

## Effect of Chronic Exposure to Acidic Environment on Radiosensitivity of Gliosarcoma Cells

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### 1. Introduction

In unconventional radiotherapy modalities, such as stereotactic radiosurgery (SRS), stereotactic body radiation therapy (SBRT) and microbeam radiation therapy (MRT), high-dose radiation delivery would cause severe damage in tumor vasculature, leading to chronic hypoxia and acidosis in tumors [1]. In this study, the chronic exposure of cells to acid culture medium, prior or posterior to irradiation, has been investigated for its effect on clonogenic cell survival.

### 2. Materials and Methods

#### 2.1. Cell preparation and irradiation

Rat gliosarcoma cells (ATCC, CRL-2200) were incubated in a Dulbecco's Modified Eagle's Medium (GIBCO) supplemented with 10% fetal bovin serum (GIBCO) at 37°C in a humidified 10% CO<sub>2</sub> atmosphere. Cells were irradiated at room temperature in the hard x-ray beam irradiation facility at Seoul National University [2].

#### 2.2. Acidity control of culture medium

The acidity (pH 6.6) of culture medium was controlled by flushing twice the hypoxia chamber (MIC-101, Billups-Rothenberg) with 20% O<sub>2</sub>, 40% CO<sub>2</sub> and 40% N<sub>2</sub> gas mixture for 7 min at a flow rate of 10 liters/min. The pH of culture medium was measured using a pH tester (HI 98203, HANNA).

#### 2.3. Clonogenic cell survival assay

Cells were incubated for different time spans in normal or acidic culture medium. Radiation exposure was made at doses of up to 10 Gy. After irradiation, cells were seeded for colony formation. After 4 days of incubation under acidic or normal medium conditions, culture medium was renewed for another 10 days of incubation. The colonies of more than 50 cells were counted for record.

### 3. Results and Discussions

#### 3.1. Effect of pre-irradiation exposure to acid culture medium on cell radiosensitivity

When cells were exposed to chronic (2 and 4 days) acidic condition before irradiation, cell radiosensitivity significantly increased in comparison to control cells, as shown in Fig. 1. But, acute (150 min) exposure of cells to acid culture medium made little difference.

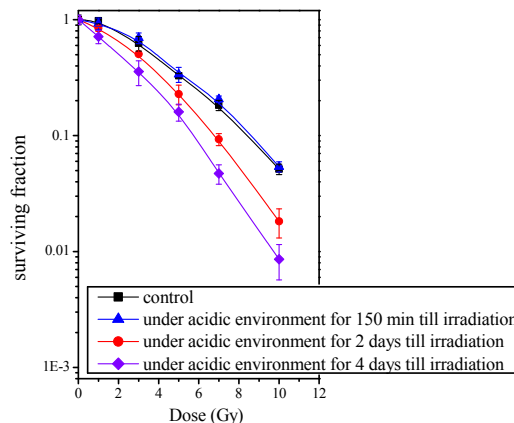


Fig. 1. Clonogenic surviving fractions of gliosarcoma cells exposed to acid culture medium for 150 min, 2 or 4 days till irradiation. In each experiment set, surviving fractions were normalized to the unirradiated cells. Each data point represents the mean  $\pm$  S.E.

Table I:  $\alpha/\beta$  ratios from the linear quadratic fitting curves in Fig. 1.

| culture condition                                     | $\alpha/\beta$      |
|---|---------------------|
| control   | $6.389 \pm 1.319^a$ |
| under acidic environment for 150 min till irradiation | $6.824 \pm 1.993$   |
| under acidic environment for 2 days till irradiation  | $8.091 \pm 0.621$   |
| under acidic environment for 4 days till irradiation  | $15.263 \pm 1.892$  |

<sup>a</sup>standard error

Data points in Fig. 1 were fitted by employing the linear quadratic model. The values of  $\alpha/\beta$  ratio from four different curves are listed in Table I. The 2 and 4 day pre-irradiation exposures to acid culture medium resulted in about 1.27 and 2.39 times of  $\alpha/\beta$  ratio, respectively, as compared to the control. According to Alpen [3], it can be suggested that chronic exposure to acidic environment before irradiation makes cells to be more susceptible to lethal damages.

We defined the dose modifying factor (DMF) as the ratio of radiation dose administrated under altered medium condition to dose delivered under normal medium condition needed to produce the same biological effect. DMF<sub>50</sub> and DMF<sub>10</sub> mean the values of DMF at 50% and 10% cell survival, respectively. According to the definition, culture medium condition specified with DMF less than 1 can be interpreted to

increase the radiosensitivity.  $DMF_{50}$  and  $DMF_{10}$  were 0.79 and 0.81 for 2 day exposure to acid medium prior to irradiation and 0.55 and 0.68 for 4 day exposure, respectively.

