Predominance of the Metastatic Phenotype in Somatic Cell Hybrids of the K-1735 Murine Melanoma

Alexander H. Staroselsky, Sen Pathak, Yuti Chernajovsky, Susan L. Tucker, and Isaiah J. Fidler

University of Texas M. D. Anderson Cancer Center, Departments of Cell Biology [A. H. S., S. P., I. J. F.], Immunology [Y. C.], and Biomathematics [S. L. T.], Houston, Texas 77030

ABSTRACT

The purpose of these studies was to determine whether the metastatic phenotype will dominate when metastatic and nonmetastatic clones of the K-1735 mouse melanoma are hybridized by somatic cell fusion. Three nonmetastatic and three metastatic clones were transfected with DNA from plasmids pSV2neo or pSV2hygro, which confer resistance to the drugs neomycin or hygromycin, respectively. The metastatic properties of the six clones were not altered by these transfections. The tumorigenicity and metastatic capacity of hybrid cells formed by somatic cell fusion of nonmetastatic and metastatic clones were examined. To do so, near-tetraploid hybrids containing a nearly complete chromosomal complement from both parental cells were injected i.v. into syngeneic mice, and the number of metastatic nodules in the lung was determined at 45 days or when the mice became moribund. Seven of nine hybrids produced from the fusion of metastatic and nonmetastatic clones exhibited a highly metastatic phenotype, although in most cases the metastatic potential of the hybrids was lower than that of the metastatic parent cells. Very similar results were obtained in athymic nude mice. The metastatic potential of the hybrids was directly correlated with their growth in the subcutis of nude mice. These results indicate that the metastatic capacity of K-1735 cells predominates in somatic cell hybrids between nonmetastatic and metastatic cells. When fusion of nonmetastatic and metastatic cells yields a hybrid with nonmetastatic properties, it may be due to suppression of growth.

INTRODUCTION

Although most neoplasms result from the expansion of a single cell (1–3), by the time of diagnosis malignant tumors contain heterogeneous subpopulations of cells with differences in such properties as growth rate, antigenic and immunogenic status, cell-surface receptors and products, response to cytotoxic agents, invasiveness, and metastatic potential (4). To produce metastasis, tumor cells must complete a series of sequential and highly selective steps (2, 6). Failure to complete any one of the steps of the process eliminates the cells from the metastatic cascade. For this reason, the failure of tumor cells to produce a metastasis can be due to different single or multiple deficiencies (7–9).

Our understanding of the pathogenesis of metastasis has been advanced considerably by the ability to isolate subpopulations of tumor cells with different invasive and metastatic properties from heterogeneous rodent and human neoplasms (5, 8, 10). The availability of these selected subpopulations with known quantitative and qualitative differences in metastatic potential has facilitated numerous studies of the biochemical characterization of metastatic cells (2, 6, 11). Recent attention has also been focused on identifying the genetic control of the metastatic phenotype and the genes responsible for regulating the numerous discrete steps in the process (2, 6, 9, 11, 12). Many of these studies follow the precedent set by investigations of tumorigenicity. In some systems, tumorigenicity has been shown to be due to the activation of dominant genes (3, 13), whereas in other systems somatic cell hybridization (14–17) and molecular biology techniques revealed that tumorigenicity is a recessive trait (14, 15) manifested after the loss or inactivation of tumor suppressor genes (16–22). Thus, the genetic regulation of tumorigenicity is determined by the interaction of activated dominant genes or the loss or inactivation of recessive tumor suppressor genes or both (23).

To determine whether the metastatic phenotype is dominant or recessive, several laboratories have examined somatic cell hybrids between metastatic and nonmetastatic or even nontumorigenic cells (24–29). The results depended on the nature of the fused cells. In some systems the hybrids were nontumorigenic and, hence, nonmetastatic (24–26). In others using nontumorigenic lymphoid cells the hybrids were invariably metastatic (30–37). Efforts to answer this question definitively require a model that fulfills several criteria: (a) metastatic and nonmetastatic clones must be isolated from the same parental tumor, be nonantigenic, and be of similar tumorigenic and antigenic properties; (b) somatic cell hybrids with altered tumorigenicity and antigenicity should be identified; and (c) since a failure to produce metastasis can be due to different deficiencies (9), several nonmetastatic and metastatic clones should be studied.

