Exceptional Lethality for Nude Mice of Cells Derived from a Primary Human Melanoma

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ABSTRACT

BRO human melanoma cells, obtained from a biopsy of a highly aggressive and malignant primary tumor, were grown as xenografts in nude mice and in cell culture. These cells were exceptionally tumorigenic and malignant for nude mice. NIH-II nude mice survived 11.0 ± 0.4 (S.E.) and 14.1 ± 0.4 days after i.p. inoculation of 10^5 or 10^6 BRO cells, respectively, and lethal tumors developed in all mice inoculated i.p. with only 10^3 cells. The doubling time (2.3 days) of the volume of tumors formed in nude mice inoculated i.p. with only 10^3 cells. The doubling time (2.3 days) of the volume of tumors formed after s.c. inoculation was comparable to the doubling time of these cells in culture. After i.p. or s.c. inoculation, BRO cells metastasized to the diaphragm and lungs, causing respiratory failure in most of the host mice. The original tumor and the cell line derived from it had undifferentiated structures with prominent nuclei and very large nucleoli. Karyotype abnormalities included a gigantic A group chromosome, a large D group chromosome, and an unusual double centromere chromosome not found typically in human melanoma cells. Due to the short and reproducible survival times of nude mice inoculated i.p. with BRO cells, this model system may be useful for rapidly determining the effects of experimental treatment on the survival of hosts bearing human tumor cells.

INTRODUCTION

In the past few years, many human tumors have been implanted successfully in nude athymic mice. These xenografts retain important features of the original tumor and are useful models for the study of human cancer (4, 16). Human melanoma xenografts may be of particular interest because tumor xenografts and the parent tumor respond similarly to chemotherapy (8) and because most melanomas are highly refractory to known therapies. We report here on melanoma cells derived from an untreated primary tumor of a patient who died of highly aggressive and malignant melanoma. These cells are highly tumorigenic, metastatic, and rapidly lethal for nude mice.

MATERIALS AND METHODS

Tumor. The BRO melanoma is a cell line derived from a primary melanoma which was remarkable for its malignancy and aggressiveness. The patient from whom the specimen was obtained was a 34-year-old white male who had a growth on his left shoulder which had developed rapidly into a large fungating and ulcerating mass. After wide radical excision, the lesion was diagnosed as an invasive malignant melanoma. Microscopic examination showed no evidence of tumor in the surgical margins or of metastases in the adjacent lymph nodes. However, 2 new lesions appeared in the same region 7 weeks later. In spite of further surgery, treatment with 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide and Corynebacterium parvum, and hyperthermia, the tumor metastasized and grew rapidly in both lungs and in other organs, and the patient expired from the disease less than 6 months after removal of the primary tumor. The primary tumor was propagated in nude mice after inoculating s.c. a mince of the biopsy specimen according to described procedures (6).

Inoculation of Cultured Cells into Nude Mice, NIH-I (Swiss), NIH-II, and NIH-III (beige) nude mice were bred and maintained in our laboratory in pathogen-free conditions (5). Adult mice of the same sex and approximate age (3 to 5 months) were used in each experiment. BRO cells in a volume of 0.5 ml were inoculated with a 1/4-inch 25-gauge needle i.p. in the midline of the abdomen or s.c. in the upper midback. The skin perforations were closed with a thermocautery (V. Mueller, Chicago, IL) to prevent leakage. Aliquots of the cell suspensions used for these inoculations were replated routinely in vitro to check for sterility and reproductive viability of the cells.

RESULTS

Histology. The histological features of the malignant melanoma from both the patient (Fig. 1) and from the transplanted melanoma in the nude mouse (Fig. 2) are similar. The neoplastic cells have moderately large rounded vesicular nuclei and prominent, often single, nucleoli. The neoplastic cells have abundant pale eosinophilic cytoplasm and are arranged in groupings but without evidence of intercellular cohesion. The cells contain no melanin but have a positive dopamine reaction. These histological findings are characteristic of an amelanotic malignant melanoma and are retained in tumors of nude mice inoculated with BRO cells passaged for at least 64 generations.

Morphology. In culture (Fig. 3), the triangular or elongated ovoidal BRO cells exhibit the clear nuclei and the prominent nucleoli observed in melanomas. Giant cells, multinucleated or with a very large, abnormal nucleus, are not uncommon. Numerous mitotic figures are present.
Electron Microscopy. Cultured BRO cells have large rounded nuclei with finely dispersed chromatin and large prominent nucleoli (Fig. 4). Within the cytoplasm are moderately numerous organelles, primarily mitochondria, free ribosomes, and occasional profiles of endoplasmic reticulum. The surface of the cells frequently has small filopodia. No melanosomes or premelanosomes are identified.

