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Relative Biological Effectiveness Values of Spot-scanning Proton Beam Therapy at Shonan Kamakura General Hospital

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Abstract. *Background/Aim: This study aimed to confirm the relative biological effectiveness (RBE) values of the proton beam therapy (PBT) system installed in Shonan Kamakura General Hospital. Materials and Methods: Clonogenic cell-survival assays were performed with a human salivary gland (HSG) cell line, a human tongue squamous-cell carcinoma cell line (SAS), and a human osteosarcoma cell line (MG-63). Cells were irradiated with proton beams and X-rays with different doses (1.8, 3.6, 5.5, and 7.3 Gy for proton beams, and 2, 4, 6, and 8 Gy for X-rays). Proton beam irradiation used spot-scanning methods and three different depths (at the proximal, center, and distal sides of the spread-out Bragg peak). RBE values were obtained from a comparison of the dose that resulted in a surviving fraction of 10% (D_{10}). Results: D_{10} of proton beams at the proximal, center, and distal sides and X-rays in HSG were 4.71, 4.71, 4.51, and 5.25 Gy, respectively; those in SAS were 5.08, 5.04, 5.01, and 5.59 Gy, respectively; and those in MG-63 were 5.36, 5.42, 5.12, and 6.06 Gy, respectively. The RBE_{10} values at the proximal, center, and distal sides in HSG were 1.11, 1.11, and 1.16 respectively; those in SAS were 1.10, 1.11, and 1.12, respectively; and those in MG-63 were 1.13, 1.12, and 1.18, respectively. Conclusion: RBE_{10} values of 1.10-1.18 were confirmed by in vitro experiments using the PBT system. These results are considered acceptable for clinical use in terms of therapeutic efficacy and safety.*

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Key Words: Relative biological effectiveness, proton beam therapy, human salivary gland cell, human tongue squamous-cell carcinoma cell, human osteosarcoma cell.

Particle therapies, including proton beam therapy (PBT) and carbon-ion radiotherapy (RT), have become more widespread in recent years, and have shown favorable clinical outcomes in various cancers (1-4). Proton and carbon-ion beams have higher dose localization properties than X-ray RT owing to the distal fall-off due to the Bragg peak and sharp lateral penumbra (5-8). This physical advantage enables the administration of high-dose to tumors while sparing normal tissues, which may have resulted in favorable outcomes.

Administration dose is the one of the most important factors of tumor control. Doses of PBT are expressed as relative biological effectiveness (RBE) weighted dose [Gy (RBE)], which is defined as the physical dose multiplied by the RBE value of the PBT. The RBE value of PBT is defined as 1.1 for all cancers in clinical settings, and the average RBE value of PBT is 1.05 for human salivary gland (HSG) cell line according to *in vitro* experiments (9). However, RBE value may not always be constant depending on the RT system of the facility, type of tumor, and depth in spread-out Bragg peak (SOBP) (10). Therefore, it is important to confirm the RBE value when the PBT system is installed. Additionally, there are insufficient studies on the identification of RBE values in the spot-scanning method. Therefore, the RBE of PBT is of interest to radiation oncologists.

In Shonan Kamakura General Hospital (SKGH), PBT using the spot-scanning method has been started since January 2022. Although physical measurements are performed in the PBT system installed at SKGH, confirmation of biological effect using *in vitro* experiments, which measure the RBE values of PBT, has not been performed. Here, we reported the RBE confirmation of PBT at three different depths within the SOBP of the spot-scanning irradiation method in several cancer cells.

Table I. *Survival parameters and biological equivalent doses.*

Cell lines	Beam	Position	D_{10}	D_{37}	SF_2	RBE_{10}	RBE_{37}
HSG	Proton	PP	4.71	2.46	0.46	1.11	1.16
	Proton	IC	4.71	2.47	0.50	1.11	1.16
	Proton	DP	4.51	2.36	0.44	1.16	1.21
	X-ray		5.25	2.86	0.53		
SAS	Proton	PP	5.08	2.63	0.53	1.10	1.07
	Proton	IC	5.04	2.77	0.56	1.11	1.01
	Proton	DP	5.01	2.82	0.57	1.12	1.00
	X-ray		5.59	2.81	0.51		
MG-63	Proton	PP	5.36	2.95	0.55	1.13	1.10
	Proton	IC	5.42	2.77	0.54	1.12	1.17
	Proton	DP	5.12	2.87	0.58	1.18	1.13
	X-ray		6.06	3.24	0.58		

D_{10} and D_{37}: Dose that resulted in a surviving fraction of 10% and 37%, respectively; DP: at the distal 95% physical dose point to the spread-out Bragg peak center; HSG: human salivary gland tumor; IC: at the center of the spread-out Bragg peak; SAS: human tongue squamous-cell carcinoma; MG-63: human osteosarcoma; PP:, at the proximal 95% physical dose point to the spread-out Bragg peak center; RBE_{10} and RBE_{37}: relative biological effectiveness that calculated from the D_{10} and D_{37}, respectively; SF_2: survival fraction values after 2 Gy exposure.

