with approximately 2.5- and 3.5-cm diameters, respectively. Specimens were taken 0.5 hr after the last \([^{3}H]dThd\) injection for autoradiographic examination. The percentage of labeled cells and labeled mitotic cells in a population of over 1000 cells was determined in several areas of each tumor nodule.

In vitro cell cycle kinetics of SqCaVu-1H cells was analyzed from cultures grown on the microscope slides described above. Twenty thousand cells were inoculated into each of 4 chambers/slide with 0.5 ml tissue culture medium. After 3 days in culture, \([^{3}H]dThd\), 2 μCi, 6.7 Ci/mmol, was added to each chamber. After 10 to 35 min of labeling, the medium was removed, and the cultures were immediately washed with 37°C
medium containing 5 × 10⁻⁵ M dThd plus 10⁻⁵ M cytosine (Sigma) as a label "chase." The chase medium was removed, and the cultures were incubated with fresh chase medium for the duration of the experiment. At various times after the pulse label, cultures were rinsed with Hanks’ balanced salt solution to remove the serum in the media and were fixed in freshly prepared 75% methanol:25% glacial acetic acid. The fixed cultures were prepared for autoradiography with NTB-3 emulsion and developed with D-19 developer after a 24-hr exposure. Criteria for labeled in vitro cultured cells were determined as the total incorporation of [6-³H]dUrd into DNA in units of dpm/µg DNA.

RESULTS

Xenografts

After a typical period of 2 weeks, more than 80% of the implants produced visible growing tumors. Histological examination of the xenografts after the second, third, and sixth passages revealed no noteworthy changes from the histopathology of the patient’s biopsy. The tumor was comprised of very poorly differentiated cells approximately 15 µm in diameter, with poorly defined cytoplasmic margins. Nuclei appeared to be 7 to 10 µm in diameter, with large and multiple nucleolar regions and diffuse chromatin. The tumors consisted of well-vascularized regions which penetrated irregularly into areas of necrosis. Although the histopathology of the xenografts was generally consistent with that of the patient’s tumor, the nuclei in the xenografts appear very much smaller than those in the patient’s biopsy (Figs. 1 and 2). The xenografts have undergone more than 7 serial passages in nude mice.

The growth data of the tumor nodules conformed to the Gompertzian growth equation. Ranges for α and β were 10⁻⁶ to 0.06 and 0.04 to 0.37, respectively. The best-fit values for α were less than 0.025 for 7 of the 12 tumors measured; thus, the growth approached an exponential rate of increase in over one-half of the tumors measured. Approximate exponential growth is a consequence of the relationship

\[ \exp(-at) \sim 1 - at \]

when \( at \) is much smaller than 1 (Chart 1). No significant differences in the growth patterns were detected within the first 3 passages of this tumor or between host strains of mice. The effective size of the surviving portion of the implant (\( S_0 \)) ranged over 10,000-fold in the tumors from 0.001 to 18.1 cu mm. The interrelationships of \( S_0 \), α, and β given in the Gompertzian equation give rise to the calculated doubling time. At the arbitrarily chosen 35 days after implantation, the doubling time ranged from approximately 5 to 30 days. The 2 methods of fitting the data were virtually equivalent.

Tissue Culture

SqCaVu-1H cells in culture uniformly consist of epithelial-like cells, characterized by a thin, laminar, agranular cytoplasm with a poorly visible cytoplasmic membrane. The nuclei are relatively large and have very prominent and often multiple nucleoli. The chromatin is finely granular (Fig. 3). The volume distribution of the whole cells typically resembled a skewed Gaussian curve. The modal volume ranged from 1364 to 1878 µm³, while the mean volume ranged from 1784 to 2132 µm³, depending upon the growth phase of the culture (Chart 2). Sixty metaphase spreads were analyzed. The modal chromosome number was 64, with a range of 44 to 68. Most of the chromosomes were definitely of human origin. Some were apparently normal, while others had deletions, translocations, or inversions (Fig. 4). The origin of marker chromosomes designated XVII through XVIII could not be identified positively and are assumed to be complex rearrangements of human chromosomes. No chromosome or obvious rearrangement of
Cell Cycle Kinetics

**Xenografts.** Not surprisingly, tumors from which PLM data were derived were heterogeneous with respect to histological and labeling characteristics. Large areas of necrotic cells were often juxtaposed to regions of apparently intact and radiolabeled tumor cells. In general, the mean grain count per labeled mitosis was 7 to 9 grains, with S.D. of approximately 5 grains. After several sequential biopsies from the same tumor, hemorrhagic regions were apparent in histological sections of the tumors. Some biopsies did not contain 100 mitoses in over 4 entire sections; data from such specimens were not included in the analysis.

