Growth and Metastasis of Hamster Melanoma following Transplantation into Athymic Mice

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ABSTRACT

Melanotic hamster malignant melanoma (MM1) is a transplantable, locally invasive tumor which metastasizes widely in syngeneic hosts. We have established three clones, HM1, HM3, and HM4 of the original MM1 line in culture. Inoculation (s.c.) into 4- to 5-week-old male athymic mice produced highly vascular, melanotic, locally invasive tumors in 100% of mice inoculated with a latency of 4–7 days. Karyotype analysis of HM cells revealed modal chromosome numbers of 39–41 (43% HM1), 43 (22% HM3), and 44–47 (61% HM4). Sixty-one percent of HM1 cells were hypodiploid, 4% diploid, and 5% hyperdiploid. HM1, -3, and -4 cells also exhibited aneuaphyly, endoreduplication, translocation exchanges, additions, deletions, dicentromeric and ring chromosomes, and double minutes although not all cells exhibited all abnormalities. Initial metastasis was to regional lymph nodes with eventual progression to lung and liver. Mice inoculated with HM1, -3, and -4 cells were dead with metastatic disease within 57, 63, and 64 days, respectively, following s.c. inoculation (5 x 10⁵ cells) when the mice were 90–100 days old. Mortality rate was highest in line HM3 with 50% of the mice dead within 33 days postinoculation. Metastatic potential of HM1 and HM3 cells rose significantly when successive generations of HM1 and HM3 cells cultured from isolated lung metastases were reinoculated. Metastasis to lymph nodes and liver was not observed with increasing passage generations of lung metastasis. Our observations provide evidence that hamster melanomas are clonally heterogeneous, locally invasive, and exhibit rapid growth and metastasis following s.c. inoculation into adult athymic mice. Transplantable malignant hamster melanoma cells also exhibit a significant preferential metastasis to lung following culture and sequential reinoculation of lung metastases in athymic mice. As such, they appear to provide a reproducible model of metastasis in an immunocompromised host.

INTRODUCTION

The biology and histopathology of spontaneous malignant melanoma of hamsters suggest that this tumor closely resembles human melanoma (1–3), particularly in its ability to locally invade surrounding tissues and metastasize to distant sites (4, 5). Melanotic and amelanotic cell lines derived from these tumors exhibit a significant overall incidence of metastasis in hamsters as well as a difference in metastatic pattern following s.c. transplantation in syngeneic hosts (4–6). Although a significant incidence of metastasis to lymph nodes, liver, and kidney is present, melanotic cells (MM1) metastasize chiefly to lung suggesting a hematogenous route of metastasis (4–6). In hamsters, the number of metastatic sites increases directly with time postinoculation (4). Other lines derived from this pigmented and amelanotic hamster melanomas exhibit different patterns of metastasis (1–3, 7, 8). Collectively, these observations suggest that the original neoplasms are clonally heterogeneous.

The metastatic potential of hamster melanoma in hamster and outside its syngeneic host and its attendant immune system has received little experimental attention. Limited data on other malignant hamster tumors following inoculation into athymic mice suggest that although hamster neoplasms are tumorigenic in athymic mice, they grow more slowly and less invasively than in hamsters, metastasize only when primary tumors are large, and spontaneously regress in germ-free athymic mice (9).

As part of a larger investigation on clonal selection of transplantable tumors in athymic mice, we characterized the growth and metastasis of three clonal variants of malignant hamster melanoma cell line MM1 following inoculation into athymic mice. We were particularly interested in whether tumorigenicity and growth rate would be limited and tumor heterogeneity maintained when inoculated into adult athymic mice with significant levels of natural killer cell activity (10, 11). Our data suggest that cell lines derived from hamster melanoma (MM1) can be successfully carried in adult athymic mice where they exhibit a significant, reproducible, organ-specific metastasis.

MATERIALS AND METHODS

Animals. Four to 5-week-old male athymic (BALB/c) nude mice were obtained from the animal production area of the National Cancer Institute-Frederick Cancer Research Facility. They were maintained in filter cages on sterile wood shavings at 24° C and constant humidity under a 12-h light/12-h dark lighting regimen and received sterile mouse chow and water ad libitum. Male Syrian hamsters 4 and 6–7 weeks of age were supplied by Engle Laboratories (Farmersberg, IN) and housed 2/cage under similar environmental conditions as athymic mice. Food and water were supplied ad libitum.

