ABSTRACT

In the present study, the human colonic adenocarcinoma cell line LoVo was established as a xenograft system in BALB/c athymic mice, and its biological and cell kinetic properties were examined. Line LoVo produced a 100% tumor incidence with inocula of $10^6$ to $10^7$ cells i.p. or $10^7$ to $10^8$ cells s.c., while inocula of $10^8$ cells s.c. produced tumors in 50% of the cases. No tumor development was noted following i.v. inoculations of $10^7$ to $10^8$ cells after 120 days.

Tumors arising i.p. grew as discrete nodules without ascitic fluid near vascular sources throughout the peritoneal cavity but did not induce new vascularization. Tumors arising s.c. grew with a typical Gompertzian pattern with growth characteristics expected for human colonic adenocarcinoma material. Tumor-doubling times ($T_d$) were 7 days for $10^4$ and $10^5$ inocula between days 10 to 40 increasing to >13 days thereafter. Inocula of $10^9$ cells grew slower ($T_d = 13$ days) and did not exhibit a significant plateau region. Tumors arising s.c. were extensively vascularized from the murine epidermis with no encapsulation by the host connective tissue. Using lethally irradiated cells or brain extract, the tumor growth pattern did not exhibit a Révész effect. All tumors exhibited a well-differentiated morphology with extensive glandular formations. Focal necrosis was evident in early tumors, which coalesced to form a central mass at larger sizes. No metastatic involvement was noted in any normal tissues, including the proximal lymph nodes.

Tumors arising s.c. from $10^7$ cells were classified as early (0.4 to 0.6 cu cm) or late (1.4 to 1.6 cu cm). The cell kinetic profiles were determined using the percentage of labeled mitoses and in vitro $[3H]$ and $[14C]$ thymidine-labeling procedures, as well as the primer-available DNA polymerase assay to estimate tumor growth fraction. Results indicated a slowly proliferating nature for early LoVo xenografts (growth fraction, 34%) with a characteristic human generation time ($T_g$), ∼65 hr; S-phase duration ($T_s$), 18 hr and a low cell loss factor (10%). With increasing tumor size, the major kinetic changes noted were a reduced growth fraction (23%) and an increased cell loss factor (63%).

A colony formation assay was established using collagenase (150 IU/ml) and mechanical manipulations to achieve single-cell suspensions. Mean plating efficiency was 18.9% and was lower than 100 and 1000 cells.

These results suggest that LoVo xenografts possess many properties expected for human colonic adenocarcinomas in situ and may provide an in vivo screening system for potential antitumor agents.

Introduction

Carcinoma of the large bowel is one of the most frequently occurring cancers in this country with an estimated incidence of 101,000 new patients in 1977 (27). Despite the availability of a spectrum of antiproliferative agents, chemotherapy of surgically unresectable tumors has remained largely unsatisfactory, as evidenced by the lack of a significant increase in 5-year survival over the last 20 years (12, 17, 32). Presently, new, more effective antitumor agents and more aggressive strategies are being sought for the management of advanced large bowel cancers. Cell cultures of human cancers provide a rapid and economical system to screen agents for cytotoxicity and mode of action. Although these studies constitute a fundamental stage in the development of antitumor agents or procedures, a logical extension is the development of complementary in vivo screening systems. Murine tumor systems, while convenient, have the disadvantage of being pharmacologically distinct, thereby limiting their use in predicting clinical responsiveness. This is evidenced by screening studies using the drugs anguidine and N-(phosphonacetyl)-L-aspartate, which both show considerable efficacy against murine colon carcinoma cells but fail to elicit lethal effects on cultured human colonic carcinoma cells or show significant clinical responses (5, 13).

One way of circumventing these problems is to use human tumor xenografts in athymic mice. This system has the advantages of the in vivo situation and the biological responses of human cells, although modified by the murine physiology. Previous reports have indicated the efficacy of these models as screening systems for potential antitumor agents (20, 21). Although xenografts can be directly established from primary tumors, long intervals are usually necessary for the production of sufficient numbers of tumor-bearing animals for experimental manipulations. In addition, serial transplantation in vivo can result in progressive changes in tumor growth kinetics and biological properties (2).