### 3.2. Effect of post-irradiation exposure to acid culture medium on cell radiosensitivity

As shown in Fig. 2 and 3, gliosarcoma cells became more radioresistant by post-irradiation exposure to acid culture medium no matter how long the pre-irradiation exposure to acid medium was maintained.

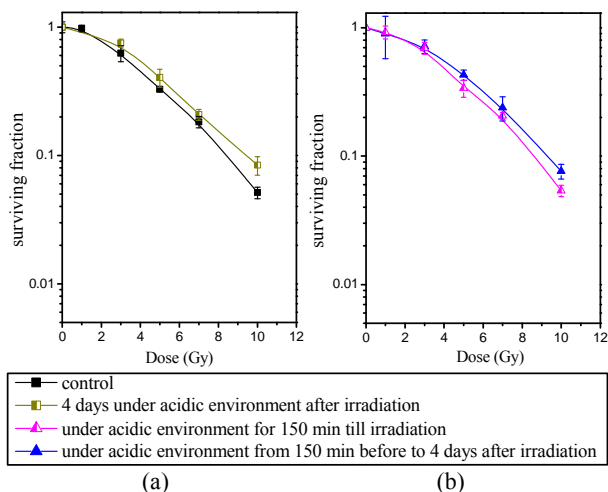


Fig. 2. Comparisons of radiosensitivity of gliosarcoma cells, in terms of clonogenic cell survival, with and without post-irradiation exposure to acid medium: (a) no and (b) 150 min pre-irradiation exposure to acid medium. Clonogenic cell surviving fractions were normalized to unirradiated cells in each experimental condition. Each data point represents the mean  $\pm$  S.E.

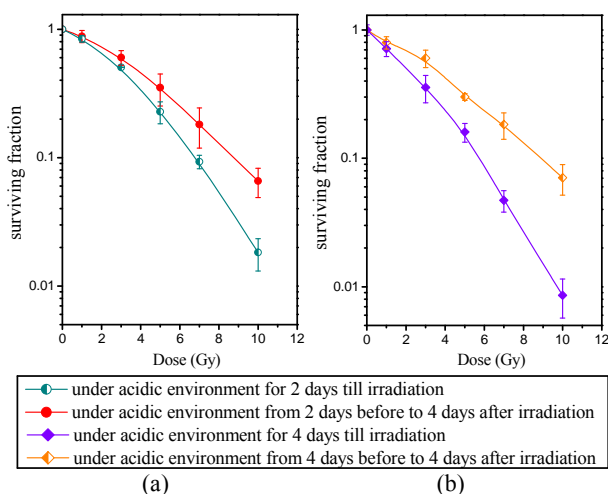


Fig. 3. Comparisons of radiosensitivity of gliosarcoma cells, in terms of clonogenic cell survival, with and without post-irradiation exposure to acid medium: (a) 2 day and (b) 4 day pre-irradiation exposure to acid culture medium. Clonogenic cell surviving fractions were normalized to unirradiated cells in each experimental condition. Each data point represents the mean  $\pm$  S.E.

It is known that the post-irradiation exposure to acid culture medium prolongs the  $G_2$ -arrest of irradiated cells and thus increases the repair of potentially lethal damage (PLD) [4-6]. The 150 min pre-irradiation exposure to acid medium (Fig. 2(b)) resulted in quite similar gain in surviving fraction via PLD repair by post-irradiation exposure to acid culture medium to that with no pre-irradiation exposure to acid medium (Fig. 2(a)). The gain in surviving fraction via PLD repair was more efficient for cells with longer pre-irradiation exposure to acid medium (Fig. 3(a) and (b) as compared to Fig. 2(a) and (b)). The post-irradiation exposure to acid medium itself led to the similar level of radiation responses regardless of the duration of pre-irradiation exposure to acid culture medium.

## 4. Conclusion

Unconventional high-dose radiation therapy, such as SRS, SBRT and MRT, may cause severe vascular damage in tumors, thereby a number of tumor cells facing chronic hypoxia and thus acidosis. According to our observation, gliosarcoma cells become more vulnerable to radiation damage by chronic exposure to acidic condition before irradiation. The longer the pre-irradiation exposure is, the more vulnerable to radiation damage the cells become. However, the repair of PLD by post-irradiation exposure to acid medium is efficient enough to eliminate the difference in number of the cells carrying PLDs due to different durations of pre-irradiation exposure to acidic condition.

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