Enhanced Metastatic Potential of Murine Fibrosarcomas Treated in Vitro with Ultraviolet Radiation

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ABSTRACT

The purpose of this study was to determine whether repeated treatment of tumor cells in vitro with mutagenic doses of ultraviolet (UV) radiation could influence the metastatic behavior of these cells in vivo. Three cloned lines of UV-2237, a fibrosarcoma induced in a C3H mouse by chronic irradiation with UV, and SF-19, a spontaneous C3H fibrosarcoma, were grown in culture. These cell lines varied from low to high metastatic potential in an in vivo lung colony assay. Cell lines treated in vitro with UV radiation produced more experimental metastases than the counterpart unirradiated cultures. We conclude that, in all four tumor lines, exposure of tumorigenic cells to mutagenic doses of UV radiation can alter their biological behavior and that this may contribute to the progression of tumors from low to high metastatic capability.

INTRODUCTION

Clinical observations of cancer have suggested that nonmetastatic benign tumors may progress with time to become metastatic (16, 17). The process of tumor progression is thought to consist of a step-by-step development of the tumor through qualitatively different stages that evolve due to the emergence of new cell variants that have a selective advantage. Nowell (24, 25) has postulated that these variants arise because of genetic variability within the developing tumor, which then allows for selection of more aggressive sublines. This genetic lability of neoplastic cells, in some circumstances, may reflect the continued presence of the carcinogen or oncogen (7, 25).

Even though several investigations (15, 30, 31) have shown that repeated exposure of animals to chemical carcinogens or UV radiation increases the number, size, and invasiveness of the primary tumors in the autochthonous host, it is difficult to determine if the repeated exposure of the carcinogen affected the host or the developing tumor or both.

The purpose of this study was to determine whether repeated treatment of tumor cells in vitro with mutagenic doses of UV radiation could influence the subsequent malignant behavior of these cells in vivo as determined by the formation of experimental lung metastases.

MATERIALS AND METHODS

Animals. Specific-pathogen-free female C3H/HeN mammary tumor virus-negative (C3H−) mice and athymic nude NIH-Swiss (nu/nu) mice were obtained from the Animal Production Area of the Frederick Cancer Research Center. The mice were conventionally housed, 5/cage, and fed water and Charles River Autoclavable Agway Feed (rodent chow) ad libitum. Within a single experiment, all mice were age matched and were 8 to 12 weeks old at the beginning of the experiment.

Thymectomy. Four- to 5-week-old mice were given i.p. injections of 0.5 ml atropine sulfate and anesthetized with sodium nembutal. The thymus was removed with gentle vacuum suction through a mediastinal incision. The incision was closed with metal wound clips.

Cell Cultures. The UV-2237 is a fibrosarcoma that was induced in a female C3H− mouse by chronic UV irradiation. The tumor was established in culture from the first in vivo passage in immunosuppressed syngeneic mice. Cloned cell lines were obtained as described previously (22). SF-19 is a fibrosarcoma that arose spontaneously in a C3H− mouse and was established in tissue culture. All cell lines were grown as monolayers on tissue culture plastic in Eagle's minimal essential medium (Flow Laboratories, Rockville, Md.) supplemented with 5% fetal bovine serum, glutamine, nonessential amino acids, and vitamins (Grand Island Biological Co., Grand Island, N. Y.). The cultures were maintained at 37° in a humidified incubator in an atmosphere containing 5% CO2. All cell lines were examined for and found free of Mycoplasma and the following murine viruses: reovirus type 3; pneumonia virus of mice; K-virus; Theiler's virus; Sendai virus; minute virus of mice; mouse adenovirus; mouse hepatitis virus; lymphocytic choriomeningitis virus; ectromelia virus; and lactate dehydrogenase virus (M. A. Bioproducts, Walkersville, Md.).

Cell lines that were irradiated with UV were transferred to tissue culture flasks following the final UV irradiation. After 2 weeks in culture, the cell lines were frozen at −70° in medium containing 30% fetal bovine serum and 10% dimethyl sulfoxide. In order to ensure reproducibility of in vivo and in vitro assays, the cultures were tested within 2 weeks after recovery from frozen stocks. Thus, the cells were injected approximately 3 weeks after they were exposed to UV radiation.