The cell kinetic data are presented in Table 1. Three of the PLM experiments did not yield a second wave of mitosis; accordingly, only G2 and S phases were calculated for these experiments. Estimates of phase durations were 6.3, 7.4, and 9.8 hr for G2 and 13.6, 10.7, and 13.4 hr for S. A fourth experiment utilized 3 mice with 5 tumors and was extended to detect a second wave of labeled mitoses. Cell cycle durations calculated from the latter experiment were G2 phase, 6.7 hr; S phase, 15.2 hr; and cell cycle time, 46.6 hr. The data from the 4 experiments were pooled and analyzed, and resulting estimates are G2 phase, 8.7 hr; S phase, 12.2 hr; and cell cycle time, 42.8 hr (Chart 4). The labeling indices ranged from 4.9% in the first-passage tumor biopsied 50 days after implantation to 20.5% in a second-passage tumor 34 days after implantation. The mitotic index ranged from 1.2 to 3.1%. The calculated growth fractions from the pooled data are 0.20 and 0.82 when observed labeling indices of 4.9 and 20.5%, respectively, are assumed.

Assays of the percentage of labeled cells after repeated injections of [3H]dThd for 52.5 hr yielded 79.8 ± 9.3% labeled cells in the 2.5-cm-diameter (approximately 3.8-cu cm) tumor and 56.9 ± 13.2% labeled cells in the 3.5-cm-diameter (approximately 21.8-cu cm) tumor. The percentage of labeled cells varied from region to region depending upon the proximity of necrotic cells and blood supply. After repeated administration of [3H]dThd in one host, a section was cut along the axis of a single capillary which was surrounded by a coaxial cord of tumor cells which was in turn surrounded by a large necrotic mass. The percentage of labeled cells less than 40 µm from the capillary was 60.7%, while only 32.4% of the cells were labeled in the remaining distance to the necrotic mass, i.e., cells lying between 40 and 125 µm from the capillary. The...
Table 1

Cell cycle kinetic data

<table>
<thead>
<tr>
<th>Source</th>
<th>Passage level</th>
<th>Time postimplant (days)</th>
<th>Tumor size (ml)</th>
<th>Labeling index (%)</th>
<th>Mitotic index (%)</th>
<th>Duration of G2 phase (hr)</th>
<th>Duration of S phase (hr)</th>
<th>Duration of cell cycle (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xenografts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor 1</td>
<td>1</td>
<td>1</td>
<td>50</td>
<td>2.6</td>
<td>4.9</td>
<td>9.8 ± 0.2</td>
<td>13.4 ± 0.6</td>
<td>—</td>
</tr>
<tr>
<td>Tumor 2</td>
<td>2</td>
<td>34</td>
<td>1.8</td>
<td>15.4</td>
<td>1.4</td>
<td>7.4 ± 0.8</td>
<td>10.7 ± 1.0</td>
<td>—</td>
</tr>
<tr>
<td>Tumor 3</td>
<td>2</td>
<td>34</td>
<td>6.5</td>
<td>20.5</td>
<td>3.1</td>
<td>6.3 ± 0.3</td>
<td>13.6 ± 0.3</td>
<td>—</td>
</tr>
<tr>
<td>Tumors 4–8</td>
<td>5</td>
<td>36</td>
<td>2.1</td>
<td>ND</td>
<td>ND</td>
<td>6.7 ± 0.6</td>
<td>15.2 ± 1.0</td>
<td>46.6 ± 6.2</td>
</tr>
<tr>
<td>Pooled data</td>
<td>1, 2, 5</td>
<td></td>
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<td></td>
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<tr>
<td>In vitro</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td>51.7</td>
<td>1.2</td>
<td>5.6 ± 0.6</td>
<td>8.6 ± 0.9</td>
</tr>
</tbody>
</table>

* Mean ± S.E.
* Extrapolated from growth curve for this tumor.
* Average for 5 tumors.
* ND, not done.

