Radiation Response of Vulvar Squamous Cell Carcinoma (UM-SCV-1A, UM-SCV-1B, UM-SCV-2, and A-431) Cells in Vitro

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ABSTRACT

Standard therapy for squamous cell carcinoma (SCC) of the vulva consists of radical surgery and inguinal node dissection. Radiation therapy has been used for preoperative treatment in advanced cases to reduce the size of the tumor, and also as the only treatment in inoperable or recurrent disease. To study the inherent radiation sensitivity of vulvar carcinoma, we tested three new vulvar SCC cell lines and the long-established cell line A-431 by using a 96-well plate clonogenic assay, earlier shown by us to be suitable for survival studies of SCC. SCC and adenocarcinoma cell lines derived from other sites were used as a reference. Cells were irradiated with a 4-MeV linear accelerator at a dose rate of 2.0 Gy/min. The vulvar cell lines were found to be highly resistant to radiation with the average mean inactivation dose of 3.44 ± 0.34 Gy as calculated from the area under the curve. The results were consistent in repeated experiments and for all cell lines. The average value for area under the curve was 1.79 ± 0.30 for the other SCC lines tested. The values for area under the curve differed significantly (P < 0.0001) between the vulvar lines and reference SCC lines. These results indicate that vulvar SCC cells in vitro express exceptional inherent radiosensitivity, and thus development of new therapeutic approaches would be more advantageous in advanced cases.

INTRODUCTION

Vulvar carcinoma accounts for 3 to 5% of gynecological malignancies. The majority (85 to 90%) of these tumors are of squamous cell origin, and the average age of the patient is over 60 yr. Vulvar carcinoma typically grows and metastasizes relatively slowly; Stage 1 carcinomas have subclinical lymph node involvement in about 20% of the cases, and Stage II in about 45% of the cases. The first sites of metastases are the inguinal nodes, and at this stage the therapy of choice is radical surgery and lymph node dissection resulting in a 5-yr survival rate of 90%. The additional involvement of pelvic lymph nodes reduces survival rate to about one-third. Other factors associated with poor prognosis are advanced local spreading of the carcinoma, recurrent growth after surgery, and advanced age or poor physical condition of the patient (1–3).

Additional forms of therapy have been tried in such advanced cases with poor prognosis. Response to chemotheraphy has not been good; however, predictive testing with subrenal capsule assay has improved the possibility of finding individual sensitive tumors (4). With radiotherapy alone, the 3-yr survival rates have been as low as 10% (5), but using radiotherapy or chemoradiotherapy combined with radical surgery, promising results have been obtained (6–15). The use of supervoltage machines has made it possible to direct greater doses than earlier deeper into the tissue, and thus reduce the effects of radiation on the skin and mucosa. The tumor doses commonly used vary between 40 and 60 Gy (3, 6, 7, 9, 16).

The present knowledge of the effect of radiation therapy is mostly based on therapy trials. The in vitro testing of radiation sensitivity of vulvar SCC has been hampered by the low number of cell lines available. Clonogenic assays have also been difficult to perform with SCC, because it usually does not grow in a semisolid medium. We have recently published a new 96-well plate clonogenic assay, which has proven suitable in testing the radiosensitivity of SCC of the head and neck (17). We have also recently established three new vulvar SCC lines. These new cell lines and the long-established line A-431 (18) have been used in this study to evaluate the radiation response of vulvar SCC in vitro.

MATERIALS AND METHODS

Cell Lines. The long-established vulvar SCC cell line A-431 was obtained from the American Type Culture Collection (Rockville, MD). The well-characterized breast cancer cell line MCF-7 (19) was obtained from Dr. C. McGrath from Michigan Cancer Foundation. All the other cell lines used in this study have been established under the supervision of Dr. T. E. Carey in the Cancer Research Laboratory of the University of Michigan. The donors of UM-SCV-1A, UM-SCV-2, and UM-SCV-1B cell lines had an advanced poorly differentiated SCC of the vulva.

The first two cell lines were established from the primary tumor and the third one from a malignant pleural effusion (S. G.). All vulvar SCC lines were aneuploid with DNA indexes over 2.0 in flow-cytometric DNA analysis. UM-SCC-35 and 13 other SCC cell lines, which have been tested so far using the same assay, were used as reference material. They were established from tumor tissue of patients with SCC of the head and neck (T. E. C.). All these cell lines were found to have the morphological and antigenic characterstics known to be typical for human SCC (20, 21). UM-EC-1 was derived from an advanced, poorly differentiated endometrial adenocarcinoma (22). Prior to the experiments the cells were grown in complete Eagle’s minimal essential medium with 2 mm glutamine, 1% nonessential amino acids, 100 units/ml of penicillin, 100 µg/ml of streptomycin, and 15% FBS. The cells remained in logarithmic growth by passing on weekly or biweekly bases.

