Growth Kinetics of Primary Versus Metastatic Human Melanoma Xenografts in Nude Mice

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Primary and metastatic tumors from a single patient with fatal malignant melanoma were heterotransplanted and maintained in BALB/c/Nu mice. Kinetic parameters were determined using tritiated thymidine as a single injection for percent labeled mitoses studies and continuous infusion for growth fraction studies. Tumor growth characteristics in mice were also examined. Karyotyping and ouabain studies showed that the human chromosomes were maintained in serial transplantation and did not form hybrid cells with the mice. Results suggest that while the metastatic cells have longer cell cycle and duration of S phase, they possess other characteristics which permit faster growth and easier transplantation. The possible role of an "angiogenesis factor" in this situation is discussed. J Invest Dermatol 87:537–539, 1986

Malignant melanoma, once metastatic, is a devastating disease highly refractory to chemotherapy or radiation therapy. One may ask if the metastatic lesion represents a clone of more "malignant" cells from the primary or if it is the same tumor, only present in a vital location such as the brain or liver. The athymic nude mouse has previously been shown to be a valid model for studying human melanoma kinetics [1]. We have measured 2 cell kinetic parameters, cell cycle and growth fraction, for tumors xenografted in nude mice to compare a skin primary and brain metastasis taken from a single patient with fatal malignant melanoma. In addition, tumor growth characteristics in tumor transplanted in athymic nude mice were examined. The results of these studies are the subject of this report.

MATERIALS AND METHODS

Xenografts The primary and metastatic lesions were removed during a single operative procedure from a 61-year-old male. The primary tumor was nodular and histopathology showed primarily spindle cells, although some areas were more pleomorphic. Breslow levels were not measured since the primary was a pedunculated mass extending upward for 4.0 cm. The cultured tumor was taken from the vertical growth phase. The brain metastasis was composed of epithelial-looking cells some of which contained brown pigments.

Surgical specimens were placed in McCoy's 5A tissue culture medium with 10% fetal calf serum added during transportation to the laboratory. The tumors were then immediately minced and surgically implanted subcutaneously into male BALB/c nude mice, 6–8 weeks of age (National Cancer Institute, Frederick, Maryland) [2]. Tumors were serially passaged when they reached a size of 1.0–1.5 cm. While female mice were used to carry the tumors, only male BALB/c nude mice were used in the kinetic studies. Animals were maintained in a laminar flow environment. Tumors were measured with calipers at selected intervals and volume was calculated as length × (width)² × ⅓ [3]. Tumor volume was plotted as a function of time after implantation.

Cell Cycle Kinetics: Percent Labeled Mitoses Mice bearing tumors from the 5th, 6th, and 7th transplantation were given i.p. injections of 50 μCi (2 μCi/g) 20 Ci/mmol [3H]thymidine (dThd) (Amersham, Arlington Heights, Illinois) for percent labeled mitoses (PLM) analysis.

At various times after injection, mice were anesthetized with chloral hydrate and sample tumors were removed and placed in Bouin's fixative for 6–18 h. Each mouse carried 1–5 tumors. At least 2 tumors from different animals were taken for each point. Care was taken to use tumors not previously traumatized. All animals were autopsied with examination of brain, lung, and abdominal organs.

Microscopic slides of the tumors were deparaffinized and dipped in NTB-2, nuclear track emulsion (Kodak, Rochester, New York). At 2, 4, and 6 weeks of exposure, slides were developed with D-19 (Kodak) and stained with hematoxylin and eosin. For the PLM curves, 4 weeks' exposure showed optimal labeling and were used for determination. Background grain counts were typically less than 1 grain per cell, and more than twice background was the minimum used for counting a cell as labeled. At least 200–500 mitoses were scored for each point on the PLM curve. Labeling index (LI) was determined from the 1-h specimens in the assay. At least 1000 cells were counted to determine the LI for each specimen.

Growth Fraction Animals bearing 1–5 tumors from the 5th, 7th, and 8th transplantation passages were used for the growth fraction assay. The mice were anesthetized with chloral hydrate and implanted with Alzet pumps (Alza Pharmaceutical, Palo Alto, California) containing approximately 0.2 ml of [3H]dThd, 1 mCi/ml, 20 Ci/mmol (Amersham) into the peritoneal cavity. This pump provides continuous infusion of a solution over 170 h. Thus, it can be used to estimate the growth fraction [1,4]. At selected intervals after pump implantation, tumors were removed and placed in Bouin's fixative for 6–18 h. At least 2 tumors were taken at each time point except 36 and 72 h for the metastasis where only 1 tumor was available. At least 1000 cells were counted per time point. All animals were autopsied with examination of

Manuscript received December 24, 1985; accepted for publication May 8, 1986.

Supported in part by United States Public Health Service grant AM27110 from the National Institutes of Health and by the Southern California Dermatology Foundation.

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Abbreviations:
LI: labeling index
PLM: percent labeled mitoses

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0022-202X/86/$03.50

537
brain, liver, and abdominal organs. Microscopic slides were dipped in emulsion and developed as described for the PLM determination. Six-week development times were used in this study.

**Cell Cycle Times** The S-phase was determined according to the method of Quastler [5] as equal to the time between the 37% intercepts of the PLM curve. The G2 phase equals the time from [3H]dThd injections to the 37% intercept on the ascending limb of the curve. If a second wave of labeled mitoses is not seen on the PLM curve, an estimate of generation time (Tc) can be made by the formula, Tc = (Ts × GF)/LI, where Ts is the duration of the S-phase, GF is the growth fraction, and LI is the labeling index [6].

