Combined Use of Hyperthermia and Irradiation Cause Antiproliferative Activity and Cell Death to Human Esophageal Cell Carcinoma Cells - Mainly Cell Cycle Examination -

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<Abstract> In clinically hyperthermia and irradiation therapy for malignant neoplasms are known that they have antiproliferative activity and cell death (including apoptosis) inducing activity. However not only mechanisms of cell death induction but treatment effects of them still have been unclear. In this time we showed that cell cycles from G0/G1 phase to S-G2/M phase were delayed by hyperthermia and G2/M phase accumulation were caused immediately by irradiation. And we also demonstrated that the combination treatments of hyperthermia and irradiation induced synergistic antiproliferative effects and strong effects of cell death to human esophageal carcinoma cell lines. Although treatments of hyperthermia and irradiation were mild individually, combination treatment of hyperthermia and irradiation were useful for esophageal carcinoma treatment.

Key words: esophageal carcinoma, cell cycle, hyperthermia, irradiation.

Introduction

Human esophageal carcinoma is one of the most malignant neoplasms. The use of treatments such as radiation therapy, hyperthermia, chemotherapy, immunotherapy including surgery have not improved the long-term survival rates. The 5-year survival rate for cases that undergo esophageal carcinoma excision is 41.9% and that inoperative case is only 5.7%.

Hyperthermia and/or irradiation therapy for malignant tumors has shown antiproliferative effects and improvement of patient prognosis. In vitro antiproliferative tumor effects using hyperthermia and irradiation have been reported, and it is assumed that these effects will occur in clinical cases. Furthermore, clinical analyse is of human esophageal carcinoma have been performed and antiproliferative tumor effects and improvement of patient prognosis have been reported. Since these treatments are found to induce apoptosis in tumor cells, the mechanisms and treatment effects have been examined. But cell death of esophageal carcinoma cells has been some obscure point.

We have employed hyperthermia and irradiation therapy alone or along with surgery and chemotherapy for esophageal carcinoma patients since 1985, and the treatment effects of operative and inoperative cases have improved. We analyzed the cell cycle using an in vitro assay with human esophageal carcinoma
cell lines to clarify the combined treatment of hyperthermia and irradiation as well as the synergistic effect of antiproliferative activity on tumors and induced cell death (including apoptosis) using protocols of a clinical model in our laboratory.

Materials and Methods

1) Cell culture

6 cell lines of human esophageal carcinoma, SGF-3,-4,-5,-7,-8 and -9 were established from human esophageal carcinoma in our laboratory since 1980 (Table 1). They were maintained in RPMI Medium 1640 (Gibco) and Ham’s F-12 Nutrient Mixture (Gibco) at the ratio of 1:1 supplemented with 10% fetal calf serum (Mitsubishi Kasei Corp.). Cells were cultured in the flasks (25cm²/Tissue Culture Flask Slim Type, IWAIU) at 37°C under 5% CO₂ component humidified incubator, and the exponentially growing cells were employed for the experiment. And SGF-8 cells that was most sensitive of hyperthermia and irradiation among them was mainly used our main experiment.

2) Hyperthermia and irradiation

A water bath (Racomace model HT-100, Iuchi Co.) was set at 43.5°C. The flasks with cells were placed to float on the water bath within a thermometer (Model TMd4, Internova Inc.), and were warmed at 43.5°C for 1 hour. The temperature of the medium in the flasks reached 43.5°C within 5 minutes and did not differ by more than 0.1°C. Concerning X-ray irradiation, Hitachi MBR-1505R2 (Hitachi Medical Corporation) was employed as a generator. The radiation dose were adjusted from 2 Gy to 5 Gy / min by changing the exposure time in some experiments.

3) MTT assay

Cells were seeded in a 96-hole micro-culture plate (F96 Micro Well, DOJINDO) at a density of 1x10⁴ cells/0.2ml / well. After incubating at 37°C for 24 hours, hyperthermia (at 43.5°C for 1 hour), radiation (2 Gy / day x 5 days) and combination treatments were performed. 72 hours later, 0.05mg (50μl of 1mg/ml) 3-(4, 5-dimethyl-2-thiazolyl) 2, 5-diphenyl-2H-tetrazolium bromide (MTT) solution (Wako) was added to each well and incubated at 37°C for 4 hours. The medium was then aspirated, and 150 μl of dimethyl sulfoxide was added to each well. The optical absorbance was determined for each well at 570 nm using an SJeia autoreader (Model-8,000, Sankyo Junyaku Co.)⁰⁰. The survival rates (% survival) were calculated using the following formula:

\[
\% \text{ survival} = \left( \frac{\text{mean optical absorbance of the treated group}}{\text{mean optical absorbance of the control group}} \right) \times 100
\]

These analyses were performed in quadruplicate about each cell of 6 cell lines.

4) Inspection of cell morphology

Cells were treated with hyperthermia or irradiation and then observed by electron microscopic inspection followed by Sawataishi. The pellet was made and immediately fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer solution.

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Table 1: Established cell lines of human esophageal carcinomas (SGF series).

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Age</th>
<th>Sex</th>
<th>Starting date of culture</th>
<th>Origin</th>
<th>Histology (differentiation)</th>
<th>Doubling time (hours)</th>
</tr>
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<tbody>
<tr>
<td>SGF-3</td>
<td>44</td>
<td>M</td>
<td>Jul.22 1980</td>
<td>primary tumor</td>
<td>s.c.c. moderately</td>
<td>33</td>
</tr>
<tr>
<td>SGF-4</td>
<td>64</td>
<td>M</td>
<td>Feb.17 1981</td>
<td>primary tumor</td>
<td>s.c.c. moderately</td>
<td>26</td>
</tr>
<tr>
<td>SGF-5</td>
<td>78</td>
<td>M</td>
<td>Dec.13 1983</td>
<td>LN metastatic lesion</td>
<td>s.c.c. well</td>
<td>30</td>
</tr>
<tr>
<td>SGF-7</td>
<td>71</td>
<td>M</td>
<td>Apr.14 1987</td>
<td>primary tumor</td>
<td>s.c.c. moderately</td>
<td>35</td>
</tr>
<tr>
<td>SGF-8</td>
<td>52</td>
<td>M</td>
<td>Jun.9 1988</td>
<td>LN metastatic lesion</td>
<td>s.c.c. poorly</td>
<td>38.5</td>
</tr>
<tr>
<td>SGF-9</td>
<td>58</td>
<td>M</td>
<td>Jun.13 1992</td>
<td>primary tumor</td>
<td>s.c.c. well</td>
<td>28</td>
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