Experimental flexibility can be markedly improved through the use of established human cell lines as the primary xenograft material. This provides for the large-scale production of genetically equivalent tumors in a relatively short period of time without sequential in vivo passage. In the present study, we describe the biological and cell kinetic characteristics of one such system using the human adenocarcinoma cell line LoVo in the BALB/c athymic mouse.
Materials and Methods

Cell Line. LoVo cells were propagated as monolayer cultures in 16-oz Owen's bottles containing Ham's F-10 media supplemented with 20% fetal calf serum, vitamins, glutamine, and antibiotics (7). Cell cultures were routinely examined for evidence of Mycoplasma contamination using morphological and biochemical methods. Under these conditions, line LoVo exhibits many morphological and functional features expected of human GI3 cancers, including the formation of acinar structures, signet ring cells, and the synthesis of carcinoembryonic antigen. Cells were harvested from confluent bottles, using hyaluronidase (100 IU/ml) for 5 min at 37° followed by trypsin (2.5% in Hanks' balanced salt solution) for 5 min at 37°. These conditions provide for maximum numbers of single viable cells. Cell counts were performed with a Model ZBI electronic particle counter (Coulter Electronics, Inc., Hialeah, Fla.).

Athymic Mice. Six- to 8-week-old female BALB/c athymic mice (Sprague-Dawley, Madison, Wis.) were used throughout. Mice were housed 5/cage in isolated rooms maintained at 28.5° with a 12-hr light cycle. All bedding, housing, and food material were autoclaved prior to use, and the cages were covered with sterile fiberglass filters. All manipulations were performed under laminar-flow hoods with sterile materials. Under these conditions, mice have been maintained in excellent physical condition for longer than 18 months.

Tumor Growth. Aliquots of 103 to 106 LoVo cells suspended in 0.3 ml of Hanks' balanced salt solution were inoculated i.p., i.v., or s.c. (right flank). Mice were followed for 120 days to document tumor development. For mice inoculated s.c., tumor growth was monitored by caliper measurements in 2 dimensions (w2), and volumes (V) were estimated by the formula for prolate ellipsoid [V = (4/3)πw2]. Tumor growth curves were constructed, lines were fit using the least-squares technique, and the tumor-doubling times (Td's) were determined.

Tumor growth dynamics were also analyzed using mathematical models, based on Gompertzian growth kinetics, which provided the best least-square estimates for the alpha and beta Gompertz parameters. These parameters were then analyzed for linear relationships as described previously (4, 19), and a growth equation was determined.

In an attempt to modify LoVo tumor growth characteristics via the Révész effect (22), 107 LoVo cells were inoculated s.c. with an equal number of lethally irradiated LoVo cells (7.5 kilorads 137Cs; 23°) or in a vehicle containing a brain extract (7.5 mg/ml) prepared from BALB/c athymic mice as described in detail elsewhere (22). Control mice received 107 lethally irradiated cells at different sites.

In all experiments, animals were sacrificed by cervical dislocation. For animals inoculated i.v., careful macro- and microscopic examination for tumor nodules was performed. In animals inoculated i.p. and s.c., careful examination was made for metastatic involvement. In tumor-bearing animals, tissue samples were obtained from different areas of the cancer. In all animals, the following whole organs were prepared for histological examinations: liver, spleen, lungs, intestines, brain, inguinal and axillary lymph nodes, and femoral and vertebral bone marrow. All tissues were fixed in neutral buffered formalin for 48 hr and embedded in paraffin. Routine histological sections were prepared and stained with hematoxylin and eosin.

PLM Analysis. At early (0.4 to 0.6 cu cm; Days 30 to 40) and late (1.3 to 1.6 cu cm; Days 60 to 70) periods in the growth of s.c. tumors, animals received i.p. injections of 4 μCi [3H]-dThd/g body weight (20 Ci/mmol; New England Nuclear, Boston, Mass.). At 3-hr intervals beginning 30 min after the injection, animals were sacrificed, and the tumors were excised and fixed in neutral buffered formalin for 48 hr. Three-μm histological sections were cut, and autoradiographs were prepared as described below. Following development, slides were stained with Harris hematoxylin and eosin and PLM curves were constructed using the criteria described by Steel (29).