Karyotype. The modal chromosome number of BRO cells is 62 (range, 42 to 72). The chromosomal abnormalities found most consistently (Fig. 5) are a gigantic A group chromosome (23 of 23 metaphases) and a large abnormal D group chromosome (22 of 23 metaphases). Also, an unusual chromosome with a double centromere is present in about half (12 of 23) of the metaphases examined. This last abnormality has been observed in only one of over 70 other melanomas we have studied.3

BRO Cells in Culture. BRO cells grow readily in culture and have been passaged 90 times to date. Cells plated at density of 8 × 10^6 cells/25-sq cm flask doubled every 1.9 ± 0.2 days during logarithmic growth. The plating efficiency of 10^6 cells plated per 25-sq cm flask was 4.1%.

Tumor Growth after i.p. Inoculation of BRO Cells. BRO cells were highly malignant and rapidly lethal for nude mouse hosts after i.p. inoculation (Table 1). The median survivals of NIH-II mice inoculated with 10^7 or 10^6 cells were 11 and 14 days, respectively. The survivals of NIH-I mice similarly inoculated were comparable. NIH-II mice (14) inoculated with 10^6 cells all developed tumors and had a median survival of 32 days (range, 26 to 51 days). As few as 100 BRO cells inoculated i.p. were sufficient to induce tumors and kill most (12 of 18) NIH-II mice. As shown in Table 2, BRO cells were lethal to host mice much more rapidly than were several other human tumor lines tested, although BRO cells in vitro divided more slowly compared to these other human cells. The tumors used for comparison were selected for their rapid growth after inoculation in nude mice.

A day or so prior to death, most mice bearing BRO cells had evident respiratory distress with labored and rapid breathing and marked inactivity. Gross evidence of tumor in the diaphragm and/or of hemorrhagic lungs was observed in autopsies of 65% of the animals. Multiple tumor foci were also observed in other internal organs, but there was minimal or no apparent distention in 90% of the animals. In the other cases, the bulk of the tumor localized near the intestines or elsewhere in the lower abdomen with concomitant distention. No ascites was ever found.

Tumor Growth after s.c. Inoculation of BRO Cells. The tumor growth after s.c. inoculation of BRO cells was rapid and lethal in nude mice. The median survivals of NIH-I and NIH-II mice inoculated with 10^7 cells were 45 and 55 days, respectively. The rates of tumor growth were similar for mice inoculated with 10^6 cells, although it took longer (20 days) for tumors to appear in all the animals. With 10^5 cells inoculated, about 20% of the NIH-I and NIH-II mice developed tumors compared to 70% of the NIH-III nude mice (Table 3). These latter mice are deficient in natural killer cells (14) as well as T-lymphocytes. No tumor regressions were noted in any mice inoculated with BRO cells.

Autopsy of 10 s.c.-inoculated mice at random showed that 8 of these animals had internal metastases, including 3 mice in which tumors did not appear at the injection site after inoculation of 10^3 to 10^4 cells. In all 8 mice with metastases, the diaphragm and/or the lungs were heavily involved with tumor.

**DISCUSSION**

BRO melanoma cells were obtained from a primary tumor of a patient who had had no prior anticancer treatment. The patient's primary tumor had grown rapidly into a large ulcerating mass, and the tumor recurred locally within 7 weeks after radical surgery. The rapid recurrence of tumor despite the apparent absence of melanoma cells in the margins of the surgical wound and in the adjacent lymph nodes indicates that the tumor regrew rapidly from a very small number of cells. Subsequently, the tumor metastasized to both lungs and to other organs, and death ensued shortly thereafter. The exceptional tumorigenicity and lethality of BRO tumor cells after inoculation into nude mice parallel in important respects the clinical course of this disease. Relatively few cells are required to form lethal tumors. After s.c. inoculation, the tumor cells double approximately every 2 days, and the tumor ulcerates after a few weeks. The cause of death in most nude mice inoculated i.p. is respiratory failure arising from tumor metastases to the diaphragm and lungs, and some of the s.c.-inoculated mice expire with similar pathology.

Compared to most human tumor xenografts, BRO cells are highly malignant and aggressive. The majority of human melanomas implanted in nude mice form transplantable tumors, but metastases are relatively rare, especially when biopsies from primary tumors are implanted in adult mice (4, 13, 17). Lung metastases have been observed in nude mice after i.p. inoculation of human tumors (7, 13, 17). When established human tumor cell lines have been used to form tumors in nude mice, the inocula usually have contained 10^6 to 10^7 cells (1, 9, 19). With BRO cells, lethal tumors are formed after inoculation of as few as 100 cells, and although they arise from a primary tumor, these cells metastasize readily. In addition, the doubling time during logarithmic growth of BRO cells in vivo is about the same as the rate in vitro, in contrast to results obtained with other human melanoma xenografts, for which tumor growth was significantly slower in vivo (19). The tumorigenicity and rapid in vivo growth of BRO cells suggest that the host cytotoxic immune response,