Tumors. Three melanoma cell lines (HM1, -3, and -4) derived from a Syrian hamster malignant melanoma (MM1; courtesy of Dr. Joel Fortner, Memorial Sloan-Kettering Institute) and cloned in vitro by dilution were used in this study. All lines were maintained on plastic in MEM-H (GIBCO, Grand Island, NY) plus 15% fetal bovine serum containing penicillin, streptomycin, and fungizone at 37° C in a humidified atmosphere. Cell lines and mice were routinely checked and found free of Mycoplasma, reovirus type 3, mouse adenovirus, murine hepatitis virus, ectromelia virus, and pneumonia virus of mice (Microbiological Associates, Bethesda, MD). To insure hamster origin during passage through mice, samples from initial cultures and cultures of metastatic nodules were karyotyped and subjected to isoenzyme analysis (Authenti Kit, Corning Medical, Corning, NY), respectively, against hamster liver and MM1 standards. Tumor cells were harvested from culture according to Kozlowski et al. (12) and 5 x 10⁵ cells/0.2 ml MEM-H inoculated s.c. into the right flank. All animals were killed over solid CO₂, and specimens of primary tumor were taken for histological examination.

Assay for Metastasis. At autopsy, internal organs were immediately examined for gross metastases. Lungs were removed, perfused, and

Received 8/25/86; revised 2/6/87, 5/1/87; accepted 5/20/87.

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1 Supported in part by CA 31046, the Louis A. Lerner Memorial Fund, the Community Chest of Dwight, and the P. F. Stathus Memorial Cancer Research Fund.

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* The abbreviations used are: MEM-H, minimum essential medium with Hanks' basal salts; DT, doubling time.

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fixed in neutral formol: saline and examined under a dissecting microscope for the presence of metastasis by two independent investigators. Previous reports suggest that highly metastatic tumor cell variants preexist in a parental population of tumor cells, and subclones of both low and highly metastatic clones derived from heterogenous tumors show a wide variation in the production of experimental metastasis (13–15). When coupled with a rapid primary tumor growth rate and significant incidence of early morbidity and mortality, the use of total number of metastatic lesions to determine alterations in metastatic potential becomes difficult. We therefore set an arbitrary postinoculation cutoff time to initialize metastatic potential. The presence of at least one metastatic deposit 1–2 mm in diameter was taken as positive for metastasis. Since morbidity and mortality were significant by 40 days postinoculation of all cell lines, the majority (>90%) of mice were killed between 30 and 35 days postinoculation in studies determining the metastatic potential of successive generations of lung metastasis. This provided a narrow window with which to assess whether a significant increase in metastasis was associated with selection pressure and obliterated assessing metastatic potential on a significantly reduced population of mice with significant morbidity. Representative sections of all tissues were fixed in formal: saline and embedded in paraffin. Paraffin blocks were cut at 6 μm in stepwise sections and stained with hematoxylin and eosin for histological verification of metastasis and presence of micrometastasis.

Selection Procedure. The selection procedure was adapted from one previously described for murine B16 melanoma (16). Cell lines HM1 and HM3 were inoculated s.c. into the right flank of athymic mice or hamsters. Solitary lung metastases designated HM1-F1 and HM3-F1 were dissected free of surrounding normal tissue prior to fixation, mechanically dispersed in Hanks' balanced salt solution, and pressed through a 60-mesh stainless steel screen. The cells were centrifuged, resuspended twice in MEM-H, and plated into 25-cm² culture flasks. Nonadhering cells were washed off 24 h later with fresh MEM-H. Penicillin-containing flares were established and passaged twice in vitro prior to an isoenzyme assay to assure that each line remained uncontaminated. After isoenzyme and histological analysis metastatic cells (F1) were harvested and 5 x 10⁵ cells reinoculated s.c. into additional mice or hamsters. Thirty to 35 days later, mice or hamsters were killed and a pulmonary metastasis became adapted to grow in culture as described above. This procedure was repeated four times with HM1, twice more with HM3 in athymic mice, and twice more with HM1 in hamsters. All organs were examined for gross metastasis and specimens were taken for histological confirmation of metastasis after each passage generation in athymic mice and hamsters.