In the present study we analyzed the dominance of the metastatic phenotype in somatic cell hybrids of the K-1735 murine melanoma (38). This tumor system has been well characterized for studies of metastasis (8, 9), and many clones with different metastatic potentials have been isolated by in vitro and in vivo techniques (39, 40). We have chosen the classic method of somatic cell genetics because, unlike DNA-mediated gene transfer, whole-cell fusion combines the genomes of both partners in one hybrid cell, preserves the normal chromosome location of the relevant genes, and allows a direct determination of dominance or recessiveness of metastasis.

MATERIALS AND METHODS

K-1735 Melanoma Lines. The original K-1735 melanoma induced in a C3H/HeN mouse by exposure to UV light followed by painting with croton oil was the gift of Dr. Margaret L. Kripke (38) (University of Texas M. D. Anderson Cancer Center). The parental tumor was cloned in vitro by a double-cloning method (8). Of the large number of clones thus isolated, clones 3, 10, and 19 (designated C-3, C-10, and C-19) were classified as nonmetastatic or low metastatic (8, 9). Clone 4 (designated C-4) is highly metastatic and produces melanotic tumor foci in the lungs of syngeneic mice. K-1735 clones M-2 and X-21 were derived from solitary spontaneous lung metastases produced by the K-1735 parental line growing s.c. (39); both were shown to be clonal in origin (41, 42) and to be highly metastatic in syngeneic recipients (42).

In Vitro Culture of K-1735 Cells. All tumor cell lines were maintained in tissue culture in Dulbecco’s modified Eagle’s medium supplemented...
with 10% fetal bovine serum, sodium pyruvate, nonessential amino acids, l-glutamine, and 2-fold vitamin solution (Gibco, Grand Island, NY). Cell cultures were maintained on plastic and were incubated in 5% CO₂/95% air at 37°C. Cultures were free of Mycoplasma and the following murine viruses: reovirus type 3; pneumonia virus; K virus; Theliler’s encephalitis virus; Sendai virus; minute virus; mouse adenovirus; mouse hepatitis virus; lymphocytic choriomeningitis virus; ectromelia virus; and lactate dehydrogenase virus (assayed by M. A. Bioproducts, Walkersville, MD). To minimize problems of biological stability (1), all in vivo studies and cytogenetic analyses were carried out with cultures at passages 6 to 10. Cultures were maintained for no longer than 4 weeks after recovery from frozen stocks.

Transfection of K-1735 Cells. Transfection of DNA was carried out by the calcium phosphate procedure as described by Graham and Van der Eb (43). K-1735 cells were transfected with pSV2neo plasmid encoding for resistance to G418 (44) or pSV2hygromycin B encoding for resistance to hygromycin B (45). Resistant cells were selected in medium containing 500 units/ml G418 (Gibco) or 250 units/ml hygromycin B (Boehringer-Mannheim, Indianapolis, IN). After 3–4 weeks, the colonies were pooled and expanded into confluent cultures.

Cell Fusion. Cells were fused by the method of Davidson and Gerald (46). Briefly, 1 × 10⁶ cells from each cell line were plated into a 60-mm culture dish containing 4 ml of supplemented medium. After 24 h of incubation at 37°C, the culture medium was removed and the cells were treated with 2 ml of 50% (w/v) polyethylene glycol (Mr ~1500) in serum-free medium for 1 min at room temperature. The polyethylene glycol solution was removed and the cells were washed four times with HBSS, refed with medium, and incubated for an additional 24 h. The cells were then harvested with 0.25% trypsin and 0.02% EDTA and resuspended in medium with 100 units/ml (active concentration). Two to 3 weeks after initiation, growing colonies were pooled and grown in the double-selection medium. The hybrid nature of the cells was confirmed by karyotype analysis.

