

# Evaluation of the relative biological effectiveness of spot-scanning proton irradiation *in vitro*

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## ABSTRACT

Variations in relative biological effectiveness (RBE) from a fixed value of 1.1 are critical in proton beam therapy. To date, studies estimating RBE at multiple positions relative to the spread-out Bragg peak (SOBP) have been predominantly performed using passive scattering methods, and limited data are available for spot-scanning beams. Thus, to investigate the RBE of spot-scanning beams, Chinese hamster fibroblast V79 cells were irradiated using the beam line at the Hokkaido University Hospital Proton Therapy Center. Cells were placed at six different depths, including the entrance of the proton beam and the proximal and distal part of the SOBP. Surviving cell fractions were analyzed using colony formation assay, and cell survival curves were obtained by the curve fitted using a linear–quadratic model. RBE<sub>10</sub> and RBE<sub>37</sub> were 1.15 and 1.21 at the center of the SOBP, respectively. In contrast, the distal region showed higher RBE values (1.50 for RBE<sub>10</sub> and 1.85 for RBE<sub>37</sub>). These results are in line with those of previous studies conducted using passive scattering proton beams. Taken together, these data strongly suggest that variations in RBE should be considered during treatment planning for spot-scanning beams as well as for passive scattering proton beams.

**KEYWORDS:** relative biological effectiveness, spot scanning, radiotherapy, proton therapy

## INTRODUCTION

Proton therapy allows the delivery of high-radiation doses to tumors without damaging the surrounding healthy organs [1], and an increasing number of patients have been treated using this therapy [2]. Although most patients are treated using passive scattering methods, modern proton therapy centers often adopt pencil beam scanning because it offers more flexible and conformal dose distributions compared with passive scattering methods, and minimizes overall exposure.

Hokkaido University Hospital started spot-scanning proton therapy (SSPT), a type of pencil beam scanning proton therapy, in March 2014. During SSPT, several thousand small-sized, nearly mono-energetic proton beams are used to administer a planned dose to the target volume. The depth position of the spots are adjusted by

changing the acceleration energy, whereas their lateral positions are moved using a pair of scanning magnets [3, 4]. This technique does not require collimators or compensators, and can thus reduce neutron contamination from interactions of protons with these materials.

Relative biological effectiveness (RBE) is defined as the ratio of the absorbed dose of a reference radiation to that of a test radiation that produces the same biological effect, and RBE is an essential consideration during treatment planning for proton therapy. Treatment planning in proton therapy is generally based on a constant RBE of 1.1, according to Report 78 of the International Commission on Radiation Units and Measurements (ICRU78). In line with ICRU78, determinations of RBE have been performed widely, using passive scattering methods [5, 6], and show RBE values ranging from 1.1 to

1.2 at the beginning of the flat top portion of the spread-out Bragg peak (SOBP). However, recent studies warrant cautious use of this RBE value for treatment planning because it may lead to lower estimates of biological doses in organs at risk (OARs) that are located close to the distal fall-off of SOBP [7–9].

Many studies have revealed that RBE tends to increase with increasing linear energy transfer (LET) towards the distal region of the SOBP [10, 11], and Paganetti reviewed this RBE characteristic of proton beam comprehensively [12]. However, it remains unclear whether these RBE values for passive scattering methods are relevant to spot-scanning systems. This is because, first, the proton energy spectra, the fluence, and the LET of SSPT proton beams may differ from those of conventional passive scattering systems, resulting in differences in the RBE [13]. Second, smaller neutron contamination from SSPT may contribute to differences in RBE between these systems [14].

While Gueulette *et al.* determined RBE for SSPT at three depth positions according to the intestinal crypt regeneration in mice and showed RBE values of 1.11 for the initial plateau, 1.16 for the center, and 1.21 for the distal region of the SOBP [15], RBE values could not be estimated at positions with high-dose gradients, such as the distal fall-off of the SOBP, reflecting poor estimates of precise positions with *in vivo* experiments. The goal of this study is to derive an RBE suitable for SSPT, including the value at the distal fall-off of the SOBP. We performed a clonogenic survival assay using a V79 cell line and estimated the RBE values at six different depths (a 5 mm depth point from the primary plane, three points in the SOBP plateau and two points in the distal fall-off).