For in vivo studies, the tumor cells were harvested from cultures in their exponential growth phase by overlaying the
cells with a thin layer of 0.25% trypsin-0.02% EDTA for 2 min. The cells were then washed and resuspended in Hanks' balanced salt solution. Tumor cell viability was assessed before injection by trypan blue exclusion, and only suspensions with single cells of >95% viability were used in the studies.

Clones 15 and 38 (low metastatic potential) and clone 25 (high metastatic potential) of the UV-2237 fibrosarcoma were used. They were classified on the basis of their behavior in 3 in vivo tests (22). In the first test, mice were given i.v. injections of tumor cells, and the number of lung colonies was counted 3 weeks later (quantitative lung colony assay). In the second test, mice were given s.c. injections of tumor cells, and the number and site of spontaneous metastases were determined at necropsy. In the third test, mice were given i.v. injections of tumor cells, and the number and site of extrapulmonary tumors were determined. The results of the 3 tests were similar: clones judged to be of high or low metastatic potential in one test usually exhibited the same behavior in the other 2 tests. The clones were ranked in order of increasing metastatic potential. By adding the rank of each clone in each of the 3 tests, we were able to measure how metastatic each clone was in relation to the others.

**Experimental Pulmonary Metastasis.** Unanesthetized mice were inoculated i.v. with 1 x 10^5 tumor cells in 0.2 ml Hanks' balanced salt solution in the lateral tail vein. All mice were killed 21 days after tumor cell injection, and their lungs were removed, rinsed in water, and fixed overnight in Bouin's solution (22). The number of tumor colonies was determined by counting parietal metastases under a dissecting microscope. The majority of such experimental metastases in mice are found on the surface of the lungs (12). The lung colonies were counted in a blind fashion.

**Spontaneous Metastasis and Tumorigenicity.** Syngeneic normal and immunosuppressed mice were given s.c. injections of 1 x 10^6 tumor cells of UV-2237, clone 25, and 1 x 10^6 tumor cells of UV-2237 clone 15. All s.c. tumors were measured weekly, and the mice were inspected 3 times per week for healthy appearance. Eleven weeks following s.c. injection, the mice were necropsied. The number of s.c. tumors was recorded, and all tissues with suspected metastases were fixed in Bouin's solution for histological examination.

**In Vitro Growth Rate Determinations.** Cell lines were plated at a density of 10^4 cells per 60-mm plastic Petri dish (Falcon Plastics, Oxnard, Calif.) in complete minimal essential medium. Triplicate cultures were trypsinized, and the number of cells per dish was determined every 24 hr for 4 days using a Coulter Counter (Coulter Electronics, Hialeah, Fla.).

**Detection of Ouabain-resistant Mutants.** Cell lines were plated in triplicate at a density of 10^6 cells per 100-mm plastic dish in complete minimal essential medium containing 3 mM ouabain (Sigma Chemical Co., St. Louis, Mo.). Eleven to 14 days later, the medium was decanted from the plates, the cells were fixed with methylene blue in 50% methanol, and the colonies were counted.

**UV Irradiation.** Twenty-four hr after plating 1 x 10^3 tumor cells in 60-mm dishes, the medium was removed, and the monolayers were washed in phosphate-buffered saline and exposed to UV radiation from one Westinghouse FS40 sunlamp which delivered an average incident dose rate of 0.43 J/sq m/sec. The dose killed 40% of the plated cells. This was determined by plating 200 cells per dish and counting the number of colonies surviving 14 days after UV irradiation. Control dishes were washed with phosphate-buffered saline but were not exposed to UV radiation. Survival was determined by the ratio of the number of colonies in the treated dishes to the number of colonies in the untreated dishes.

UV radiation exposures were given at 3- to 5-day intervals for a total of 5 treatments. Irradiated and unirradiated cultures were tested subsequently in the quantitative lung colony assay (22).

**Statistical Analyses.** Differences in the number of lung colonies between test groups were analyzed with the Mann-Whitney U test (2-tailed).