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**Table 1**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Mice</th>
<th>Sex</th>
<th>Median ± S.E.</th>
<th>Mean ± S.E.</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>NIH-II</td>
<td>M</td>
<td>12.0 ± 0.2</td>
<td>14.0 ± 0.4</td>
</tr>
<tr>
<td>2</td>
<td>NIH-II</td>
<td>F</td>
<td>11.2 ± 0.6</td>
<td>12.0 ± 0.2</td>
</tr>
<tr>
<td>3</td>
<td>NIH-II</td>
<td>M</td>
<td>12.0 ± 0.7</td>
<td>16.0 ± 0.4</td>
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<tr>
<td>4</td>
<td>NIH-II</td>
<td>F</td>
<td>9.0 ± 0.6</td>
<td>14.0 ± 0.9</td>
</tr>
<tr>
<td>5</td>
<td>NIH-II</td>
<td>M</td>
<td>10.0 ± 0.9</td>
<td>13.0 ± 0.4</td>
</tr>
<tr>
<td>Overall</td>
<td>NIH-II</td>
<td></td>
<td>11.0 ± 0.4</td>
<td>14.0 ± 1.1</td>
</tr>
<tr>
<td>6</td>
<td>NIH-I</td>
<td>M</td>
<td>14.0 ± 0.8</td>
<td>16.0 ± 1.5</td>
</tr>
</tbody>
</table>

* B. C. Giovanella and S. O. Yim, unpublished results.
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Table 2
Survival of Mice (5/group) Inoculated I.p. with Human Tumor Cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>Tumor type</th>
<th>No. of cells inoculated</th>
<th>Mice</th>
<th>Sex</th>
<th>Median survival (days)</th>
<th>In vitro doubling time (hr)</th>
<th>Ref.</th>
</tr>
</thead>
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<tr>
<td>BRO</td>
<td>Melanoma</td>
<td>10⁷</td>
<td>NIH-I</td>
<td>M</td>
<td>14</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>BEL</td>
<td>Melanoma</td>
<td>10⁶</td>
<td>NIH-I</td>
<td>M</td>
<td>16</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>FIN</td>
<td>Melanoma</td>
<td>10⁵</td>
<td>NIH-II</td>
<td>M &amp; F</td>
<td>14</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>FOS</td>
<td>Melanoma</td>
<td>10⁴</td>
<td>NIH-II</td>
<td>F</td>
<td>40</td>
<td>38</td>
<td>7</td>
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<tr>
<td>SHO</td>
<td>Melanoma</td>
<td>10³</td>
<td>NIH-II</td>
<td>M</td>
<td>25</td>
<td>ND</td>
<td></td>
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<tr>
<td>DOY</td>
<td>Lung carcinoma</td>
<td>2.5 × 10⁷</td>
<td>NIH-II</td>
<td>M</td>
<td>35</td>
<td>24</td>
<td>11</td>
</tr>
<tr>
<td>HT-29</td>
<td>Colon carcinoma</td>
<td>4 × 10⁶</td>
<td>NIH-II</td>
<td>M</td>
<td>41</td>
<td>24</td>
<td>3</td>
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<tr>
<td>KIE</td>
<td>Breast carcinoma</td>
<td>2.6 × 10⁵</td>
<td>NIH-II</td>
<td>F</td>
<td>45</td>
<td>ND</td>
<td>2</td>
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<tr>
<td>PAX</td>
<td>Teratocarcinoma</td>
<td>10³</td>
<td>NIH-II</td>
<td>F</td>
<td>61</td>
<td>28</td>
<td>10</td>
</tr>
</tbody>
</table>

* See Table 1.

ND, not determined.

Chart 1. Growth of BRO tumor in individual mice after s.c. injection of 10⁴ cells in NIH-I and NIH-II mice. Tumor volume was calculated as the product of the 3 major dimensions.

Most malignant human melanomas. However, electron microscopic examination shows that these cells have a very rudimentary structure which is even more simplified than that of most highly undifferentiated melanoma cells. The most prominent features are the large nucleus and nucleoli. BRO cells are hypotriploid, and most of the chromosomal abnormalities are consistent with those of other human melanomas (7). The most unusual feature of the karyotype is the large double centromere chromosome found in about half of the metaphases.

BRO cells are grown easily in culture medium and as xenografts in at least 3 different strains of nude mice. Of particular interest are the short and reproducible survival times of nude mice inoculated i.p. with BRO melanoma cells. The effects of experimental treatment on the survival of host mice inoculated with these cells can be determined usually within 2 to 5 weeks (12).

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References

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