## MATERIALS AND METHODS

### Cell culture

V79 cells were obtained from the RIKEN Cell Bank (Tsukuba, Ibaraki, Japan) and cultured in  $\alpha$ -MEM (Life Technologies, Carlsbad, CA) supplemented with 10% fetal bovine serum (BioWest, Nuaille, France) at 37°C in 5% CO<sub>2</sub>/95% air.

### Irradiation

Cells were seeded in a chamber slide flask (Thermo Scientific/Nunc, Penfield, NY) at  $1.8 \times 10^6$  cells/flask, incubated for 6 h and then filled with  $\alpha$ -MEM immediately prior to irradiation.

Proton beams were generated using a ProBeat RT (Hitachi, Tokyo, Japan), with an SOBP width of 6 cm, an energy range of 156.7–182.8 MeV, spot spacing of 5 mm and a field size of  $10 \times 10$  cm. The isocenter plane was matched with the center of the SOBP, and dose flatness of the SOBP was  $\pm 2.5\%$  compared with the center of the SOBP in the depth direction. The average dose rate was 2.68 Gy/min. As reference photon beams, 6 MV X-rays were generated using a linear accelerator (Mitsubishi Electric Co., Tokyo, Japan) with a field size of  $10 \times 10$  cm, and the dose rate was  $\sim 2.5$  Gy/min. According to the protocol of Japanese Standard Dosimetry 12 [16], the measurement of the dose and dose-rate was conducted with a PTW Markus Chamber (Type 34045; PTW, Freiburg, Germany) and an electrometer (Type 10021; PTW, Freiburg, Germany).