**RESULTS**

**Number of Ouabain-resistant Colonies following UV Irradiation In Vitro.** Cell lines were exposed to UV radiation in vitro every 3 to 5 days for a total of 5 treatments. The results shown in Table 1 confirm that UV irradiation was mutagenic to the fibrosarcoma cells. We reached this conclusion by determining the number of ouabain-resistant cells in the control cell lines and in those that received multiple treatments with UV radiation. The number of ouabain-resistant colonies increased with exposure to UV radiation in vitro in all 3 UV-2237 clones and the spontaneous fibrosarcoma.

**Ability of Tumor Cells Exposed to UV Radiation In Vitro to Form Experimental Lung Metastases.** Next, we assessed the in vivo behavior of tumor cells exposed in vitro to UV radiation. Two cell lines were tested for experimental lung metastasis in 3 types of recipients: syngeneic normal mice; syngeneic ATX mice; and 3-week-old allogeneic nu/nu mice. Successful experimental lung metastasis occurs in 3-week-old, but not 6-week-old, nu/nu mice. This has been correlated with low levels of natural killer cell activity (18). Following the UV irradiations in vitro, both clones 15 and 25 showed an increased ability to form experimental lung metastases in the ATX mice (Table 2, Experiment 1). Similar results were obtained when 3-week-old nu/nu mice were given injections of the same cells. There was a small increase in the number of lung colonies in normal mice receiving injections of the tumor cells that had been exposed to UV radiation; however, this increase was not statistically significant.

To assess the reproducibility of this finding, the experiment was repeated, but a spontaneous fibrosarcoma (SF-19) and a third UV-2237 clone (clone 38) also were included. The results of the second experiment are shown in Table 2, Experiment 2. The results were similar to those of the first experiment. Repeated exposure of clones 15 and 25 to UV radiation in vitro increased their ability to form lung metastases in ATX and nu/nu mice. However, this time, the number of experimental pulmonary tumor colonies found in normal recipients was significantly greater for both clones 15 and 25 following UV irradiation than that produced by the unirradiated controls. Clone 38 produced experimental lung metastases in ATX and nu/nu mice but not in normal mice. The increase in the number of

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3 The abbreviation used is: ATX, immunosuppressed by adult thymectomy and 450 rads of whole-body X-irradiation 10 days after surgery (3 weeks before tumor cell injection).
lungs nodules formed by UV-irradiated cells was significantly greater than that formed by unirradiated cells in ATX and nu/nu recipients. In addition, the nonmetastatic fibrosarcoma, SF-19, showed an increase in the number of experimental lung metastases following in vitro UV irradiation; these increases were statistically significant with all 3 types of recipients. No increase in metastasis formation was obtained with tumor cells treated only once with UV radiation (data not shown).

Spontaneous Metastasis and Tumorigenicity of Tumor Cells Exposed to UV Radiation in Vitro. In the previous experiments, tumor lung-colonizing ability was assayed. To determine if enhanced lung colonization actually reflected enhanced metastatic potential, we next assessed spontaneous metastasis. Two cell lines were tested for their ability to metastasize spontaneously from a s.c. site in syngeneic normal mice and syngeneic mice immunesuppressed by ATX. Following UV irradiation in vitro, both clones 15 and 25 showed an increased ability to form spontaneous metastases in normal and ATX recipients.

Table 1

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Clone</th>
<th>No UV irradiation</th>
<th>5 UV radiation exposures</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV-2237</td>
<td>15</td>
<td>0</td>
<td>11.5 ± 0.41</td>
</tr>
<tr>
<td>UV-2237</td>
<td>25</td>
<td>0.5 ± 0.5</td>
<td>34.5 ± 1.50</td>
</tr>
<tr>
<td>UV-2237</td>
<td>38</td>
<td>0</td>
<td>33.8 ± 2.29</td>
</tr>
<tr>
<td>SF-19</td>
<td>0</td>
<td>15.0 ± 1.53</td>
<td></td>
</tr>
</tbody>
</table>

a Tumor cells (10⁶) were plated in triplicate in 3 mM ouabain. Eleven to 14 days later, the cells were fixed and stained.
b The dose of UV radiation was calculated to kill 40% of the plated cells.
c Mean number of ouabain-resistant mutants ± S.E.