MTX Sensitivities

The cellular response to MTX depended partly on the culture method and growth state of the cells. Control [6-3H]dUrd incorporation rates were 7578 dpm/μg DNA (range, 6139 to 8439 dpm/μg DNA) for the in vitro cultured cells in logarithmic growth, 4787 dpm/μg DNA (range, 4569 to 4877 dpm/μg DNA) for plateau-phase cells, and 184 dpm/μg DNA (range, 140 to 208 dpm/μg DNA) from a xenograft. Chart 6 illustrates the dose response of the cells under these various growth conditions. The concentration at which MTX decreased the incorporation rate by 50% of control was 2.2 × 10^-6 M for the logarithmically growing population and 9 × 10^-6 M for the plateau-phase cells in vitro. The cells recovered from the xenograft incorporated less than 4% of the radiolabel than that incorporated by tissue culture cells. MTX inhibition of the xenograft cells was nearly dose independent.

DISCUSSION

A human squamous cell carcinoma of the vulva has been established and investigated in 2 model systems, xenografts in
nude mice and cells in tissue culture. The human origin of the xenografts is verified by the presence of human chromosomes and absence of mouse chromosomes in the cell line SqCaVu-1H, which was derived from a second-passage xenograft. The heteroploidy of SqCaVu-1H cells was anticipated, since many transformed cells grown in vitro are heteroploid. The presence of the marker chromosomes may prove to be useful in distinguishing this cell line in future experimentation, for example, in comparisons of these cells with cell lines of similar origin, such as HeLa cells. The histopathological similarity between the biopsy from the patient and the xenografts after 6 serial passages confirms the identity of the tumor (Figs. 1 and 2).

The appearance of tumors in a nude mouse inoculated with SqCaVu-1H cells demonstrated that the malignancy of these cells was not lost as a result of in vitro culture. It has been hypothesized by Wu et al. (39) that the hydrolytic activity of tumor cells facilitates local invasiveness and metastases. In particular, the secretion of plasminogen activator may be instrumental in the mechanisms of malignant invasion. Since the autopsy of the patient with this tumor revealed a remarkable degree of tumor invasiveness and unusually widespread metastases, we assayed SqCaVu-1H cultures for plasminogen activator production. Chart 3 illustrates that, during logarithmic phase growth, the cumulative amount of plasminogen activator per Petri dish increased over 30-fold. During plateau phase, the plasminogen activator decreased until the cultures were refed. Chou et al. (7) found similar results using mouse 3T3 cells, but they found that confluent cultures of 3T3 cells transformed with SV40 continued to accumulate plasminogen activator in the medium. Neither Chou et al. (7) nor we were able to demonstrate the appearance of an inhibitor in the media from plateau-phase cultures (data not shown). Several alternative hypotheses may be tested with regard to the decreasing amounts of plasminogen activator in these cultures. For example, plasminogen activator may degenerate after several days under the culture conditions. Alternatively, cells in plateau phase may metabolize plasminogen activator as the medium becomes depleted.

It is uncertain whether plasminogen activator secretion plays a major role in invasiveness and metastasis in vivo. We have not found metastatic nodules in nude mice bearing this tumor, and the tumors invariably have been encapsulated by connective tissue. However, it appears that the immune system itself may play a complex role in the establishment of metastasis. Klein et al. (18) have recently demonstrated that malignant cells are released and circulated and are present in the major organs of nude mice bearing experimental tumors. They found that only after suppression of the residual immunity of the hosts did metastatic tumors appear.

The ability to grow these cells both in vitro and in nude mice provides the opportunity to compare the characteristics and responses of the same human solid tumor cells under widely varying conditions. In the in vivo environment, the growth rate of the xenografts was variable from host to host and among different sites in the same host (Chart 1). Tumor growth rates did not correlate with the implantation site or with the sex or strain of mouse. Several factors are likely to play a role in determining the growth rate of the transplanted tissue fragments. Both the quantity and the metabolic quality of the tumor cells in the explant are likely sources of variability. Too few cells in the implant may not produce a tumor which appears rapidly, and a high proportion of nonproliferating cells may inhibit growth by competition for available nutrients. The availability of nutrients and transport of cellular waste by the microvasculature at the implant site undoubtedly affect the rate of tumor growth. Finally, the individual variation from host to host with regard to providing optimal metabolic and immunological conditions for tumor growth could affect the growth rate. The nearly exponential growth of over one half of the tumors measured was unexpected and might be interpreted as a characteristic of an aggressive tumor. The relationship of cell number and cellular growth phase to the variability of tumor nodule growth is a subject to be investigated by the injection of SqCeVu-1H cells into nude mouse hosts in future experiments.