Chromosome Preparation and Banding Induction. Culture flasks fed 24 h earlier were treated with trypsin, and dislodged cells were centrifuged, exposed to 0.4% KCl for 20 min at room temperature, and fixed in methanol:acetic acid (3:1 by volume). Fixed cells were washed in fixative three times and dropped on wet slides for air-dry preparation.

Five- to 7-day-old slides were treated for G-T-G banding following the procedure described elsewhere (47). Forty to 50 G-banded metaphase spreads were evaluated from each clone and the parental line to identify the marker chromosomes.

Mice. Specific pathogen-free female mice of the inbred strain C3H/HeN (mammary tumor virus-negative) and nude mice (NCR nu/nu) were purchased from the Animal Production Area, National Cancer Institute (Frederick, MD). The mice were 6–8 weeks old at the time of the experiments.

Animals were maintained in facilities approved by the American Association for Accreditation of Laboratory Animal Care in accordance with current United States Department of Agriculture, Department of Health and Human Services, and NIH regulations and standards.

In Vivo Studies. Tumor cells in exponential growth phase were harvested by a brief treatment with a 0.25% trypsin:0.02% EDTA solution (w/v). The flask was tapped sharply to dislodge the cells, medium was added, and the cells were washed and resuspended in Ca²⁺- and Mg²⁺-free HBSS to the desired cell concentration. Cell viability was determined by trypsin blue exclusion, and only single-cell suspensions of greater than 90% viability were used.

Tumorigenicity and Subcutaneous Growth. Aliquots of 2 × 10⁵ cells in 0.2 ml of HBSS were injected into the subcutis of syngeneic C3H/HeN and nude mice in the anterior thoracic region (n = 5). Tumor growth was monitored twice weekly, and the length and width of each tumor were recorded.

Experimental Lung Metastasis. K-1735 cells (or hybrids) at different concentrations (1–2 × 10⁶ viable cells/0.2 ml of HBSS/mouse) were injected into the lateral tail vein of unanesthetized syngeneic C3H/HeN or athymic nude mice. The mice were monitored daily and killed when moribund or 45 days after tumor injection. The lungs were fixed in Bouin’s solution, and the number of tumor nodules was determined under a dissecting microscope.

Establishment of Cell Lines from Lung Metastases. Solitary tumor nodules were teased from the lung parenchyma under aseptic conditions. The tumor nodules were minced in a small culture well containing medium with 10% fetal bovine serum. Several days later, adherent cells were harvested with 0.25% trypsin:0.02% EDTA and transferred into larger vessels. To characterize the nature of the recovered cells, they were grown in media containing G418 (500 units/ml) and/or hygromycin B (250 units/ml) for 2 weeks. The selection medium was changed every 3 days.

Statistical Analysis. The in vivo data were analyzed using the Mann-Whitney test and the Kruskal-Wallis test.

RESULTS

Biological and Metastatic Properties of Transfected K-1735 Cells

In the first set of experiments, we determined whether the transfection of K-1735 clones with plasmids containing genes that confer resistance to G418 or hygromycin B changed their biological and metastatic behavior. The differences in metastatic potential were clear. Clones 3, 19, and 23 produced a median number of 2 lung nodules, whereas clones C-4, X-21, and M-2 produced a median number of 200, 199, and 270 lung nodules, respectively. The introduction of drug resistance genes into the nonmetastatic (C-3, C-19, C-23) and metastatic (C-4, M-2, X-21) clones did not alter their metastatic potential in syngeneic mice (Table 1) or their cell cycle time and morphology in vitro (data not shown).

Metastatic Potential of Somatic Cell Hybrids

Autofusion. To rule out the possibility that cell fusion per se could alter metastatic potential, we examined the production of experimental metastasis by somatic cell hybrids of identical clones transfected with the drug-resistant genes. In the first set of these experiments, single cells of transfected clones (neomycin- or hygromycin-resistant) were admixed (1:1) and injected i.v. The mice were killed on day 45 or when moribund, and the number of lung-tumor colonies was determined. The data shown in Table 2 demonstrate that the mixture of transfected cells produced an incidence of metastasis very similar to that of parental (nontransfected) or transfected cells injected alone. In the next set of studies, the individual clones were fused and selected in medium containing both G418 and hygromycin. Although the hybrid cells were larger than either paren-