The mean grain count for labeled cells was ≥10 with a background count of <1.0 grain/cell. MF with ≥3 grains were scored as positive. For each animal, 400 MF were scored (100 in each of 4 separate tumor sections). Each slide was scored by moving completely across the section, such that the entire tumor was examined. PLM curves were fitted and cell cycle parameters were extracted by the automatic procedure of Thames and White (31) based on the Hahn model and by the asymmetry technique of Mendelsohn and Takahashi (16).

In Vitro Kinetics. Individual cell kinetic profiles were also obtained from 5 early and late tumor-bearing animals using the PDP assay to estimate tumor GF and in vitro single- and double-labeling procedures to estimate the [3H]dThd LI and Ts, respectively (3, 26). Briefly, approximately 0.5 to 1.0 cu cm of tumor was minced in Ham's F-10 media, filtered through a stainless steel screen, washed twice, and resuspended in fresh media. Aliquots of this sample were taken and cytocentrifuge preparations were made for use in the PDP assay. In this assay, the sample nuclei are provided with the necessary materials for DNA synthesis, including [3H]dTTP. When both αDNA polymerase and DNA template are present in the nuclei, DNA synthesis occurs and can be quantitated by autoradiography (18). The percentage of labeled nuclei is termed the PDP index and has been shown to provide a good estimate of the GF in murine solid tumors, as determined from mathematical calculations of PLM data (26). One thousand cells were examined for each PDP index.

The remaining tumor sample was incubated with [3H]dThd (2.5 μCi/ml, 20 Ci/mmol; New England Nuclear, Boston, Mass.) at 37° for 30 min in [3H]dThd LI studies or for 60-min double-labeling Ts determinations. In the latter case, [3H]-dThd (0.25 μCi/ml, 51 Ci/mmol; New England Nuclear, Boston, Mass.) was added after 60 min for an additional 30 min. Labeling was terminated on ice, and viable cells were isolated on a Ficoll-Hypaque gradient. The cells were then washed, and cytocentrifuge preparations were made. One thousand cells were examined for each [3H]dThd LI determination, while 200-labeled cells were evaluated for the Ts estimate. Once these kinetic parameters were determined, the Ts, the potential tumor-doubling time (Td's), and the cell-loss factor were calculated as described previously (28). Single-label slides were also used in the determination of the tumor mitotic index.

Continuous Labeling Studies. Early tumor-bearing animals were given i.p. injections of 0.25 μCi [3H]dThd/g body weight (20 Ci/mmol; New England Nuclear, Boston, Mass.) at 6-hr intervals for 120 hr. Animals were sacrificed, the tumors were removed, and portions were enzymatically dispersed (see "Col-
oncy Formation Assay") to obtain a single-cell suspension. The percentage of labeled nuclei per 1000 total cells was then determined and plotted against time. The remaining tumor sample was fixed in neutral buffered formalin, 3-μm histological sections were prepared, and the percentage of labeled MF was determined versus time as described for the PLM analysis.

Autoradiography. All studies used Ilford K-5 emulsion diluted 1:1 with triple-distilled water and applied by dipping at 45°. Slides were air dried for 1 hr and stored for 1 to 14 days at 4°. PLM and continuous-labeling slides were exposed for intervals necessary to produce a mean grain count of approximately 10 grains/labeled cell and were developed using Kodak D-19 developer and fixer. PDP, [3H]dThd LI, and T8 slides were developed using a gold activation procedure to reduce exposure times with T5 slides using double-emulsion autoradiography (3). Background counts for the in vitro kinetics using exposure times with T5 slides using double-emulsion autoradiography were determined and plotted against time. The remaining tumor was then aspirated the cells each time. This procedure provided >95% viable cells were plated in 60-mm Petri dishes containing 5 ml of Ham's F-10 media. Plates were incubated for 30 min, aspirated with 106 LoVo cells inoculated i.p. produced palpable masses after 120 days. All s.c. tumors grew by contiguity with no invasion of the underlying musculature. No metastatic involvement was noted for any tissues including the proximal lymph nodes.