In tissue culture, SqCaVu-1H cells grew classically with a logarithmic growth phase followed by a plateau phase. Measurements of cell volume spectra appear to be a sensitive indicator of the proliferative activity of these cells. The sensitivity of such measurements was indicated by a decrease of mean cell volume a full day before the cultures entered plateau phase and the media became noticeably acidic (Chart 2). Volume spectra changes have also been noted with respect to the proliferative activity of populations of mitogen-stimulated lymphocytes in culture (6, 32). In the present case, the mean volume increased, and the volume spectrum shifted toward increased sizes during midlogarithmic growth and after refeeding. The modal volume decreased during logarithmic growth and increased during plateau phase until the cultures were refed; thereafter, the modal volume decreased. Ross (29) found that modal volumes of synchronized Chinese hamster ovary V79-325 cells were found to increase nearly linearly with cell cycle progression from the beginning of the G1 phase through mitosis. He found that, depending on the experiment and media, asynchronous cells increased or decreased their modal volume during the transition from logarithmic to plateau phase. Clearly, further experimentation is warranted with respect to the size spectra and growth phases of cells in culture.

To the best of our knowledge, this is the first comparative report of the cell cycle kinetics of a human squamous cell carcinoma of the vulva grown in xenografts and in tissue culture. Lamerton and Steel (20) have studied the cell cycle kinetics of human tumors xenografted to conventional mice which were immune compromised by thymectomy and irradiation; however, they did not report data on tumors from the female genital tract. Rolfstad et al. (28) have reported the cell cycle kinetics of a human melanoma and a human fibrosarcoma xenografted to nude mice and compared their data with those in published reports of similar human tumors in situ. The PLM values of Rolfstad et al. (28) are questionable, since the labeled cells were likely to have been damaged by the radiation from the 10-μCi/g body weight, 18.8-Ci/μmol [3H]dThd dosage they used. This dosage is sufficient for a so-called suicide experiment (27). Recent data of Pollack et al. (23) suggest that an extended exposure to relatively low doses of [3H]incorporated into DNA will inhibit the progression of cells through the G2 phase and/or mitosis.

The cell cycle kinetic data are summarized in Table 1. The estimates for G2 and S phases in vivo are scattered around 8 and 13 hr, respectively. The cell cycle duration is estimated from one experiment to be 42 to 47 hr. The scatter and uncertainty of these data are probably due to the heterogeneous nature of the tumors, individual variation among tumors,
and the dispersal of the cell cycle phases. Other measures of cell kinetic variation can be seen in the ranges of the labeling indices and mitotic indices, which vary by factors of 4 and 2, respectively. Analysis of labeled tumor cells after repeated injections of label into the host must be interpreted with caution because of the possibility of (a) nonuniform delivery of label to all tumor cells; (b) labeled cells leaving the proliferative cycle; and (c) unlabeled cells entering the proliferative cycle. The calculated growth fraction from in vivo PLM data was 0.20 to 0.82, while the repeatedly labeled tumors yielded labeling indices from 32 to 88%, depending upon the region assayed. The comparison of these values illustrates that the growth fraction in solid tumors varies greatly, depending upon location of the cells assayed within the tumor. This variation lends to the cell cycle kinetic heterogeneity of the tumor. The duration of each cell cycle phase of these cells in vitro is shorter than the corresponding phase in the xenografts. The in vitro G1 phase of approximately 2.1 hr is remarkably short, especially when compared to the 21.1-hr G1 phase in the xenografts.

PLM curves derived from serial biopsies of the same tumor can be criticized on several grounds. Simpson-Herren et al. (31) have shown that surgical removal of a tumor or even sham surgery increases the labeling index of Lewis lung carcinomas in conventional C57BL × DBA/2 F1 mice. Secondly, it has been shown that the cellular kinetics of large tumors may be different from that of smaller and/or better vascularized tumors (11, 14, 36–38). Finally, sampling from a single tumor does not allow an averaging of the data because of individual variations among hosts. These criticisms notwithstanding, it is an overriding consideration for the treatment of an individual patient; the therapy is usually tailored to the patient. Hence, the tumors used for this study may be a fair comparison to clinical specimens. It is important, however, to estimate the extent of heterogeneity within individual tumors and the variability due to host differences.

A critical test of these model systems in the context of cancer research is the accuracy with which they represent the clinical presentations of solid tumors and the variability due to host differences. These criticisms notwithstanding, it is an overriding consideration for the treatment of an individual patient; the therapy is usually tailored to the patient. Hence, the tumors used for this study may be a fair comparison to clinical specimens. It is important, however, to estimate the extent of heterogeneity within individual tumors and the variability due to host differences.