<table>
<thead>
<tr>
<th>Table 1 Production of experimental lung metastasis by clones of the K-1735 melanoma with low and high metastatic potential</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low metastatic clones</strong></td>
</tr>
<tr>
<td><strong>No. of lung nodules</strong></td>
</tr>
<tr>
<td><strong>median (range)</strong></td>
</tr>
<tr>
<td>C-3</td>
</tr>
<tr>
<td>C-3N</td>
</tr>
<tr>
<td>C-3H</td>
</tr>
<tr>
<td>C-19</td>
</tr>
<tr>
<td>C-23N</td>
</tr>
<tr>
<td>C-23H</td>
</tr>
</tbody>
</table>
Table 2 Production of experimental lung metastasis by somatic cell hybrids (autofusion) of the K-1775 melanoma

<table>
<thead>
<tr>
<th>Clones</th>
<th>Cell mixture No. of lung nodules median (range)</th>
<th>Somatic cell hybrids No. of lung nodules median (range)</th>
<th>In vitro growth*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonmetastatic*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-3N and C-3H</td>
<td>0 (0–3)</td>
<td>0 (0–2)</td>
<td>NA† NA</td>
</tr>
<tr>
<td>C-19N and C-19H</td>
<td>0 (0–4)</td>
<td>0 (0–12)</td>
<td>NA† NA</td>
</tr>
<tr>
<td>C-23N and C-23H</td>
<td>0</td>
<td>0</td>
<td>NA† NA</td>
</tr>
<tr>
<td>Metastatic*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-4N and C-4H</td>
<td>208 (106–300)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>X-21N and X-21H</td>
<td>90 (80–108)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M-2N and M-2H</td>
<td>110 (108–150)</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Tumor cells were recovered from lung metastases and grown in culture with medium containing G418 and/or hygromycin B.
† Cells (1 × 10⁶) of each clone and 2 × 10⁶ hybrid cells were injected i.v. into 10 syngeneic mice/group. The mice were killed 45 days later.
‡ Cells (5 × 10⁶) of each clone and 1 × 10⁶ hybrid cells were injected i.v. into 10 syngeneic mice/group. The mice were killed when moribund (day 20–37 after injection).

A nonparametric method of data analysis (the Kruskal-Wallis test) was used to test whether the somatic cell hybrids between a specific metastatic clone and three different nonmetastatic clones produced the same numbers of lung nodules. In each case, the test showed that they did not (Table 5). The differences in assay time did not affect the results of the analyses. Subsequent pairwise comparisons using the Mann-Whitney test showed that all three hybrids formed from each metastatic clone were different in terms of colony production (Table 5).

Studies in Athymic Nude Mice

Since host immunity can prevent the establishment of experimental metastases (9), we determined whether the low-metastatic potential of C-23H/C-4N and C-23H/M-2N was due to augmented antigenicity (51, 52). The experiment shown in Table 4 was repeated in immunodeficient nude mice (Table 6). The cell mixtures or hybrids (nonmetastatic/nonmetastatic, metastatic/metastatic) produced metastatic incidences similar to those in syngeneic mice (data not shown). Next, we injected nude mice with combinations of different nonmetastatic and metastatic cell clones, confirming that in this tumor system, metastatic cells do not promote the formation of lung metastases by nonmetastatic cells (49).