Growth in Vitro. Primary cell line HM1, -3, and -4 and lung metastasis from each passage in vivo were seeded at a density of 5 x 10⁵ cells/25-cm² flask in MEM-H with 16% fetal bovine serum. Triplicate flasks were established and passaged twice in vitro prior to an isoenzyme assay to assure that each line remained uncontaminated. After isoenzyme and histological analysis metastatic cells (F1) were harvested and 5 x 10⁵ cells reinoculated s.c. into additional mice or hamsters. Thirty to 35 days later, mice or hamsters were killed and a pulmonary metastasis became adapted to grow in culture as described above. This procedure was repeated four times with HM1, twice more with HM3 in athymic mice, and twice more with HM1 in hamsters. All organs were examined for gross metastasis and specimens were taken for histological confirmation of metastasis after each passage generation in athymic mice and hamsters.

Growth in Vivo. The interval between the time of inoculation (day 0) and the appearance of a 2-mm diameter primary s.c. tumor was taken as the latent period. The largest diameter of the tumor was then measured every other day until the diameter reached 1.0 cm. At that time, the tumor was measured in three dimensions weekly and the volume calculated (17). Measurements were taken until the animal died spontaneously or at the conclusion of an experiment. Tumor doubling time was estimated from semilogarithmic plots of tumor volume.

Karyotyping. Cells were G-banded using a modification of standard technique because they were fairly resistant to hypotonic shock. Briefly, cells were grown to 80% of a confluent state, then inoculated with colcemid (0.5 μg/ml) at 37°C. Cells were detached with trypsin:EDTA, centrifuged at 500 x g (for 10 min), and swollen in 10 ml 0.075 M KCl for 20 min at 25°C, at which time they were fixed in 10 ml methanol:glacial acetic acid (3:1). They were then recentrifuged and refixed in 4.0 ml fixative and allowed to stand overnight at 4°C. Cells were centrifuged at 800 x g for 10 min at 25°C and refixed for 15 min at 25°C with fresh fixative. The cells were finally resuspended in 3-4 drops of fresh fixative and gently mixed with a Pasteur pipet. Drops of the turbid suspension were applied to glass slides, dried at 25°C, and

### Table 1 Comparison of in vitro and in vivo doubling times of successive generations of lung metastasis of malignant melanoma cell lines HM1, -3, and -4

<table>
<thead>
<tr>
<th>Cell line</th>
<th>DT in vitro (h)ᵃ</th>
<th>DT in vivo (days)ᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td>HM1</td>
<td>34</td>
<td>6.0</td>
</tr>
<tr>
<td>F1</td>
<td>40</td>
<td>5.5</td>
</tr>
<tr>
<td>F2</td>
<td>36</td>
<td>7.0</td>
</tr>
<tr>
<td>F3</td>
<td>36</td>
<td>7.0</td>
</tr>
<tr>
<td>F4</td>
<td>38</td>
<td>5.0</td>
</tr>
<tr>
<td>HM3</td>
<td>44</td>
<td>6.5</td>
</tr>
<tr>
<td>F1</td>
<td>42</td>
<td>4.8</td>
</tr>
<tr>
<td>F2</td>
<td>42</td>
<td>4.8</td>
</tr>
<tr>
<td>F3</td>
<td>36</td>
<td>5.0</td>
</tr>
<tr>
<td>HM4</td>
<td>56</td>
<td>6.5</td>
</tr>
</tbody>
</table>

ᵃ Number of cells plated, 5 x 10⁵. ᵇ 5 x 10⁵ cells inoculated s.c. into groups of 5-6 male athymic mice 5 weeks of age. ᶜ Mean of three flasks. ᵈ F1–F4 are passage (generations) numbers of each cell line. A solitary lung metastasis (F1) was isolated, cultured, and repassaged (s.c.) into mice and the process repeated with subsequent lung metastasis.

G-banded with Giemsa for 4 min following a brief digestion (10 s) with pancreatin in Hanks' balanced salt solution.

A minimum of 50 metaphase spreads was counted for each cell line and chromosomes were arranged according to Popescu and D'Apalo (18, 19).