Table 2

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Recipients</th>
<th>Cell line</th>
<th>Clone</th>
<th>No UV irradiation</th>
<th>5 UV radiation exposures</th>
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<tr>
<td>1</td>
<td>Normal</td>
<td>UV-2237</td>
<td>15</td>
<td>0 (0–0)</td>
<td>0 (0–35) NS</td>
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<tr>
<td>ATX</td>
<td>UV-2237</td>
<td>0 (0–68)</td>
<td>219 (21–275)</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>nu/nu</td>
<td>UV-2237</td>
<td>11 (14–80)</td>
<td>130 (45–263)</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>UV-2237</td>
<td>7 (0–50)</td>
<td>14.5 (0–30)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>ATX</td>
<td>UV-2237</td>
<td>115 (4–130)</td>
<td>165 (12–210)</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>nu/nu</td>
<td>UV-2237</td>
<td>49 (20–146)</td>
<td>178 (63–263)</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Normal</td>
<td>UV-2237</td>
<td>15</td>
<td>0 (0–0)</td>
<td>0 (0–12) 0.01</td>
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<tr>
<td>ATX</td>
<td>UV-2237</td>
<td>80 (19–99)</td>
<td>134 (94–167)</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>nu/nu</td>
<td>UV-2237</td>
<td>12.5 (5–96)</td>
<td>177 (65–267)</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
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<td>2 (0–7)</td>
<td>133 (44–230)</td>
<td>0.01</td>
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<tr>
<td>ATX</td>
<td>UV-2237</td>
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<td>300 (300–301)</td>
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<tr>
<td>nu/nu</td>
<td>UV-2237</td>
<td>56 (23–135)</td>
<td>296 (185–315)</td>
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</tr>
<tr>
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<td>UV-2237</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>ATX</td>
<td>UV-2237</td>
<td>7 (0–20)</td>
<td>48 (34–126)</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>nu/nu</td>
<td>UV-2237</td>
<td>7.5 (0–35)</td>
<td>211 (125–283)</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>SF-19</td>
<td>UV-2237</td>
<td>38</td>
<td>0 (0–0)</td>
<td>96 (45–206) 0.01</td>
</tr>
<tr>
<td>ATX</td>
<td>UV-2237</td>
<td>0 (0–0)</td>
<td>209 (20–232)</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>nu/nu</td>
<td>SF-19</td>
<td>0 (0–2)</td>
<td>66 (26–162)</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

a Median number of pulmonary tumor colonies, 10 mice/group.
b Probability of no difference from unirradiated control cells (Mann-Whitney U test, 2-tailed).
c Numbers in parentheses, range.
d NS, not significant.

Table 3

<table>
<thead>
<tr>
<th>Recipients</th>
<th>Cell lines</th>
<th>Clone</th>
<th>No. of UV exposures</th>
<th>Lung</th>
<th>Heart</th>
<th>Kidney</th>
<th>Other</th>
<th>p&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Tumor incidence&lt;sup&gt;b&lt;/sup&gt;</th>
<th>p&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>UV-2237</td>
<td>15</td>
<td>1/10</td>
<td>0&lt;sup&gt;b&lt;/sup&gt; (0–1)</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>3/10</td>
<td>10/10</td>
<td></td>
</tr>
<tr>
<td>ATX</td>
<td>UV-2237</td>
<td>5</td>
<td>9/10</td>
<td>5&lt;sup&gt;b&lt;/sup&gt; (0–13)</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>&lt;0.05</td>
<td>10/10</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Normal</td>
<td>UV-2237</td>
<td>25</td>
<td>9/10</td>
<td>75 (0–200)</td>
<td>0/10</td>
<td>4/10</td>
<td>8/10</td>
<td>&lt;0.05</td>
<td>10/10</td>
<td>NS</td>
</tr>
<tr>
<td>ATX</td>
<td>UV-2237</td>
<td>5</td>
<td>8/8</td>
<td>2 (0–4)</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>10/10</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Normal</td>
<td>UV-2237</td>
<td>25</td>
<td>8/8</td>
<td>58 (32–150)</td>
<td>2/8</td>
<td>2/8</td>
<td>7/8</td>
<td>&lt;0.01</td>
<td>8/8</td>
<td>NS</td>
</tr>
</tbody>
</table>