In the next set of experiments, we determined the production of experimental lung metastases by somatic cell hybrids between different combinations of nonmetastatic and metastatic clones. Early passage cells of nine different hybrids were injected i.v. into groups of syngeneic mice (Table 4). The fusions of the nonmetastatic C-3 or C-19 clones with the highly metastatic C-4, M-2, or X-21 clones all resulted in hybrids producing a high number of lung metastases. Similar results were obtained with hybrids between the nonmetastatic C-23 with the highly metastatic X-21 cells, but fusion of C-23 with the highly metastatic C-4 or M-2 cells produced a low-metastatic or nonmetastatic hybrid. Histological examination of lungs from these mice did not reveal the presence of micrometastases. The hybrid nature of the cells was demonstrated by their growth in medium containing G418 and hygromycin B (Table 4).
metastatic cells and with somatic cell hybrids between metastatic and nonmetastatic cells. The results were very similar to those obtained in normal syngeneic mice. All the mice inoculated with mixtures of clones developed lung metastases (Table 6). Hybrids C-3H/C-4N, C-3N/X-21H, C-3H/M-2N, and C-19N/X-21H were also highly metastatic in nude mice. Hybrids C-19H/C-4N, C-19H/M-2N, and C-23N/X-21H produced an intermediate number of metastases. Since hybrids C-23H/C-4N and C-23H/M-2N produced very few lung metastases in nude mice (Table 6), just as they did in syngeneic mice, their low metastatic capacity was not due to immune rejection. A comparison of the incidence of metastasis in nude and syngeneic mice revealed that some hybrids, e.g., C-3N/X-21H, produced fewer metastases in nude mice. Whether this is due to increased susceptibility to lysis mediated by natural killer cells (53) is now under investigation.

Tumorigenicity of the Somatic Cell Hybrids

Since the proliferative capacity of tumor cells is mandatory for formation of metastasis (2, 54), we determined the tumorigenic capacity of the various hybrids by injecting them into the subcutis of nude mice. In control experiments, cells from low metastatic clones (C-3, C-19, C-23) were not metastatic in either syngeneic or nude mice. The predominance of the metastatic phenotype was evident in 7 of the 9 hybrids. Two hybrids (both produced with fusion of metastatic clones with nonmetastatic C-23) were not metastatic in either syngeneic or nude mice. These two hybrids, however, also exhibited reduced tumorigenicity.

Most reports on fusion between metastatic and nonmetastatic cells have used a limited number of parental clones, and some only one metastatic and one nonmetastatic partner (24-26, 56). Since in many of these studies the fusion occurred across strain and even species lines, it is not surprising that the results were not identical to those recorded for the hybrids prior to their injection into mice (Table 7), suggesting that for the duration of this study, the K-1735 hybrids were karyotypically stable.

**DISCUSSION**

We report that somatic cell hybrids produced by the fusion of three low-metastatic or nonmetastatic clones with three highly metastatic clones of the K-1735 mouse melanoma exhibited heterogeneous metastatic properties in both syngeneic C3H/HeN and nude mice. The predominance of the metastatic phenotype was evident in 7 of the 9 hybrids. Two hybrids (both produced with fusion of metastatic clones with nonmetastatic C-23) were not metastatic in either syngeneic or nude mice. These two hybrids, however, also exhibited reduced tumorigenicity.

Most reports on fusion between metastatic and nonmetastatic cells have used a limited number of parental clones, and some only one metastatic and one nonmetastatic partner (24-26, 56). Since in many of these studies the fusion occurred across strain and even species lines, it is not surprising that the results were not identical to those recorded for the hybrids prior to their injection into mice (Table 7), suggesting that for the duration of this study, the K-1735 hybrids were karyotypically stable.

**DISCUSSION**

We report that somatic cell hybrids produced by the fusion of three low-metastatic or nonmetastatic clones with three highly metastatic clones of the K-1735 mouse melanoma exhibited heterogeneous metastatic properties in both syngeneic C3H/HeN and nude mice. The predominance of the metastatic phenotype was evident in 7 of the 9 hybrids. Two hybrids (both produced with fusion of metastatic clones with nonmetastatic C-23) were not metastatic in either syngeneic or nude mice. These two hybrids, however, also exhibited reduced tumorigenicity.

Most reports on fusion between metastatic and nonmetastatic cells have used a limited number of parental clones, and some only one metastatic and one nonmetastatic partner (24-26, 56). Since in many of these studies the fusion occurred across strain and even species lines, it is not surprising that the results were not identical to those recorded for the hybrids prior to their injection into mice (Table 7), suggesting that for the duration of this study, the K-1735 hybrids were karyotypically stable.