Histologically, s.c. tumors exhibited a well-differentiated morphology with extensive acinar formations. All tumors appeared to be well vascularized from the murine epidermis, but focal necrosis was evident at all tumor sizes. At larger tumor sizes, these regions coalesced to form a large central necrotic area. No encapsulation by murine connective tissue was noted. Survival time of animals inoculated s.c. with 106 or 108 LoVo cells was 68 ± 8 days, although some animals survived more than 3 months with tumors exceeding 20 cm. Death was usually associated with tumor ulceration. Animals inoculated with 106 LoVo cells survived for the 120-day observation period.

The growth characteristics of s.c. tumors followed a typical Gompertzian pattern (Chart 1). All tumors regressed during the first 10 to 15 days, with the 106 inoculum disappearing completely for 30 days. T0's for tumors arising from 106 and 108 cells were approximately 7 days during Days 10 to 40, increasing to greater than 13 days between Days 40 to 80. The larger cell inoculum plateaued at a volume 2 to 3 times greater than that of the 106 inocula, although plateau growth was noted at similar times for each group. Tumors arising from 106 cells appeared later (30 days) and grew at a slower rate, with a T0 of greater than 12 days throughout and no significant plateau noted.

The α0 and β Gompertz parameters represent positive values in close approximation to blood vessels but did not induce new vascularization. Histologically, i.p. tumors were well differentiated and showed little necrosis. Survival times for animals inoculated i.p. were: 35 ± 5 (S.D.) days (106 cells); 47.5 ± 4.5 days (107 cells); 58 ± 8 days (108 cells); and 81.3 ± 8.5 (109 cells).

Inoculations of 107 to 108 cells s.c. produced a 100% tumor incidence within 14 days. In 50% of the cases, 106 cells produced tumors, while inocula of less than 106 cells failed to produce palpable masses after 120 days. All s.c. tumors grew by contiguity with no invasion of the underlying musculature. No metastatic involvement was noted for any tissues including the proximal lymph nodes.

Histologically, s.c. tumors exhibited a well-differentiated morphology with extensive acinar formations. All tumors appeared to be well vascularized from the murine epidermis, but focal necrosis was evident at all tumor sizes. At larger tumor sizes, these regions coalesced to form a large central necrotic area. No encapsulation by murine connective tissue was noted. Survival time of animals inoculated s.c. with 107 or 108 LoVo cells was 68 ± 8 days, although some animals survived more than 6 months with tumors exceeding 20 cm. Death was usually associated with tumor ulceration. Animals inoculated with 106 LoVo cells survived for the 120-day observation period.

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The α0 and β Gompertz parameters represent positive values
governing the initial tumor growth rate and the timing of the plateau phase, respectively. For s.c. LoVo xenografts, individual growth curves exhibited significant, although not unexpected, variations. The \( \alpha \) and \( \beta \) Gompertzian values obtained from 25 LoVo growth curves were 0.856 ± 0.66 (range, 0.13 to 2.22) and 0.033 ± 0.027 (range, 0.003 to 0.082), respectively. These growth parameters exhibited a strong linear correlation (\( r = 0.993 \)) with a slope of 24.875 ± 0.1 38 and a y-intercept of 0.034.

Results of the LoVo growth studies using lethally irradiated cells or a thromboplastic brain extract are presented in Chart 2. As indicated, there is no significant enhancement of tumor growth rate, lag time between inoculation and onset of Gompertzian growth, or the timing of the plateau.

![Chart 2](chart2.png)

**Chart 2.** Révész effects in s.c. growth of 10^7 LoVo cells inoculated with a brain extract (A) and 10^7 lethally irradiated cells at the same site (B) or a distant site (C). Points, mean of 3 to 5 mice; bars, S.E.

Cell Kinetics Studies. Results of the PLM analysis for early and late LoVo tumors are presented in Chart 3. PLM curves for early tumors exhibited a well-defined primary peak and a partially defined second wave, while curves for late tumors showed a more limited first peak with severe fading and no apparent second wave.