<sup>a</sup> At time of death or 11 weeks after injection. Mice were given injections of 1 × 10⁶ cells of line UV-2237 (15) and 1 × 10⁵ cells of line UV-2237 (25) s.c. on the flank.
<sup>b</sup> Probability of no difference in numbers of pulmonary tumor colonies from unirradiated control cells (Mann-Whitney U test, 2-tailed).
<sup>c</sup> Tumor incidence 11 weeks after injection of tumor cells s.c.
<sup>d</sup> Median number of pulmonary tumor colonies. Values represent only those animals bearing a transplanted tumor as indicated under tumor incidence.
<sup>e</sup> Numbers in parentheses, range.
<sup>f</sup> NS, not significant.
mice (Table 3). Not only were there significant increases in the number of pulmonary metastases in mice that had received injections s.c. of UV-irradiated tumor cells, but the number of extrapulmonary metastases was also significantly increased. Extrapulmonary metastases were found in the ovary, adrenal gland, mesentary, lymph nodes, liver, diaphragm, pleural cavity, and s.c. sites.

Not only did in vitro irradiation of tumor cells affect metastatic potential, but tumorigenic potential was altered as well. Decreased tumorigenic potential was observed in mice given injections s.c. of clone 25. However, an increased tumorigenic potential was seen in mice given injections s.c. of clone 15. The change in tumorigenic potential was significant only in syngeneic normal mice and not in mice immunosuppressed by ATX (Table 3).

**Growth Rate of Tumor Cells Exposed to UV Radiation In Vitro.** We then tested the effect of repeated exposures to UV radiation on the growth of tumor cells injected s.c. Because many UV radiation-induced tumors are immunologically rejected upon transplantation to normal syngeneic mice, the growth rate of the tumor cell lines was tested in nude mice to avoid the complication of antigenicity. Nude mice, 5 per group, received injections s.c. of $5 \times 10^5$ tumor cells from each cell line. The s.c. growth of the SF-19 cells was enhanced after in vitro exposure of the cells to UV irradiation (Chart 1A). There were no statistically significant differences in the rate of s.c. growth of the radiated and unirradiated cells from UV-2237, clones 15 and 38 (Chart 1, B and C). Overall, repeated exposure to UV irradiation did not significantly enhance the growth rate of UV-2237, clone 25 (Chart 1D).

We also measured the doubling time of these tumors in vitro. The doubling times in vitro of both the unirradiated and the irradiated tumor cell lines were identical, as determined by Student's $t$ test (Table 4).

**DISCUSSION**

These studies were designed to determine whether repeated mutagenic treatment of tumor cells in vitro with UV radiation could influence their malignant behavior in vivo. Our results suggest that UV irradiation can, indeed, alter the malignant behavior of tumor cells.

An increase in the number of experimental lung metastases after repeated exposure to UV radiation was observed consistently with all 4 tumor cell lines tested. These lines were tested in 3 different recipients: normal; ATX; and 3-week-old nude mice. Previous studies (1, 2, 8-10, 13, 14, 20, 28) showed that the role of the immune system in experimental cancer metastasis varies for different tumors and that tumor immunogenicity is an important factor in the relationship between host immunity and tumor dissemination. UV radiation-induced tumors are highly antigenic and usually are immunologically rejected in normal recipients (21). Had we tested for metastatic potential in intact normal animals only, we would have failed to detect the effects of in vitro radiation on metastatic potential in vivo, at least for the 3 clones of UV-2237.

Not only did repeated exposure to UV irradiation increase metastatic potential, but it also affected the tumorigenic potential as well. With the weakly antigenic clone 25, there was a decrease in tumor cell growth s.c. as demonstrated by the ability of normal syngeneic recipients to reject the UV-irradiated tumor cells. These results parallel those of Boon and Kellerman (3), Boon and Pel (4), and Pel et al. (26), showing that decreased tumorigenic potential was observed in clones derived from tumor cells mutagenized by N-methyl-N'-nitro-N-nitroso-guanidine. Furthermore, our results extend those of Boon and show that mutagenized tumor cells, which are usually rejected by normal syngeneic mice, clone 15, have acquired an increased tumorigenic potential in the same normal recipients. Thus, mutagenesis of tumor cells not only can lead to a decrease in malignancy, but it can lead to an increase as well. The increased metastatic potential in normal intact hosts would then be explained by the fact that uncloned mutagenized tumor cells contain a mixture of cells with varying tumorigenic poten-
tials, the injection of which would lead to the selective outgrowth of the more aggressive population.