**DISCUSSION**

We report that somatic cell hybrids produced by the fusion of three low-metastatic or nonmetastatic clones with three highly metastatic clones of the K-1735 mouse melanoma exhibited heterogeneous metastatic properties in both syngeneic C3H/HeN and nude mice. The predominance of the metastatic phenotype was evident in 7 of the 9 hybrids. Two hybrids (both produced with fusion of metastatic clones with nonmetastatic C-23) were not metastatic in either syngeneic or nude mice. These two hybrids, however, also exhibited reduced tumorigenicity.

Most reports on fusion between metastatic and nonmetastatic cells have used a limited number of parental clones, and some only one metastatic and one nonmetastatic partner (24-26, 56). Since in many of these studies the fusion occurred across strain and even species lines, it is not surprising that the results were not identical to those recorded for the hybrids prior to their injection into mice (Table 7), suggesting that for the duration of this study, the K-1735 hybrids were karyotypically stable.

**DISCUSSION**

We report that somatic cell hybrids produced by the fusion of three low-metastatic or nonmetastatic clones with three highly metastatic clones of the K-1735 mouse melanoma exhibited heterogeneous metastatic properties in both syngeneic C3H/HeN and nude mice. The predominance of the metastatic phenotype was evident in 7 of the 9 hybrids. Two hybrids (both produced with fusion of metastatic clones with nonmetastatic C-23) were not metastatic in either syngeneic or nude mice. These two hybrids, however, also exhibited reduced tumorigenicity.

Most reports on fusion between metastatic and nonmetastatic cells have used a limited number of parental clones, and some only one metastatic and one nonmetastatic partner (24-26, 56). Since in many of these studies the fusion occurred across strain and even species lines, it is not surprising that the results were not identical to those recorded for the hybrids prior to their injection into mice (Table 7), suggesting that for the duration of this study, the K-1735 hybrids were karyotypically stable.
PREDOMINANCE OF THE METASTATIC PHENOTYPE

Table 6 Production of experimental lung metastasis by somatic cell hybrids of low and high metastatic potential in nude mice

<table>
<thead>
<tr>
<th>Clones</th>
<th>Pulmonary metastasis (median range)</th>
<th>Day of autopsy</th>
<th>Somatic cell hybrids</th>
<th>Pulmonary metastasis (median range)</th>
<th>Day of autopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-3H and C-4N</td>
<td>150 (50–160)</td>
<td>24</td>
<td></td>
<td>170 (47–200)</td>
<td>34</td>
</tr>
<tr>
<td>C-3N and X-21H</td>
<td>&gt;200 (all &gt;200)</td>
<td>21</td>
<td>70 (54–103)</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>C-3H and M-2N</td>
<td>&gt;200 (180–200)</td>
<td>24</td>
<td>140 (70–200)</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>C-19H and C-4N</td>
<td>100 (50–190)</td>
<td>24</td>
<td>36 (19–65)</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>C-19N and X-21H</td>
<td>&gt;200 (all &gt;200)</td>
<td>24</td>
<td>&gt;200 (all &gt;200)</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>C-19H and M-2N</td>
<td>&gt;200 (151–200)</td>
<td>24</td>
<td>34 (17–65)</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>C-23H and C-4N</td>
<td>&gt;200 (190–200)</td>
<td>24</td>
<td>0</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>C-23N and X-21H</td>
<td>&gt;200 (all &gt;200)</td>
<td>24</td>
<td>28 (4–31)</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>C-23H and M-2N</td>
<td>&gt;200 (all &gt;200)</td>
<td>24</td>
<td>0</td>
<td>45</td>
<td></td>
</tr>
</tbody>
</table>

* Cells (1 × 10⁶) of each clone were injected i.v. into syngeneic mice (10 mice/group). The mice were killed when moribund (day 21–41).

† Hybrid cells (2 × 10⁶) were injected i.v. into syngeneic mice (10 mice/group). The mice were killed when moribund (day 30–45).