Computer-derived cell cycle parameters obtained from the PLM analysis and the kinetic values obtained using the in vitro techniques are presented in Table 2. PLM values shown are based on the procedure of Thames and White (31). Values obtained by the asymmetry technique of Mendelsohn and Takahashi (16) differed by less than 10%. There is an excellent agreement between the kinetic values for early tumors obtained from the PLM and in vitro techniques.

As the tumors increase in size and the \( T_0 \) lengths then by a factor of 2 to 13.7 days, the GF decreases from 33.9 to 23.9%, while the cell loss factor increases by a factor of 6 to 62.7%. The shape of the PLM curve for late tumors curve precludes any accurate estimation of the \( T_C \). Values for \( T_S \) and \( T_{G2+M} \) indicate a shortening of these phases. The in vitro techniques indicate no change in the \( T_S \) but a marked reduction in the \( T_C \) from 61.4 to 38.2 hr. As will be discussed below, this reduction in \( T_C \) in the face of a constant \( T_S \) could account for the maintenance of the \([\text{H}]\text{dThd}\) LI with increasing tumor size. The mitotic index exhibits an approximately 3-fold increase from the early tumor size, while the calculated \( T_m \) shows only a 70% increase during the same period.

Continuous Labeling. Results of the continuous \([\text{H}]\text{dThd}\) studies for early LoVo tumors (Chart 4) indicate a plateau value of 55 to 60% attained after 72 hr that remained constant over the next 48 hr. This plateau value has been reported to estimate the tumor proliferative fraction (24); however, the plateau value obtained herein does not agree with the calculated PLM growth fraction or the PDP index. The number of labeled MF begins to increase at approximately 12 hr after \([\text{H}]\text{dThd}\) administration and reaches 100% labeling after 80 hr. This temporal sequence is consistent with a \( G_2 + M \) period of 10.4 hr as measured by the PLM technique. The time required between the appearance of the labeled MF and 100% labeling suggests a broad distribution of \( G_2 \) times.

Colony Formation Assay. Results of the colony formation assay are presented in Chart 5. There is an excellent linear relationship between the number of cells plated and the number of observed colonies (\( r = 0.998 \)). The line was fitted using the least-squares technique and has a mean slope of 0.189, reflecting the mean plating efficiency for this system.

Discussion

Previous studies in this laboratory have established line LoVo as an in vitro model for human colonic carcinoma (7, 8). This cell line possesses many characteristics of human GI cancers, including the formation of acinar structures, signet ring cells, and the production and secretion of carcinoembryonic antigen. This line is currently in use as an in vitro screening system for potential antitumor agents and procedures.

In the present study, we describe the biological and cell kinetic properties of line LoVo grown as a xenograft in the BALB/c athymic mouse. Biologically, line LoVo exhibits a relatively low tumorigenicity with strict requirements as to the route of administration. Consistent tumor production was at-

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**Table 2.** Cell cycle parameters obtained from the PLM analysis and the kinetic values obtained using the in vitro techniques.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Early Tumors</th>
<th>Late Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T_S ) (hr)</td>
<td>61.4</td>
<td>38.2</td>
</tr>
<tr>
<td>( T_C ) (hr)</td>
<td>10.4</td>
<td>9.1</td>
</tr>
<tr>
<td>( T_{G2+M} ) (hr)</td>
<td>38.2</td>
<td>25.7</td>
</tr>
<tr>
<td>( \text{GF} )</td>
<td>55 &amp; 60%</td>
<td>40 &amp; 45%</td>
</tr>
</tbody>
</table>

**Figure 4.** Continuous \([\text{H}]\text{dThd}\) studies for early LoVo tumors. Points, mean of 3 to 5 mice; bars, S.E.
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Table 2