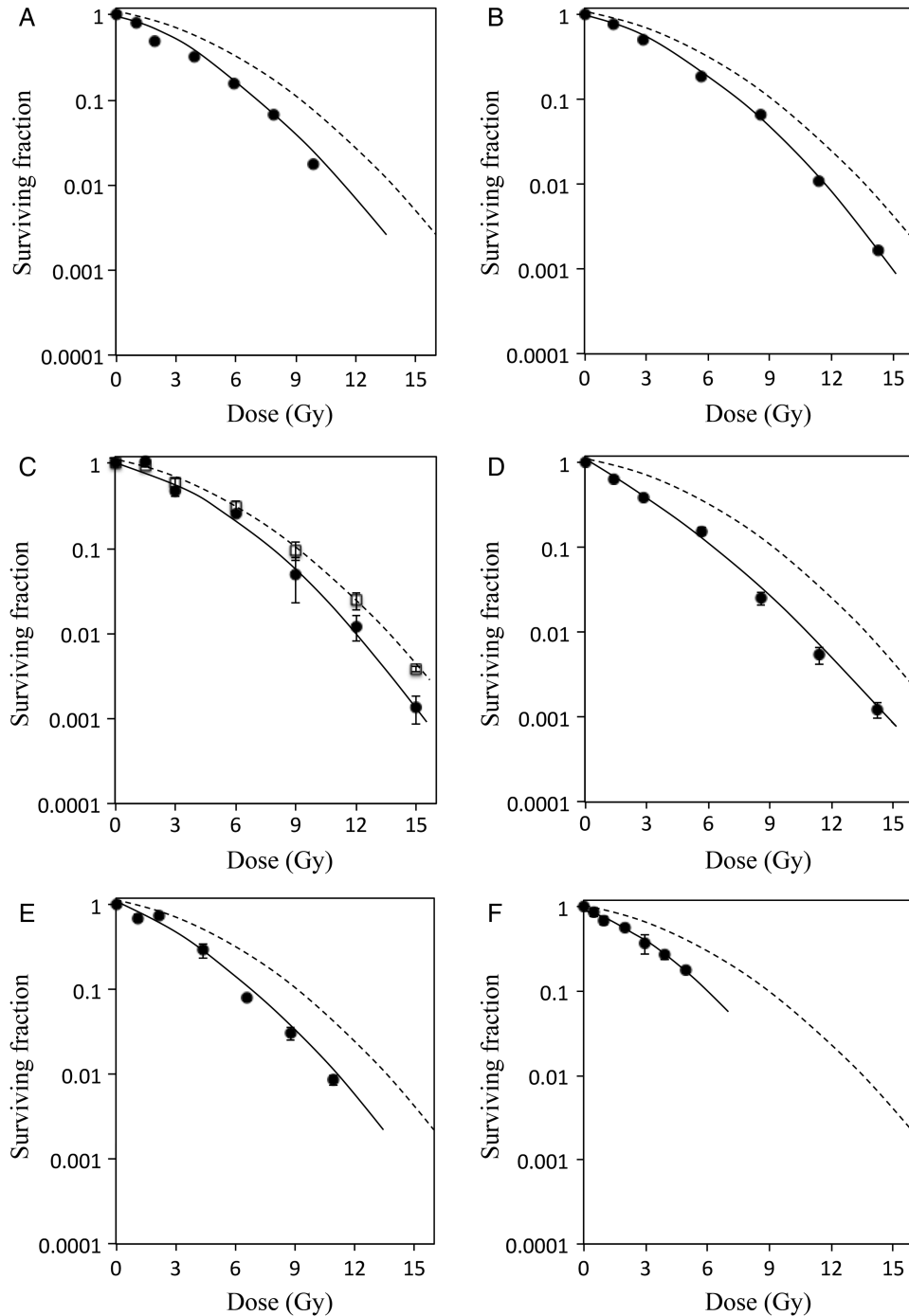
To position cells during irradiation, we designed a high-density polyethylene block with a water equivalent ratio = 1.03,  $\rho = 0.98$  g/cm<sup>3</sup> and varied the thickness of the up-front block to set depths of cells in the beams. To investigate the depth dependency of the RBE, proton beam irradiation was performed at the following six points: (a) at a 5 mm depth from the primary plane, (b) at the proximal 95% physical dose point to the center of the SOBP, (c) at the center of the SOBP, (d) at the distal 95% physical dose point to the SOBP center, (e) at the distal 73% physical dose point to the SOBP center and (f) at the distal 33% physical dose point to the SOBP center (Table 1). The high-density polyethylene block was also used during X-ray irradiation, and the buildup was 1.5 cm in water. In order to compensate back and side scatter, the blocks were located in the back or at the side of the block in which the slide flask was inserted. The flatness of the irradiated field was  $\pm 0.72\%$ .