Table 7 Chromosome number of K-1735 hybrids

<table>
<thead>
<tr>
<th>Clones</th>
<th>Fusion partners</th>
<th>Expected hybrids</th>
<th>Hybrids (median range)</th>
<th>Lung metastasis (median range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-3H, C-4N</td>
<td>39, 42</td>
<td>81</td>
<td>86 (74–86)</td>
<td>86 (77–88)</td>
</tr>
<tr>
<td>C-3N, X-21H</td>
<td>38, 41</td>
<td>79</td>
<td>82 (52–147)</td>
<td>ND*</td>
</tr>
<tr>
<td>C-3H, M-2N</td>
<td>39, 41</td>
<td>80</td>
<td>71 (58–142)</td>
<td>77 (64–157)</td>
</tr>
<tr>
<td>C-19H, C-4N</td>
<td>37, 42</td>
<td>79</td>
<td>84 (68–92)</td>
<td>ND</td>
</tr>
<tr>
<td>C-19N, X-21H</td>
<td>38, 41</td>
<td>79</td>
<td>94 (77–127)</td>
<td>ND</td>
</tr>
<tr>
<td>C-19H, M-2N</td>
<td>37, 41</td>
<td>78</td>
<td>70 (66–92)</td>
<td>ND</td>
</tr>
<tr>
<td>C-23H, C-4N</td>
<td>44, 42</td>
<td>86</td>
<td>84 (70–119)</td>
<td>ND</td>
</tr>
<tr>
<td>C-23N, X-21H</td>
<td>44, 41</td>
<td>85</td>
<td>78 (65–80)</td>
<td>75 (59–90)</td>
</tr>
<tr>
<td>C-23H, M-2N</td>
<td>44, 41</td>
<td>85</td>
<td>79 (63–102)</td>
<td>ND</td>
</tr>
</tbody>
</table>

* ND, not done.

The transfection of a single dominant oncogene has been shown to convert a tumor cell with low metastatic potential to a highly metastatic cell (60, 61). Transfection of DNA from metastatic cells of human tumors enhanced metastatic properties of different mouse tumor cells (62, 63). Molecular biology approaches have also shown significant differences between benign and malignant cells. Differential screening of complementary DNA libraries and subtractive hybridization have identified differentially expressed mRNAs in metastatic versus nonmetastatic cells. These include fibronectin (64), transin (65), murine calcium-binding protein (66), NADH dehydrogenase subunit 5 (67), human acidic ribosomal phosphoprotein P2 (68), and human elongation factor 1 subunit (69). At the same time, similar techniques have identified genes that are specifically expressed in nonmetastatic cells (70–73).

A complex process such as metastasis cannot be regulated by only one gene, but rather by the activation or deactivation of multiple specific genes (1, 8, 9, 23). The progression of cancers from the benign to the malignant state may occur by the activation of a dominantly acting regulatory gene (64–69), loss...
or inactivation of a recessive suppressor gene (72, 73), or, more likely, a combination of both. Examples of gene activation are those that code for proteins important for one or another step of metastasis such as motility, invasion, cell aggregation, and growth in specific organ environment (11). Specific examples are genes that code for proteases, receptors for extracellular matrices, motility factors, growth factors and their receptors, and angiogenesis factors and their receptors (2, 6, 7, 11, 12, 27).

In conclusion, studies using somatic cell hybrids between three low-metastatic or nonmetastatic and three highly metastatic clones of the K-1735 melanoma demonstrate the predominance of the metastatic phenotype. Genetic changes could vary among different metastatic and different nonmetastatic clones. X-21 cells are an example of highly metastatic cells in which dominant genes predominate, whereas C-23 cells are an example of nonmetastatic cells that can provide recessive (suppressor?) genes. In any event, the data suggest that, at least in this system, the progression of tumor cells from the benign to the malignant state is due to the activation of dominant genes.

ACKNOWLEDGMENTS

We thank Lola Lopez for her assistance in the preparation of the manuscript.

REFERENCES

Predominance of the Metastatic Phenotype in Somatic Cell Hybrids of the K-1735 Murine Melanoma

Alexander H. Staroselsky, Sen Pathak, Yuti Chernajovsky, et al.


Updated version Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/51/23_Part_1/6292

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/51/23_Part_1/6292. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.