Clonogenic cell surviving fractions under modulated X-ray beam fields

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1. Introduction

The modulated beam therapy aids reduction in normal tissue damage by employing spatially fractionated radiation exposure [1]. The spatially fractionated beam exposure creates areas of zero- or low-dose exposure [2]. The cellular response to non-uniform radiation exposure has been investigated for the past years in regard to intercellular communication by direct cellular contact or by factor secretion [3]. Mackonis et al. categorized the interaction between nearby cells into three types [4]. In this study, we investigated the influence of the beam modulation modes on cells *in vitro* in terms of clonogenic cell surviving fraction (SF).

2. Methods

2.1. Cell culture

Mouse endothelial cells (MECs) [Catalog No. CRL-2161, American Type Culture Collection (ATCC), Manassas, VA, USA] were cultured in T-25 flasks (Nunc, Roskilde, Denmark) with a cell growth medium which consists of Dulbecco's modified eagle medium (DMEM) (GIBCO, Grand Island, NY, USA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) (GIBCO, Grand Island, NY, USA). The cell was incubated at 37°C under humidified 10% CO_2 environment.

2.2. Clonogenic cell survival assay

Before irradiation, cells were incubated into T-25 culture flasks (NUNC) at a concentration of 1×10^6 cells per flask. Cells were dispersed to single cells with TrypLE Express (GIBCO) and detached cells were resuspended in the medium. Then, a 20μl aliquot was counted using a Muse® cell analyzer (Merck Millipore, Darmstadt, Germany). An appropriate number of cells was seeded onto a new T-25 culture flask.

Cells were irradiated with varying doses of x-rays in a single exposure. The cell culture medium was replaced with a fresh one at intervals of about three days until 10–12 days. The colonies formed were fixed with 70% ethanol and stained with 5% Giemsa staining solution (Sigma-Aldrich). The number of colonies containing more than 50 cells was counted with the naked eye.

2.3 Beam modulation and irradiation

Cells were irradiated by operating the X-ray beam tube (YXLON, Germany, 450-D08) at 200 kV and 10 mA. Dose rate was 1.5 Gy/min.

The modulated beam fields were designed to irradiate half the total area. With T-25 flask of 44 mm in width, first and second halves of the total beam field were symmetric and each consists of one 22 mm-wide beam opening. Each of the halves was further divided into two quarters consisting of 11 mm-wide beam openings. Each of the four quarters was divided into two 5.5 mm-wide one-eighths.

Fig. 1 shows dose distributions measured using the Gafchromic EBT films for beam fields with one half-, two quarter-, and four eighth-beam openings. No matter what the unit beam-opening size is, half of the total field was shielded. The shielded areas were not free from the radiation beam, but exposed to the penumbra doses. The penumbra dose is enhanced as the number of beam divisions increases.

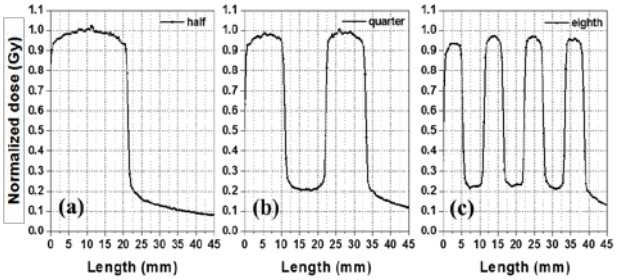

Fig. 1. Dose distribution profiles measured using the Gafchromic EBT films for beam fields with (a) one half-beam opening, (b) two quarter-beam openings, and (c) four eighth-beam openings.

3. Results

Figure 2 shows the surviving fractions of the targeted cells under the beam openings of three different types (half, quarter, and eighth). In comparison with the SF values for the targeted cells in whole beam, the targeted cells under the bean openings in half-, quarter-, and eighth-beam exposures survived to greater extents. At doses over 6 Gy, the SF values were significantly different ($p<0.05$)

Figure 3 presents the surviving fractions of the target cells under half-, quarter-, and eighth-beam exposures, normalized to that of target cells at whole-beam exposure at the same dose. As dose increased, the discrepancy in SF among the target cells under beam openings of different sizes was enhanced. Regarding the size of beam opening, the SF was the highest with the target cells under the quarter-beam opening.

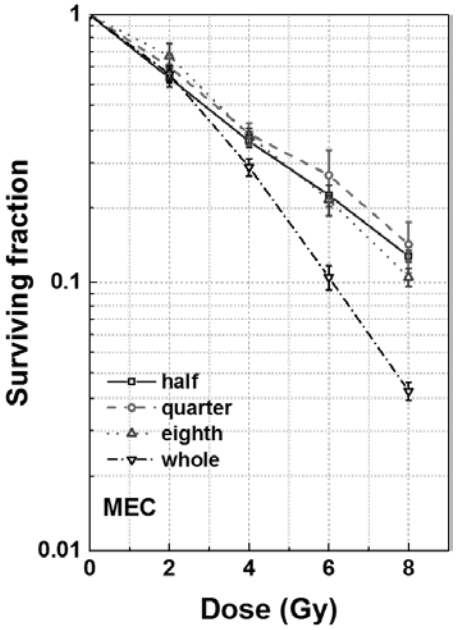

Fig. 2. Clonogenic cell surviving fractions of the targeted cells under beam openings at three different types of modulated beam modulation (square: half, circle: quarter and triangle: eighth) and at whole-beam field (nabla). Each error bar represents the standard error calculated with five individual experiments.

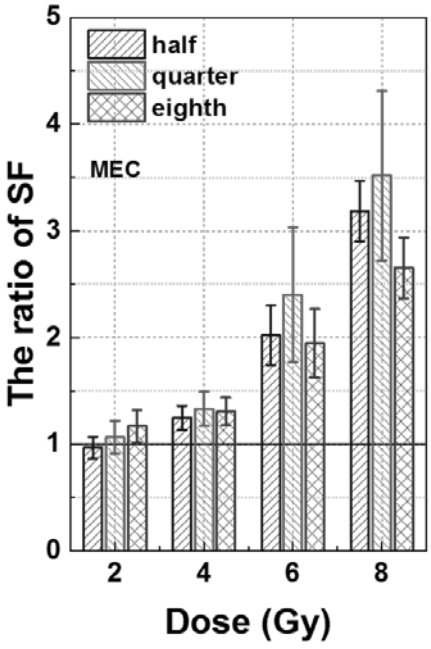

Fig. 3. Clonogenic cell surviving fractions of the targeted cells at three different types of beam modulation (half-, quarter- and eighth-beam opening), normalized to that of the cells at whole-beam exposure. The SF values are categorized according to the target dose (2, 4, 6, and 8 Gy). The error bars represent the standard errors from five individual experiments.

4. Conclusion

Spatially fractioned radiation therapy creates areas of zero- or low-dose radiation exposure by beam field modulation. This study informs that the cells respond to the same radiation dose differently depending on the size of beam opening through which they were targeted. Targeted cells under the quarter-beam exposure showed the highest clonogenic survival.

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