### Clonogenic survival assay

The clonogenic survival assay was performed according to the previous study by Matsumoto *et al.* [17]. Briefly, cells were trypsinized by incubating with 0.25% trypsin/1 mM EDTA solution at 37°C in 5% CO<sub>2</sub>/95% air for 3 min and harvested immediately after irradiation. After washing with PBS, cells were diluted with  $\alpha$ -MEM, and were seeded on 6 cm dishes at densities from 100 to 50 000 cells per dish to yield  $\sim 100$  colonies per dish depending on the radiation dose, followed by culturing for 7 days. Colonies were fixed with methanol and stained with Giemsa solution. Colonies containing  $> 50$  cells were recorded as surviving cells. Surviving fractions at each dose were

**Table 1. Survival parameters and biological equivalent doses for V79 cells irradiated with X-rays or proton beams at various depths**

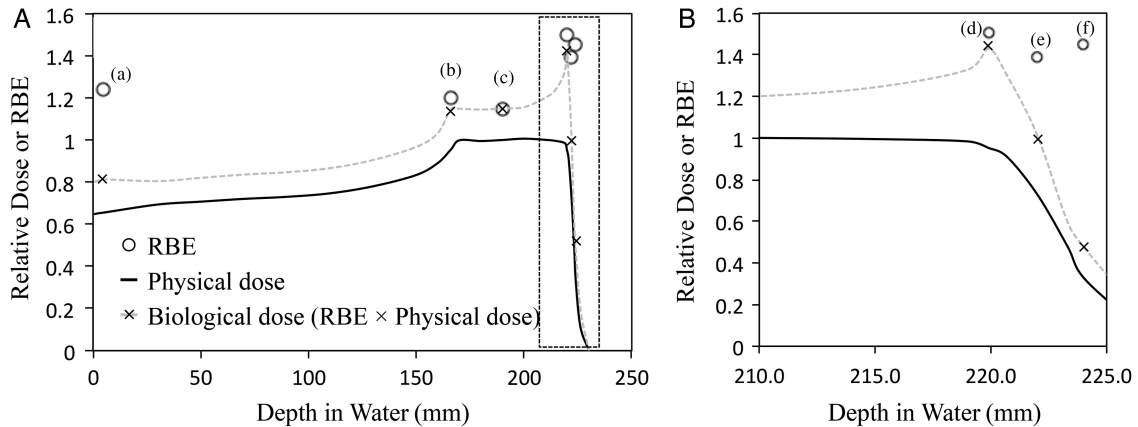
| Position | Dose fraction | Depth (mm) | $\alpha$ (Gy <sup>-1</sup> ) | $\beta$ (Gy <sup>-2</sup> ) | $\alpha/\beta$ (Gy) | D <sub>10</sub> (Gy) | RBE <sub>10</sub> | D <sub>37</sub> (Gy) | RBE <sub>37</sub> | RBE <sub>2Gy</sub> | RBE <sub>4Gy</sub> |
|----------|---------------|------------|------------------------------|-----------------------------|---------------------|----------------------|-------------------|----------------------|-------------------|--------------------|--------------------|
| A        | 0.66          | 5          | 0.18                         | 0.0191                      | 9.42                | 7.19                 | 1.24              | 3.87                 | 1.35              | 1.44               | 1.32               |
| B        | 0.95          | 165        | 0.16                         | 0.0203                      | 7.88                | 7.42                 | 1.2               | 4.09                 | 1.28              | 1.54               | 1.37               |
| C        | 1             | 190        | 0.14                         | 0.0200                      | 7.00                | 7.73                 | 1.15              | 4.32                 | 1.21              | 1.46               | 1.33               |
| D        | 0.95          | 220        | 0.32                         | 0.0117                      | 27.35               | 5.94                 | 1.5               | 2.83                 | 1.85              | 2.52               | 2.04               |
| E        | 0.73          | 222.5      | 0.24                         | 0.0191                      | 12.57               | 6.42                 | 1.39              | 3.32                 | 1.58              | 1.96               | 1.68               |
| F        | 0.33          | 224        | 0.22                         | 0.0209                      | 10.53               | 6.15                 | 1.45              | 3.3                  | 1.58              | 1.86               | 1.65               |
| X-rays   |               |            | 0.09                         | 0.0185                      | 4.86                | 8.91                 |                   | 5.23                 |                   |                    |                    |



**Fig. 1.** Survival curves of V79 cells irradiated with X-rays or with protons at different depths. Clonogenic survival of V79 cells irradiated with X-rays (white square, dashed line) or protons (closed circle, solid line) at each depth (A–F as shown in Table 1); surviving colonies were stained and counted after 7 days of incubation. Dose–response curves were fitted using linear–quadratic models as described in Materials and Methods, and  $RBE_{10}$  and  $RBE_{37}$  values were calculated from  $D_{10}$  and the  $D_{37}$  values. With regard to spatial resolution of dosimetry system, the dose error was <0.5%.

calculated with respect to plating efficiencies of non-irradiated controls and were plotted for physical doses. Survival curves were fitted using the following linear–quadratic (LQ) model:  $SF = \exp(-\alpha D - \beta D^2)$ , where SF is the surviving fraction and  $D$  is the physical dose. At  $D_{10}$

and  $D_{37}$ , cell survival was reduced to 10% and 37%, respectively, and  $RBE_{10}$ ,  $RBE_{37}$ ,  $RBE_{2Gy}$  and  $RBE_{4Gy}$  values were calculated as the ratio of the  $D_{10}$ ,  $D_{37}$  and isosurviving fraction at 2 Gy and 4 Gy to that of 6 MV X-rays. All experiments were performed at least three times.



**Fig. 2.** Relative dose–depth distributions in V79 cells. Relative physical doses (black solid line), RBE<sub>10</sub> values calculated at D<sub>10</sub> (white circle) and RBE<sub>10</sub> × physical doses (×) at each depth (a–f as shown in Fig. 2) of the proton beam; the physical dose was normalized at the SOBP center. Data are presented for (A) all positions (a–f) from 0 to 245 mm depth from water and (B) the distal region (d–f) from 210.0 to 225.0 mm depth from water.