### RESULTS

#### Tumor Growth

Mortality in mice carrying HM3 cells was 50% at 33 days with 50% of the mice dead at 48 and 54 days with HM1 and HM1 tumors, respectively (Fig. 2). All mice were dead of disease by 57, 62, and 63 days postinoculation of 5 x 10⁵ cells of lines HM3, -4, and -1, respectively.

Tumor incidence following inoculation of 5 x 10⁵ HM1 cells s.c. in 6- to 7-week-old male hamsters was ~100% with a latency of 16.6 ± 1.6 (SE) (n = 14) days. This was significantly greater than the 10.6 ± 0.8 days observed in 4-week-old hamsters (n = 10). Tumor latency of HM1 F1–F3 lung metastasis in 6- to 7-week-old hamsters was 8.7 ± 0.5, 8.0 ± 0.6, and 8.1 ± 0.5 days, respectively, all significantly (P < 0.01) shorter than primary tumor cells. Doubling times ranged from 5.5–5.7 days following reinoculation of the F1–F3 generation of lung metastasis. Inoculation of 5 x 10⁵ MM1 cells in 6- to 7-week-old male hamsters resulted in a tumor incidence of 100% with a latency of 13.3 ± 0.4 days and DT of 6 days. Animal age at inoculation significantly influenced tumor growth rate. MM1 growth in 3- to 4-week-old hamsters is three times that of animals inoculated at 15–16 weeks of age.³ Mortality was approximately 50% at 50 days postinoculation of 5 x 10⁵ HM1 through HM1-F3.

³ L. R. Stanberry and C. W. Beattie, unpublished observations.
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Table 2 Organ specific incidence of distance metastasis in athymic mice inoculated with successive generations of lung metastasis HM1, -3, and -4 hamster malignant melanoma cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Liver* no. with metastasis/total (%)</th>
<th>Lung* no. with metastasis/total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HM1</td>
<td>0/14 (0)</td>
<td>1/14 (7)</td>
</tr>
<tr>
<td>HM1-F1</td>
<td>1/30 (3)</td>
<td>8/30 (27)</td>
</tr>
<tr>
<td>HM1-F1 Li</td>
<td>1/22 (5)</td>
<td>7/22 (32)</td>
</tr>
<tr>
<td>HM1-F2</td>
<td>0/21 (0)</td>
<td>8/21 (38)</td>
</tr>
<tr>
<td>HM1-F3</td>
<td>0/20 (0)</td>
<td>10/20 (50)</td>
</tr>
<tr>
<td>HM1-F4</td>
<td>0/18 (0)</td>
<td>13/18 (72)</td>
</tr>
<tr>
<td>HM1-F5</td>
<td>0/17 (0)</td>
<td>13/17 (76)</td>
</tr>
<tr>
<td>HM3</td>
<td>0/20 (0)</td>
<td>6/20 (30)</td>
</tr>
<tr>
<td>HM3-F1</td>
<td>0/20 (0)</td>
<td>6/20 (30)</td>
</tr>
<tr>
<td>HM3-F2</td>
<td>0/14 (0)</td>
<td>5/14 (36)</td>
</tr>
<tr>
<td>HM3-F3</td>
<td>0/15 (0)</td>
<td>10/15 (67)</td>
</tr>
<tr>
<td>HM4</td>
<td>0/16 (0)</td>
<td>4/16 (25)</td>
</tr>
</tbody>
</table>

* Killed at postinoculation days 29–39; 90% of mice were killed 30–35 days postinoculation.

* Number of animals with metastasis per total number of mice inoculated with 5 x 10⁶ cells.

Fig. 1. Invasion of leg musculature by well-differentiated melanoma cells of primary cell line HM1 30 days following inoculation of 5 x 10⁶ cells s.c. to the right flank of 5-week-old male athymic mice. H & E, x 430.

Fig. 2. Incidence of mortality in athymic mice inoculated s.c. with 5 x 10⁶ hamster malignant melanoma primary cell lines HM1 (○), HM3 (□), and HM4 (△). Tumor latency was virtually identical in all cell lines.

Fig. 3. Metastatic deposit of HM1-F1 cells invading the lung parenchyma of an adult male athymic mouse. The tumor cells are spreading out from a capillary near the base of the lung. H & E, x 110.