Cytokinetic values for s.c. LoVo xenografts

<table>
<thead>
<tr>
<th>Cell [3H]dThd LI (%)</th>
<th>Mitotic index (%)</th>
<th>GF (%)</th>
<th>T_s (hr)</th>
<th>T_D (hr)</th>
<th>T_0 (days)</th>
<th>T_0, (hr)</th>
<th>T_c (hr)</th>
<th>T_c (days)</th>
<th>Cell loss factor^d (%)</th>
<th>λ^9 (x10^-3)</th>
<th>K_e (x10^-5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early (0.4 to 0.6 cu cm) PLM</td>
<td>9.5 ± 1.5^b/j</td>
<td>35.3</td>
<td>18.4</td>
<td>68.5</td>
<td>39.7</td>
<td>10.4</td>
<td></td>
<td></td>
<td></td>
<td>0.6</td>
<td>6.8</td>
</tr>
<tr>
<td>PLM and in vitro</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late (1.3 to 1.6 cu cm) PLM</td>
<td>12.9 ± 1.3^b</td>
<td>2.8 ± 0.9</td>
<td>23.9 ± 2.7</td>
<td>17.4 ± 1.8</td>
<td>38.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.0</td>
<td>13.7</td>
</tr>
<tr>
<td>PLM and in vitro</td>
<td></td>
<td></td>
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</tbody>
</table>

^α (AT_s/[^3H]dThd LI) x [ln(1 + GF)/ln 2] (Ref. 25.)
^β Mitotic index x T_c.
^γ λ_AT_s/[^3H]dThd LI (Ref. 25).
^d 1 - (T_D/T_c) x 100 (Ref. 30).
^e Correction factor for nonlinear S-phase distribution (Ref. 30). G2 - 10 hr (from T_g + M - T_a).
^f 0.693/T_c (Ref. 30).
^g Rate of cell loss. [1 - T_D/T_c] x K_e (Ref. 30).
^h in vivo.
^i Mean ± S.D.

LoVo xenografts exhibited a well-differentiated morphology characteristic of many human colon carcinomas. Vascularization of the xenografts was from the epidermis in s.c. tumors and is facilitated by the lack of murine connective tissue encapsulation. Angiogenesis does not appear to be a limiting factor in the tumor establishment, as is evidenced by the lack of a significant Révész effect using both lethally irradiated LoVo cells and a thromboplastic brain extract. Despite the extensive vascularization, areas of compression and necrosis were evident at all tumor sizes. At larger sizes, a barge central necrotic mass becomes prominent with an outer ring of cellular material.

The growth pattern of s.c. LoVo xenografts exhibited a typical Gompertian pattern. No significant differences were noted in the establishment, lag time, growth rate, or plateau timing of the 10^7 and 10^8 inocula. The computer-derived Gompertian growth parameters agree quite well with those reported previously for surgically derived colonic xenograft material (4). This suggests an equivalence between this tissue culture line and the in situ cancers as xenograft models. The disparity in the growth of the 10^8 inoculum from the 10^7 or 10^8 inoculum...
Biology and Cytodynamics of LoVo Xenografts

inocula may reflect a critical cell number at which host immune surveillance becomes significant, as discussed previously.

PLM analysis of early LoVo xenografts indicated that $T_C = 68.5\text{ hr}$, $T_S = 18.4\text{ hr}$, and $T_{G_2+M} = 10.4\text{ hr}$. These values agree well with those reported previously for human colonic tumors in situ (1, 14, 25). These early tumors also exhibit a GF of 35.3%, a $[^3\text{H}]\text{dThd}$ LI of 9.1%, and a calculated cell loss factor of 10.2%. In vitro kinetic analysis of individual tumors indicated similar kinetic profiles. Of special note is the close agreement between the tumor GF calculated from PLM data (35.3%) and the POP index (33.9 ± 2.9%). This represents the first confirmation of the POP index as an estimate of the GF in human tumors.

For late LoVo xenografts, the shape of the PLM curve indicates a broad distribution of intermitotic times. Analysis of these curves indicated a shortening of both the $T_S$ and $T_{G_2+M}$. However, the double-labeling procedure indicated that the $T_S$ remained constant. This discrepancy can be explained by the fact that the PLM analysis follows the most rapidly cycling fraction of cells which complete the S-G$_2$-M transit. The double-labeling technique measures cell movement between S and G$_2$ only, and hence should provide a more precise measurement of the $T_S$. With increasing size, the PDP index decreased by one-third, while the cell loss factor increased by a factor of 4. The $[^3\text{H}]\text{dThd}$ LI showed no significant change from the early tumor size.