**Karyotyping** Cell cultures from the primary and metastasis were incubated in McCoy's 5A medium with 10% fetal calf serum, penicillin/streptomycin 10,000 U/100 ml, and t-glutamate 29.2 mg/100 ml added. Cells were then treated with colcemide and potassium chloride, fixed in absolute alcohol and glacial acetic acid, and dropped onto slides to disperse the chromosomes.

**Ouabain Studies** Cell cultures of the primary and metastasis were incubated in McCoy's 5A with 10% fetal calf serum, penicillin/streptomycin 10,000 U/100 ml, and t-glutamate 29.2 mg/100 ml. Study flasks were then treated with media containing 5 × 10⁻⁷ M ouabain.

**RESULTS**

Figure 1 shows growth curves from typical heterotransplants for the 6th passage of the primary and metastasis. The tumors differed in several respects. Firstly, as can be seen from Fig 1, the rate of growth was greater for the metastasis. The metastasis in general was easier to transplant, with an overall percent take of 69% for the 7th passage, compared with a 42% take for the 7th passage of the primary. For both tumors, transplants of approximately 2 × 3 mm were used. Histologically, the primary tended to develop areas of necrosis at a much smaller size than the metastasis.

**Cell Cycle Kinetics** Figure 2 compares the PLM curves of the 2 tumors. Since there was no second peak of mitoses, the cell cycle time (Tc) was calculated as:

\[
Tc = \frac{\text{duration of S phase} \times \text{growth fraction}}{\text{labeling index}}
\]

Table I summarizes the data.

Autopsy revealed no visible metastasis to brain, lungs, or abdominal contents.

**DISCUSSION**

Heterotransplants of human tumors into BALB/c athymic nude mice [3,4,7,8,9] and use of [3H]dThd are established techniques to understand tumor growth [1]. We obtained both a primary and metastasis from a single patient with fatal malignant melanoma. The tumors were then established as heterotransplants and maintained in BALB/c/Nu mice for further study. The human origin of the tumor line was proven by chromosomal analysis and ouabain studies. Anaploidy was typical of our tumors, with 60–70 chromosomes per cell in the primary and an average of 81 per metastatic cell. Other studies of chromosomes in melanoma have also found anaploidy. Henry et al [10] were able to obtain chromosome smears from malignant melanoma, and found a wide range of chromosomes with a mode of 40 and 55 in 2 metastatic melanomas.

The 2 tumors differed in a variety of ways. Figure 1 shows the differences in growth rates of the 2 tumors. Histologically, areas of necrosis were seen in small primary tumor heterotransplants. A difference in ability for blood vessel formation might account for the early necrosis and lower growth rate seen in the primary tumor. This could also account for the better overall growth of the metastatic tumor despite a longer cell cycle time. The cell

![Figure 1](attachment:image1.png)

**Figure 1.** Growth curves for primary (diamonds) and metastatic (circles) melanoma heterotransplants.

![Figure 2](attachment:image2.png)

**Figure 2.** PLM curves for primary (solid line) and metastatic (broken line) melanoma.

**Table I. Cell Kinetic Parameters**

<table>
<thead>
<tr>
<th>Tumor</th>
<th>LI(%)</th>
<th>Ts(h)</th>
<th>G2(h)</th>
<th>GF(%)</th>
<th>Tc(h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>10.7</td>
<td>8</td>
<td>7</td>
<td>80</td>
<td>60</td>
</tr>
<tr>
<td>Metastasis</td>
<td>16.5</td>
<td>33</td>
<td>3</td>
<td>94</td>
<td>188</td>
</tr>
</tbody>
</table>

Key: LI = labeling index
Ts = duration of S
G2 = duration of G2
GF = growth fraction
Tc = cell cycle time
cycle kinetic data show that both the S phase and cell cycle times were longer for the metastasis. Most published cell cycle studies concerning malignant melanoma have dealt with metastatic lesions, and our results can be compared with them. The longer S phase of the metastasis compares favorably with that determined by others [6,11]. The labeling index of 16.5% is essentially the same as that found by Young and DeVita [6]. While some authors have found shorter cell cycle times [12,13], others have determined times similar to ours [14]. Interestingly, Herlyn et al [15] have found that the exponential growth phase of a primary behaves more like a metastasis than the radial growth phase of the same tumor. This regional variation and the spread of a more aggressive clone may account for the differences we found between the primary and metastasis. We did not find any metastasis to the tumor-bearing mice. This concurs with the previously reported work of Trope et al [9] who followed tumors for up to 2.5 months, although some authors have found metastasis in carrier mice [8].

To the best of our knowledge, this is the first time a primary and metastasis of malignant melanoma from the same patient have been compared using the athymic nude mouse model. Herlyn et al [15] studied 3 primary and 16 metastatic lesions taken from 4 patients using cell culture, but did not examine cell kinetics. From our work it appears that there are some inherent differences in primary and metastatic tumors in this patient which may account for the more aggressive behavior of metastatic melanoma. Additional studies with other primary–metastatic tumor pairs will be necessary before any generalization can be made. Hopefully, further research in this area will help better understand and treat this devastating disease.

REFERENCES