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cells s.c. in 6- to 7-week-old hamsters with at least 10% morbidity by 40–42 days postinoculation.

Tumor Metastasis. All primary cell lines produced a significant incidence of local and distant metastasis in athymic mice. The majority of lesions were located in the lung. A low incidence (3%) of liver metastasis was observed with line HM1 (Table 2) with limited involvement of regional lymph nodes (10%). Cell line HM-3 with the highest initial rate of gross lung metastasis (30%) was also associated with the most rapid mortality (Fig. 2). By the F2 generation of lines HM1 and HM3 regional lymph nodes were devoid of tumor cells unless immediately adjacent to or invaded by the primary tumor. In addition to invading lymph nodes proximal to the primary tumor, lines HM1, -3, and -4 spread hematogenously. This was clearly evident in lung metastasis where tumor cells invaded lung parenchyma directly from capillaries and veins (Fig. 3).

A statistically significant increase in the incidence of lung metastasis was observed between the primary and F2-F5 passages of cell line HM1 following s.c. inoculation of 5 x 10⁶ cells with significant increases between generations (Fig. 3). A similar phenomenon was observed in line HM3 with successive passages of lung metastases. The low incidence of liver metastasis...
tasis in the initial passage of line HM1 was not observed following re inoculation of cells from the liver metastasis. This metastasis was also not homogeneous for cells expressing a preference for liver, as a significant incidence of lung metastasis was observed following re inoculation of HM1-F1 LI (Table 2).

Metastases to lung were present in 31 (4 of 13), 54.5 (5 of 11), 62 (5 of 8), and 60% (6 of 10) of hamsters bearing HM1 through HM1-F3 lung metastasis and autopsied between days 35 and 42 post inoculation when morbidity was approximately 10%. The results were not significantly different. Liver metastases were also present in 4 of 13 (31%) of hamsters inoculated with HM1 cells with no detectable liver metastasis in F1–F3 generations. Unlike athymic mice, metastatic spread to cervical and axillary lymph nodes was present in 9–13% of syngeneic hamsters inoculated s.c. with the F1–F3 generations of HM-1 cells metastatic to lung.

Karyology. Karyotypic analysis of clones HM1, -3, and -4 suggested that virtually all cells expressed an abnormal karyotype with a significant number of aneuploid cells in all lines. Chromosomal number was highly variable in all cell lines and ranged from 38 to greater than 350 in line HM1 with modal numbers of 39–41, 43, and 44–47 for lines HM1, -3, and -4, respectively (Table 3). The normal number of chromosome in Syrian (golden) hamsters is 44 with 21 somatic pairs. The animals used in this study had a normal karyotype by lymphocyte G banding (data not shown). Numerous chromosomal abnormalities were also observed in all cell lines although all identified abnormalities were not present in each cell. The most common abnormality included dicentric and ring chromosomes, endoreduplication, minutes, and double minutes (Table 3; Fig. 4). A diploid number did not ensure the presence of a normal chromosome complement. An increased passage number (F2) in athymic mice was not associated with any statistically significant change in chromosome modal number or type of aberration in HM1 lung metastasis in vitro and in athymic mice (data not shown). In spite of individual variability, several chromosome patterns were present (Table 3). A significant percentage of metaphase spreads had a chromosome number greater than 3n. Only line HM4 had a significant percentage of diploid cells (17%) which may be reflected in the slower in vitro growth and slight increase in initial survival of mice inoculated with these cells. There was no discernible relationship between modal number or chromosome pattern in the primary cell lines and degree of invasiveness, growth in vivo or metastatic potential of any of the lines tested.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Predominant karyotypic features of metastatic hamster melanoma cell lines HM-1, -3, and -4</th>
</tr>
</thead>
<tbody>
<tr>
<td>HM-1, modal no. 39–41 (43%)</td>
<td>Dicentric chromosomes</td>
</tr>
<tr>
<td>Ring chromosomes</td>
<td>Endoreduplication</td>
</tr>
<tr>
<td>Minuten</td>
<td>Large D group chromosomes</td>
</tr>
<tr>
<td>Metaphase spreads ≥ 3n (25%)</td>
<td></td>
</tr>
<tr>
<td>HM-3, modal no. 43 (22%)</td>
<td>Dicentric chromosomes</td>
</tr>
<tr>
<td>Endoreduplication</td>
<td>Double minutes</td>
</tr>
<tr>
<td>Large D group chromosomes</td>
<td>C group size metacentric chromosomes</td>
</tr>
<tr>
<td>B group with shortened p arm</td>
<td>Metaphase spreads ≥ 3n (18%)</td>
</tr>
<tr>
<td>HM-4, modal no. 44–47 (61%); 44, 47 (17%)</td>
<td>Dicentric chromosomes</td>
</tr>
<tr>
<td>Large very submetacentric (A group type)</td>
<td>Metaphase spreads ≥ 3n (13%)</td>
</tr>
</tbody>
</table>