Irradiation and Clonogenic Assay. The cells were grown in T25 culture flasks till midlogarithmic phase (40% to 60% confluency), and they were fed with fresh medium on the day before plating for the experiments. The cells were harvested by trypsin-EDTA (315 units/ml of trypsin activity and 0.2 m EDTA), counted, and diluted to a standard stock solution containing 4167 cells/ml. Further dilutions of this single cell suspension were made in 6 ml of Ham’s F-12 medium with 15% FBS.

The cells were irradiated in suspension using a linear accelerator (Clinac 4/100; Varian, CA) with 4 MeV photon irradiation at a dose rate of 2.0 Gy/min. During irradiation the cells were resuspended after each 2.5 Gy to avoid sedimentation and hypoxia, but otherwise the irradiation was given as a single fraction. The expected cell kill was estimated using data from our previous work (17), and the number of cells per well was adjusted accordingly as follows: control, 2 cells/well; 0.75 Gy, 3 cells/well; 1.25 Gy, 4 cells/well; 2.50 Gy, 10 cells/well; 5.0 Gy, 20 cells/well; and 7.50 Gy, 100 cells/well.

The abbreviations used are: SCC, squamous cell carcinoma; FBS, fetal bovine serum; AUC, area under the curve.

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RADIATION SENSITIVITY OF VULVAR CARCINOMA

Table 1 Source of cell lines and results of radiation experiments

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Origin</th>
<th>Histology of tumor</th>
<th>Site of specimen</th>
<th>Prior treatment</th>
<th>Passage</th>
<th>Plating efficiency</th>
<th>Area under curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>UM-SCV-1A</td>
<td>Vulva</td>
<td>SCC</td>
<td>Primary</td>
<td>None</td>
<td>12-17</td>
<td>0.06</td>
<td>3.13</td>
</tr>
<tr>
<td>UM-SCV-1B</td>
<td>Vulva</td>
<td>SCC</td>
<td>Pleural effusion</td>
<td>None</td>
<td>13-17</td>
<td>0.06</td>
<td>3.63</td>
</tr>
<tr>
<td>UM-SCV-2</td>
<td>Vulva</td>
<td>SCC</td>
<td>Primary</td>
<td>None</td>
<td>8-11</td>
<td>0.08</td>
<td>3.23</td>
</tr>
<tr>
<td>A-431</td>
<td>Vulva</td>
<td>SCC</td>
<td>Primary</td>
<td>Not known</td>
<td>5-7*</td>
<td>0.20</td>
<td>3.77</td>
</tr>
<tr>
<td>UM-EC-1</td>
<td>Uterus</td>
<td>Adeno carcinoma</td>
<td>Primary</td>
<td>None</td>
<td>14-21</td>
<td>0.20</td>
<td>2.07</td>
</tr>
<tr>
<td>MCF-7</td>
<td>Breast</td>
<td>Adeno carcinoma</td>
<td>Pleural effusion</td>
<td>Radiation + hormone therapy</td>
<td>211-14*</td>
<td>0.45</td>
<td>2.07</td>
</tr>
<tr>
<td>UM-SCC-35</td>
<td>Tonsillar fossa</td>
<td>SCC</td>
<td>Primary</td>
<td>None</td>
<td>17-19</td>
<td>0.06</td>
<td>1.79</td>
</tr>
</tbody>
</table>

* In our laboratory.

Immediately after irradiation the cells were diluted into 44 ml of culture medium and plated into 96-well culture plates in duplicates. The plates were placed in an incubator with a water vapor-saturated atmosphere containing 5% CO₂ at 37°C. After 4 wk the plates were read using an inverted phase-contrast microscope. Wells with colonies consisting of 32 cells or more were considered positive. The experiments were made at least twice with every cell line.

Data Analysis. Plating efficiency (PE) was calculated using the formula

\[ PE = \frac{\ln(\text{no. of negative wells/total no. of wells})}{\text{no. of cells plated/well}} \]

(23). Fraction survival data as a function of the radiation dose were found to be fitted either by a linear quadratic or monoexponential equation. A microcomputer program was written to fit data to \( F = e^{-aD - bD^2} \) or \( F = Ae^{-aD} \), and to obtain the AUC by numerical integration. AUC, equivalent to mean inactivation dose, was used to compare the radiation sensitivity of individual cell lines (24). The mean AUC values were calculated for the four vulvar cell lines and for the 14 SCC lines from head and neck cancer, and compared using the 2-sample t test with pooled variances.