The experimental lung colony assay used to examine the metastatic potential of tumor cells following UV irradiation bypasses the need for initial detachment of tumor cells from the primary tumor and invasion into a blood vessel. Elimination of these initial steps may have introduced circumstances in which noninvasive tumor cells would form metastases when injected i.v. but would be unable to metastasize spontaneously when implanted s.c. This question has been addressed by comparing the metastatic behavior of the UV-2237 clones after i.v. or s.c. inoculation. In most cases, clones that were judged to be of low metastatic potential by one test were also low by the other test (22). Furthermore, the results of the spontaneous metastasis assay following UV irradiation of tumor cells in vitro support our conclusion that the lung colonization assay is a reasonable approximation of cellular metastatic potential. Thus, the formation of experimental metastases by UV-2237 tumor cells injected i.v. is a reasonable approximation of cellular metastatic potential and parallels rather well the results obtained in the more tedious and time-consuming assay of spontaneous metastasis formation.

Because the injection of tumor cells into the circulation bypasses the first steps of metastasis, i.e., invasion and detachment, we measured only the later steps, which include transport and survival in the circulation, arrest in a capillary bed, extravasation into the lung parenchyma, establishment of a microenvironment, and tumor cell multiplication (11). At present, it is not clear which step(s) in the process of metastasis were altered by repeated in vitro treatment with UV radiation. One possibility is that the tumor cells were superficially damaged by UV exposure. However, this does not seem tenable for two reasons. The acquired metastatic potential appears stable after the UV-irradiated tumor cells have been in culture for over 1 month (data not shown), and the results of the assay for spontaneous metastasis also suggests that the tumor cells were not superficially damaged. Another possibility is that the tumor cells exposed to UV radiation increased in size or had an increased tendency to clump. Following UV irradiation, the clones did not differ with regard to cell size (data not shown). Cell size, however, is probably not a phenotypic characteristic that determines whether metastasis will occur; rather, the capacity of tumor cells to deform determines their lung-colonizing ability (19, 27, 29, 32). We also observed no increase in the tendency for homotypic clumping following the exposure of tumor cells to UV radiation in vitro (data not shown). Another explanation is that an increase in growth rate could have been responsible for the increased number of pulmonary metastases seen with tumor cells that received multiple exposures of UV radiation. We tested this possibility by measuring the growth rates in vitro and in vivo. No consistent correlation was detected between growth rate in vitro and in vivo between tumor cells receiving repeated exposures to UV radiation and control cells. Furthermore, there was no simple relationship between metastatic behavior of the cell lines and their s.c. growth rate. In one instance, increased metastasis was associated with enhanced growth in vivo. However, Cifone et al. (6) found no correlation between growth rate in vitro or in vivo and the ability of tumor cells to produce experimental lung metastasis. This suggests that the mechanism(s) which ultimately lead to increased metastatic potential may not be the same for all tumors.

Cains (5) and Loeb et al. (23) have suggested that highly tumorigenic cells are hypermutable or have increased propensity to undergo mutation. If this hypothesis is correct, then increased metastatic potential after treatment with UV radiation, which is a known mutagen, could have been induced by the mutagenic action of UV radiation. Tumor cell cultures that survived 5 consecutive UV irradiations contained a higher number of ouabain-resistant cells than the untreated cultures carried in parallel. Whether the increased number of mutations induced by UV radiation was causally related to increases in the number of experimental lung metastases or just associated with this phenomenon cannot be determined from this study. However, a single UV irradiation was insufficient to enhance the metastatic potential of cells with low metastatic capability (data not shown).

In summary, these studies show that repeated exposure of tumorigenic cells in vitro to UV radiation increased the metastatic potential of the irradiated cells. There was no correlation between increasing metastatic potential and tumor growth rate in vitro. However, in one case, increased metastasis was associated with enhanced growth in vivo. This approach offers an excellent opportunity to study the changes in tumorigenic cells associated with progression from a benign to a malignant phenotype.

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REFERENCES


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