DISCUSSION

Our data clearly demonstrate that malignant melanoma of hamsters is an aggressive, locally invasive neoplasm with a short latency, rapid growth rate, and significant incidence of metastasis following s.c. inoculation in young adult (~5-week-old) male athymic mice. Rosenberg et al. (4) reported a survival rate of 32–118 days following inoculation of MM1 tumors cells into adult hamsters with a significant incidence of lung and other visceral metastasis at the time of death. Additional studies suggest that axillary lymph node and lung metastasis arise first, with metastasis to visceral organs appearing later (5, 6). We have used this observation to develop a reproducible model for metastasis and select for organ preference and metastatic potential using athymic mice.

Hamster melanoma, unlike other hamster malignancies, has a similar exponential rate of growth in athymic mice and prepubertal (4-week-old) or postpubertal (6- to 7-week-old) hamsters. The significantly shorter latency of HM1 tumors in hamsters 4 weeks of age compared to those inoculated at 6 to 7 weeks suggests that an immunological or hormonal component may be altering initial growth in syngeneic hosts. The significant decrease in latency with F1–F3 generations (passages) of lung metastasis inoculated s.c. in 6- to 7-week-old hamsters suggests that genotypic or phenotypic alterations in tumor cells may be associated with increased passage. This could be expressed as changes in tumor cell antigenicity or differentiation. The latter does not appear to be a viable explanation because no changes in cellular anatomy were noted at the light or electron microscopic level or in the level of tyrosinase activity (data not shown). Initial results from our laboratory also suggests that HM1, -3, and -4 cells are highly antigenic and do not lose a variety of cell surface antigens during sequential passage in vitro and in athymic mice. Alternatively, host immune response could change during this interval and alter tumor biology. The lack of difference in tumor latency following s.c. inoculation of HM1 and HM3 cells in adult athymic mice suggests that these cells may rapidly overcome any inhibitory influence placed on them by higher levels of circulating natural killer cells in adult athymic mice (10, 11).

Tumor cell growth rates in vitro and in vivo were not predictive of metastatic potential of individual cell lines. A decrease in the in vitro DT of line HM3 was associated with an overall decrease in DT in vivo, shorter survival, and significant increase in metastatic rate by the third passage of lung metastasis in athymic mice. A similar increase in metastasis was also observed in line HM1-F3 without any noticeable decrease in either in vitro and in vivo DT, however. This finding suggests that the metastatic potential of these cell lines is comparable and independent of primary tumor growth rate, which supports previous observations of a lack of relationship between primary tumor growth rate and invasive or metastatic potential (20, 21). The degree of local invasiveness has been suggested to be directly correlated with metastasis in a number of cell lines (20), particularly after inoculation into athymic mice (22). This suggestion appears to be confirmed by the present study. All tumors were locally invasive and exhibited metastasis independent of primary tumor growth rate. Transplantable murine B16 and K1735 (11, 23) melanoma and a hamster sarcoma (9) have also been reported to exhibit limited metastasis to distant sites in athymic mice while human tumors rarely metastasize to distant sites following s.c. inoculation (24, 25). The major difference between species and tumor type again appears to be related to
the degree of local invasiveness (26). Although rapidly dividing
anaplastic lesions have been reported to be more invasive than
better differentiated tumors (15, 20, 27), this does not appear
to be true in all cases (28), particularly with human tumors
transplanted into athymic mice. All tumors in this study were
composed of well differentiated melanoma cells capable of
synthesizing and packaging melanin. Reports on phenotypic
changes in transplantable tumors suggest that dedifferentiation
can occur during repeated passage in athymic mice (29, 30).
Differentiated function was not lost during intermittent recycling
tumor cells through culture and athymic mice in this
study. Light and electron microscopic examination of F1–F5
generations of lung metastasis suggest that this did not occur
during the course of this study (data not shown).