RESULTS

Information about the origin, tumor type, location, prior treatment, passage numbers used, plating efficiencies, as well as the mean inactivation dose (AUC) for individual cell lines is listed in Table 1. The plating efficiency for the vulvar cell lines expressed as fraction varied between 0.06 and 0.20 corresponding to 6-20%, which is fully sufficient for reliable results in the 96-well plate clonogenic assay. The fraction survival curves for the vulvar cell lines UM-SCV-1A and UM-SCV-1B are seen in Fig. 1A, and the ones for UM-SCV-2 as well as A-431 in Fig. 1B. Using the linear quadratic equation, the AUC values ranged from 3.13 to 3.77 (Table 1). Comparison of the fraction survival curves for the vulvar cell lines, the adenocarcinoma line UM-EC-1, as well as a curve representing an average fraction survival curve for the reference head and neck SCC lines is seen in Fig. 2. The statistical analysis is summarized in Table 2. The mean AUC value (± 1 SD) for vulvar SCC lines was 3.44 ± 0.34, and for the 14 reference SCC lines of the head and neck cancers, 1.79 ± 0.30. The group variances were equal according to Levene’s test \( (P = 0.9055) \). The vulvar SCC cell lines were highly radiation resistant as compared with the head and neck SCC cell lines (2-sample t test with pooled variance, \( P < 0.0001 \)).

DISCUSSION

Radiation therapy has been considered to be indicated as an additional or substituting form of treatment in vulvar SCC in

![Fig. 1. Radiation sensitivity of vulvar squamous carcinoma cell lines. The figures show the fraction survival curves as the function of radiation dose for UM-SCV-1A (●) and UM-SCV-1B (□) in A and UM-SCV-2 (●) and A-431 (□) in B.](image)

![Fig. 2. Comparison of radiation sensitivity of different cell lines. The figure shows the fraction survival curves of vulvar SCC cell lines (UM-SCV-1A, UM-SCV-1B, UM-SCV-2, A-431), an endometrial adenocarcinoma cell line (UM-EC-1), and UM-SCC-35, which well represents the average curve for the 14 head and neck SCC cell lines used as reference.](image)

Table 2 Statistical comparison between radiation responses of vulvar and head and neck squamous cancer cell lines

<table>
<thead>
<tr>
<th></th>
<th>Vulvar SCC lines</th>
<th>Head and neck SCC lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cell lines</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>Mean area under curve</td>
<td>3.44*</td>
<td>1.79*</td>
</tr>
<tr>
<td>SD</td>
<td>0.34</td>
<td>0.30</td>
</tr>
<tr>
<td>Range</td>
<td>(3.13; 3.77)</td>
<td>(1.29; 2.63)</td>
</tr>
<tr>
<td>95% confidence intervals</td>
<td>(2.95; 3.93)</td>
<td>(1.55; 1.99)</td>
</tr>
</tbody>
</table>

* Levene’s test for group variances: \( P = 0.9055 \).

* Two sample t test with pooled variance: \( P < 0.0001 \).
cases with poor prognosis due to advanced local disease or the patients' poor condition. In this group the usually advantageous therapy of radical surgery alone has not resulted in best possible surviving rates. With relatively large radiation doses of 40 to 60 Gy, the results have been promising in small series, although severe local side effects have reduced its usefulness. Previous in vitro studies have shown that SCC as a group is moderately radiation resistant, and that the sensitivity of SCC to irradiation is highly variable (17, 25–27).

There has been remarkably little knowledge on the inherent radiation sensitivity of vulvar SCC in vitro because of the rarity of existing vulvar cancer cell lines. To our knowledge only two cell lines have been available. This is due to both the low incidence of the disease and the difficulties in growing SCC cells in culture. Another problem in assessing the in vitro radiation sensitivity of SCC has probably been the difficulty in establishing a reliable method for estimating the surviving fraction of cells after radiation in these anchorage-dependent, migrating cells. We have recently published a 96-well plate clonogenic assay for radiation studies of head and neck SCC. This method has given reproducible survival curves, as well as reliable plating efficiencies with tens of cell lines tested so far. With this method the number of clonogenic cells has varied from 2–45%. The corresponding value of the vulvar lines varied from 6–20%, which is significantly higher than earlier results achieved with soft agar assay (28, 29).

We found all four vulvar SCC cell lines tested in this study highly radiation resistant. The mean inactivation dose was 3.44 Gy, which was significantly greater than the ones for SCC cell lines of head and neck as well as for adenocarcinoma cell lines. Both of these groups are commonly considered to be of limited radiation sensitivity (30). The results were consistent in repeated experiments. The survival curves of head and neck SCC lines were consistent with those obtained previously with a cobalt-60 photon beam radiation using the same assay (17).

The fraction survival curves were usually found to be fitted well with the linear quadratic equation. Instead of the survival curve parameter D,, (reciprocal of the slope of the survival curve), we calculated the mean inactivation dose using the area under the curve. This value expresses more accurately the fraction survival curves were usually found to be fitted well with the linear quadratic equation. Instead of the survival curve parameter D,, (reciprocal of the slope of the survival curve), we calculated the mean inactivation dose using the area under the curve. This value expresses more accurately the continuously curving survival line, whereas D,, is easily influ

REFERENCES


4878
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