This report is unique with respect to the comparison of MTX sensitivity of human squamous cell carcinoma cells derived from a common source and grown both in vitro and in vivo. The results raise basic questions regarding the interpretation of cell cycle kinetic data. Based on cell cycle kinetics, differences between tissue culture and xenograft control values of [6-3H]dUrd incorporation into DNA were anticipated. Since [6-3H]dUrd is incorporated into DNA only during S phase (excluding repair replication of DNA), the incorporation of this radio-label (in units of dpm/µg DNA) should correlate with the percentage of S-phase cells in the population. A comparison of the numbers of logarithmically growing and xenograft cells in S phase yielded the cell cycle kinetic conclusion that there were at least 2 times as many S-phase cells in logarithmically growing cultures as in the xenografts. Furthermore, if growth fractions and relative synthesis rates were estimated, the predicted control uptake of [6-3H]dUrd in logarithmically growing cells was 3.5 to 15 times that of the xenograft cells (Table 1). Experimentally, the incorporation ratio of the respective controls was over 40 (Chart 6). It seems likely that the discrepancy between the predicted and the experimental incorporation ratios was due to differences in the relative activities of the de novo and the salvage pathways for TTP incorporation into DNA.

The cell cycle kinetic data were based upon the salvage pathway radiotracer, [3H]dThd; the de novo pathway radiotracer, [6-3H]dUrd, was used for the MTX sensitivity experiments. It is hypothesized that the de novo pathway is relatively more active in the logarithmically growing tissue culture cells than in the xenografts. Conversely, the salvage pathway appears to predominate in the xenografts. This hypothesis will be tested with further experiments. Similarly, the different 50% inhibitory doses for the logarithmic and plateau-phase tissue culture cells and the insensitivity of the xenografts to MTX could be a result of biochemical differences, such as: (a) differences of drug transport leading to different intracellular concentration of MTX; (b) compensation for dihydrofolate reductase inhibition by increased levels of thymidine kinase; (c) variable induction of newly synthesized dihydrofolate reductase; and (d) variable pool sizes of TMP, TDP, and TTP. The results serve to illustrate that the cell cycle kinetic behavior and, in all likelihood, many biological characteristics of solid tumor cells, such as their response to therapeutic agents, depend strongly on the microscopic environment of which those particular cells are a part. Accordingly, several reports serve as cautionary examples: (a) the growth of rat-derived Walker 256 tumors was found to be slower in nude mice than in rats (5); (b) rat mammary adenocarcinomas passaged in nude mice for 20 generations changed in chemotherapy response characteristics (4); and (c) the DNA distribution of neoplastic rat brain cells shifted to near diploid when heteroploid in vitro cells were transplanted to nude mice.
The shifts in DNA distributions were reversible depending upon the culture conditions (19). In conclusion, SqCaVu-1H cells in tissue culture provide an excellent model to explore the phenotypic potentials of a human squamous cell carcinoma, but they do not necessarily express the same characteristics in vivo as in vitro. Similarly, the cell cycle kinetics of the xenografts was highly variable, but the xenografts more closely represent the cell cycle kinetics of squamous cell carcinomas of the human female genital tract in situ than do the SqCaVu-1H cells in vitro. It is suggested that therapies must ultimately be tailored to the entire spectrum of cellular biological states both in the tumor and in the normal tissues limiting therapy. To this end, model systems must be carefully chosen to provide accurate answers for the scientific questions posed.

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REFERENCES

Fig. 1. Histological section of patient’s original tumor which was diagnosed as a very poorly differentiated squamous cell carcinoma of the vulva. × 508.

Fig. 2. Histological section of the sixth passage of the patient’s tumor in nude mice. These tumors are invariably encapsulated by connective tissue. Black grains over nuclei resulted from autoradiography of this repeatedly labeled tumor. × 508.

Fig. 3. SqCaVu-1H cells grown on a glass microscope slide. These cells were derived from the second passage of the original tumor in nude mice. Black grains are from autoradiography. Note mitotic figures. × 508.
Fig. 4. Karyotype of SqCaVu-1H cells after approximately 175 passages in tissue culture. Apparently normal human chromosomes lie above the Arabic numerals. Recognizable rearranged chromosomes are to the right of their "normal" counterparts and are labeled by Roman Numerals I through XVI. The 2 marker chromosomes at the top of the karyotype, labeled XVII and XVIII, are of uncertain origin but probably represent complex rearrangements of human chromosomes.
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