The karyotype of each subline also did not provide additional
cues for the apparent increase in metastatic potential. Karyo-
typic analysis of clonal variants of murine melanoma (31–33)
suggest that increasing metastatic potential is associated with
genetic instability. Within murine lung metastasis, chromo-
somal abnormalities have been observed in virtually all cells
examined, although most metastases differed from one another
in that they exhibited characteristic combinations of chromo-
somal markers (33). While other reports suggest that karyotype
is not a marker for phenotypic diversity (34), present results on
hamster malignant melanoma support these observations (31–
33). The significant degree of aneuploidy associated with each
primary cell line indicates that this characteristic may be asso-
ciated with aggressive, locally invasive malignancies (35).

Analysis of the F1 generation of HM1 and HM3 cells suggests that
these cells had similar modal number and chromosomal abnor-
malities to the parent line (data not shown). This may not be
ture for the F2–F5 generation. An increase in genetic instability
in the presence of selection pressure could lead to more malign-
ant variants appearing as an overall increase in metastatic
potential. Tumor (metastatic) progression has been ascribed, in
part, to a genetic mechanism which occurs as a result of
acquired genetic alterations in developing tumors cells (36).
While epigenetic mechanisms cannot be ruled out (37, 38),
further karyotypic analysis of these lines would provide addi-
tional information on the relationship between genetic instabil-
ity and metastatic potential. Since repeated passage of human
tumor cells through athymic mice apparently does not alter
tumor cell chromosomal origin (39), this should not provide an
impediment to successful analysis.

The apparent increase in metastatic potential of lines HM1
and HM3 in athymic mice and preference for lung compared
to syngeneic hosts may center on the means used to analyze
metastatic incidence. Das Gupta and Terz (5, 6) have reported
that metastatic spread is wider following inoculation of MM1
cells if hamsters are examined late in the course of the disease.
We killed athymic mice prior to the onset of significant mor-
bidity and mortality, which selected for those metastasis show-
ing a preference for lung. This latter argument does not appear likely, however. When mice were examined for metastasis 40–45 days postinoculation,
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a limited incidence of regional lymph nodes close to the invading tumor was found to be involved, but there was no visceral metastasis. In addition, the incidence of melanoma metastatic to lymph nodes in mice decreased to zero with increasing passage of lung metastasis. Finally, when hamsters were killed at early postinoculation (30 days) of HMI cells and F1-F3 generations of lung metastasis, prior to significant morbidity, metastases to liver and lymph nodes were present.

It is clear from our observations that the preference of the original melanotic cell line MM1 and its sublines HMI-1, -3, and -4 for lung tissue in hamsters continues to be expressed in athymic mice. Selection of single tumor cells in vitro and reinoculation of sequential passages of lung metastasis may explain the relative inability of the F1–F5 generations of lines HMI and HM3 to metastasize to other organs. The original tumor, like those of other rodent melanomas, is apparently clonally heterogeneous. Since metastatic incidence is not yet at 100% even after five selections even though one site is preferred our observations provide evidence that the process of metastasis is selective and possibly stochastic (40).

In summary, our use of cloned cell lines exhibiting a preference for lung and increasing selection pressure by subculturing lung metastasis which appear early in the disease course has provided us with relatively phenotypical, homogeneous cell lines which are locally invasive and metastasize rapidly and selectively to an organ following s.c. inoculation in adult athymic mice. This provides a reproducible model to examine aspects of the metastatic process, particularly the role of gene expression, e.g., activated oncopgenes, genetic stability, and innate host defense mechanisms in an immunocompromised adult host.

ACKNOWLEDGMENTS

Special thanks to R. C. Moon, Ph.D. for photomicrography and many helpful discussions, and R. Macke for editorial assistance and manuscript preparation.

REFERENCES

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