It is of interest to note that the calculated rate of cell production ($K_D$) in late tumors is approximately 18% greater than that for early tumors. In addition, the calculated $T_C$ (38 hr) shows a marked reduction from the 61 hr observed in early tumors. Given the histological makeup of this tumor, with a large central necrotic mass and a peripheral cellular ring near the epidermal vascular supply, these kinetic events may suggest the loss of a subpopulation of more slowly cycling cells. With the loss of the slower "diluting" population, the measured $T_C$ would decrease and, in relation to a constant $T_S$, would result in the maintenance of the $[^3\text{H}]\text{dThd}$ LI with increasing tumor size. This hypothesis is supported by recent models of vascular compression in solid tumors (15).

These concepts are also supported by the increased mitotic index in late tumors which greatly exceeds the observed increase in $T_S$. These results are consistent with a reduced $T_C$.

Estimates of the GF obtained from the continuous-labeling curve show a marked discrepancy from the values obtained with both the PLM and PDP techniques. This discrepancy probably reflects the expansion of the labeled cell population through mitosis. The labeled daughter cells may or may not remain in cycle, thus producing an overestimate of the GF. It is of interest to note that after approximately one cell cycle, no unlabeled MF are observed in this unperturbed system. This suggests that no significant numbers of cycling cells are being sequestered in the G$_2$ period with a slow turnover time, as suggested recently by Gelfant (9). It should be noted, however, that the long interval between the first labeled MF and 100% labeling (~60 hr) implies a large variability in the G$_2$ cycle time which can be interpreted to have a G$_0$ component.

The delay between 0% and 100% MF labeling in the continuous-labeling studies, as well as the shape of the PLM curves, raises the possibility of $[^3\text{H}]\text{dThd}$ damage. However, the autoradiographic exposure times (10 to 21 days) necessary to attain approximately 10 grains/labeled cell do not support the concept of excessive $[^3\text{H}]\text{dThd}$ accumulation. A more likely explanation for the shape of the PLM curves would be cell loss occurring randomly throughout the cell cycle coupled with a range of intermitotic times. Similarly, the shape of the continuous-labeling curves can be explained by a broad distribution of G$_2$ times.

The cell kinetic profile for the in vivo LoVo model shows a marked variance with the cytodynamics of the in vitro system. Here, a $T_C$ of 29.3 hr, a $T_S$ of 10.7 hr, and a $T_{G_2+M}$ of 4.8 hr were noted (8). The lengthening of the LoVo generation time and the concomitant cell-cycle phases probably reflects a cellular adaptation to the in vivo environment. It is of interest to note that in this kinetic adaptation the LoVo cell exhibits characteristically human cytodynamics as opposed to a more rapidly cycling murine kinetic profile.

Results of the colony formation assay suggest that the LoVo xenograft system may be readily applied to drug and/or therapeutic evaluations. Previous studies have shown the efficacy of the colony formation assay over other techniques to measure tumor cell kill in that it monitors immediate kill as well as cellular damage manifest over longer periods of time (23). This capacity offers the means to precisely monitor tumor cell kill following treatment with therapeutic agents and/or strategies.

In summary, in vivo LoVo possesses many characteristics which make it an excellent in vitro-in vivo screening system for human colonic carcinoma. In vitro, the LoVo cell line is easily maintained and possesses several biological characteristics expected for human GI cancers. The kinetic profiles for the LoVo xenografts are such that these tumors are kinetically similar to the clinical situation in terms of a low cell production, high cell loss factor, and long cell cycle times. As such, these tumors would be useful in kinetically based protocol studies or in monitoring kinetic responses following the initial course of therapy.

In terms of monitoring therapeutic responses, the life span of athymic mice in our colony and the predictable nature of LoVo growth allows for the evaluation of therapy in terms of increased life span and/or tumor deviation from ideal growth. The colony formation assay with a mean plating efficiency of 18.9% would also provide an excellent means of monitoring tumor cell kill. The use of this in vitro-in vivo screening system would allow, therefore, for the evaluation of large numbers of potential antitumor agents with a high degree of resolution in a clinically relevant colonic adenocarcinoma model.

Acknowledgments

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Biological and Cell Kinetic Properties of a Human Colonic Adenocarcinoma (LoVo) Grown in Athymic Mice


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