## RESULTS AND DISCUSSION

V79 cells were irradiated at multiple irradiation positions with X-rays or proton beams and survival curves were calculated (Fig. 1). Dose–response curves of proton irradiation were lower than those of X-rays at all positions. Although positions (b) and (d) received the same 95% physical doses relative to that at the center of the SOBP, the shapes of survival curves differed and the curve of (d) was close to linear compared with that of (b), indicating greater cell death at this position.

As shown in Table 1, RBE<sub>10</sub> and RBE<sub>37</sub> values were 1.15 and 1.21, respectively, at the center of the SOBP (c). The measured RBE<sub>10</sub> and RBE<sub>37</sub> values were in close agreement with previous studies of V79 cells that were performed in other proton beam facilities with passive scattering systems [18, 19]. In addition to V79 cells, RBE values were determined at the center of the SOBP in human salivary gland (HSG) cells, with RBE<sub>10</sub> of 1.02 and RBE<sub>37</sub> of 1.04 (data not shown). These values of HSG cells were also similar to those made at facilities employing passive scattering methods [6], suggesting that different irradiation systems produce similar RBE values.

Biological doses (RBE × physical doses) are critical for treatment planning and those obtained in this study were 0.81, 1.2, 1.15, 1.4, 1.01 and 0.52 (×; Fig. 2) at positions (a), (b), (c), (d), (e) and (f), respectively. The biological dose was enhanced at the distal edge of the SOBP, and the biological proton range (90% position of the biological dose) was extended by 2.2 mm compared with the physical range. This result is in good agreement with the theoretical report by Wilkens *et al.* [20] and the experimental data using the passive scattering method by Matsumoto *et al.* [17]. Therefore, these results indicated that treatment planning should take into consideration the biological dose distribution, although whether the variation in RBE is directly linked to clinical results has not been clarified [21, 22]. Parameters of  $\alpha$ ,  $\beta$  and  $\alpha/\beta$  were calculated at each depth (Table 1). The  $\alpha$  values in proton beams ranged from 0.14 to 0.32 and were higher than those of X-rays. Moreover,  $\alpha$  values of protons tended to increase with depth of the SOBP, with a maximum value of 0.32 at the distal edge (d). However, the  $\beta$  values showed smaller variation,

with values of  $0.02 \pm 0.01$  at all positions. These observations suggest that RBE values reflect  $\alpha$  coefficients rather than  $\beta$  coefficients.

Wouters *et al.* [23] reported that cells with low  $\alpha/\beta$  values, such as V79 cells, showed low a dose-dependent increase in RBE, especially below 4 Gy, and 4 Gy was the minimum dose indicating the stable RBE. To test this dose dependence of RBE, we calculated RBE<sub>4Gy</sub> and RBE<sub>2Gy</sub> values at each irradiation point from the survival curves (Table 1). The RBE<sub>4Gy</sub> values obtained in the SOBP were >1.37 and higher than the RBE<sub>10</sub> and RBE<sub>37</sub> values at all positions, but the increase in RBE was modest. On the other hand, RBE<sub>2Gy</sub> values were significantly higher than RBE<sub>4Gy</sub> values, indicating the low-dose dependence of RBE in accordance with the results of Wouters *et al.* This result suggested that radiation oncologists or medical physicists should give consideration to the increase in biological effect at the distal region of the SOBP in the treatment planning of practical fractionated SSPT, especially if the normal tissue consists of cells with low  $\alpha/\beta$  values positioned near the target region. Biological optimization, which has been proposed by several authors [24], may be a promising approach that incorporates the variable RBE into the inverse planning process of SSPT treatment planning.

In summary, we measured the RBE of V79 cells in the SSPT system at the Hokkaido University Hospital Proton Therapy Center. In agreement with previous studies of passive scattering systems, RBE<sub>10</sub> and RBE<sub>37</sub> values at the center of the SOBP were 1.15 and 1.21, respectively. Moreover, RBE values at the distal region increased with increasing  $\alpha$  values. The present data suggest close attention should be paid to variable RBE values during treatment planning for humans.

## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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