Materials and Methods

Cell culture. An HSG cell line, a human tongue squamous-cell carcinoma cell line (SAS), and a human osteosarcoma cell line (MG-63) were used in this study, which were obtained from the Japanese Collection of Research Bioresources Cell Bank (JCRB). HSG is a standard reference cell line for RBE calculation in carbon-ion RT and is also used in PBT (8, 9). SAS and MG-63 were used as different radiosensitive cells. Cells were seeded in 6-cm tissue culture plates, cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated fetal bovine serum, and 1% penicillin-streptomycin, and incubated at 37˚C in a humidified atmosphere with 5% CO_2. The medium and serum were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Cells were passaged before confluence and were used for all experiments within 10 passages after purchase from the JCRB to obtain stable results.

X-ray and proton beam irradiation. X-ray irradiation was performed at the Shonan iPark (MBR-1520R-4, Hitachi, Japan). The dose rate and energy of X-ray irradiation were 1.7 Gy/min and 150 keV. Proton beam irradiation was performed at SKGH (PROBEAT-M1, Hitachi, Japan) using the spot-scanning methods with an SOBP width of 6 cm, an energy range of 130.2-165.5 MeV, a spot spacing of 5 mm, and a field size of 15×15 cm. To investigate the depth dependency of the RBE, proton beam irradiation was performed at three different depths: (i) at the proximal 95% physical dose point to the SOBP center, (ii) at the center of the SOBP, and (iii) at the distal 95% physical dose point to the SOBP center (Figure 1). X-ray irradiation doses were 2, 4, 6, and 8 Gy. Proton beam irradiation doses were 1.8, 3.6, 5.5, and 7.3 Gy, and these doses correspond to 2, 4, 6, and 8 Gy (RBE) in clinical practice, respectively. All experiments were performed at least thrice.

Clonogenic cell-survival assay and calculation of relative biological effectiveness. The effect of treatment on cell survival was evaluated using the clonogenic cell-survival assay. Cells were seeded into six-well tissue culture plates and exposed (or not) to X-ray or proton beam irradiation. After incubation for a further 10-14 days, the cells were fixed with methanol and stained with crystal violet. Colonies consisting of at least 50 cells were counted. Survival fractions were calculated as the ratio of surviving colonies per number of plated cells. Cell survival fractions were normalized to the survival fraction in the absence of irradiation (controls). The dose that resulted in a surviving fraction of 10% and 37% (D_{10} and D_{37}) was calculated using the linear-quadratic model (11). RBE values were obtained from a comparison of the D_{10} and D_{37} values for proton beams and X-rays. Additionally, the survival fraction values after 2 Gy exposure (SF_2) with proton beams and X-rays were evaluated to assess radioresistance.

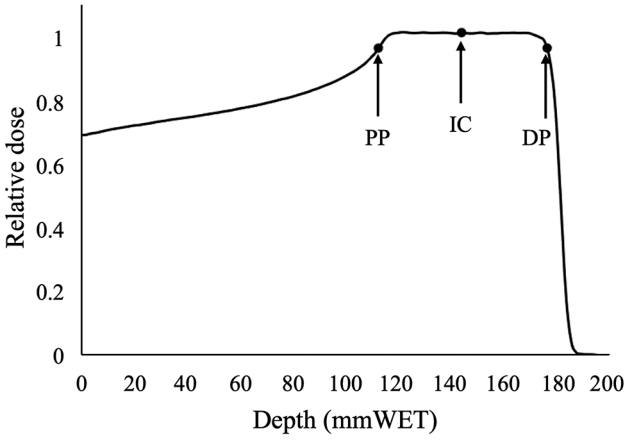

Figure 1. *Depth-dose distribution of the spread-out Bragg peak (SOBP) of proton beams. Black arrows indicate the three depths at which the sample was placed: (i) at the proximal 95% physical dose point to the SOBP center (PP), (ii) at the center of the SOBP (IC), and (iii) at the distal 95% physical dose point to the SOBP center (DP).*

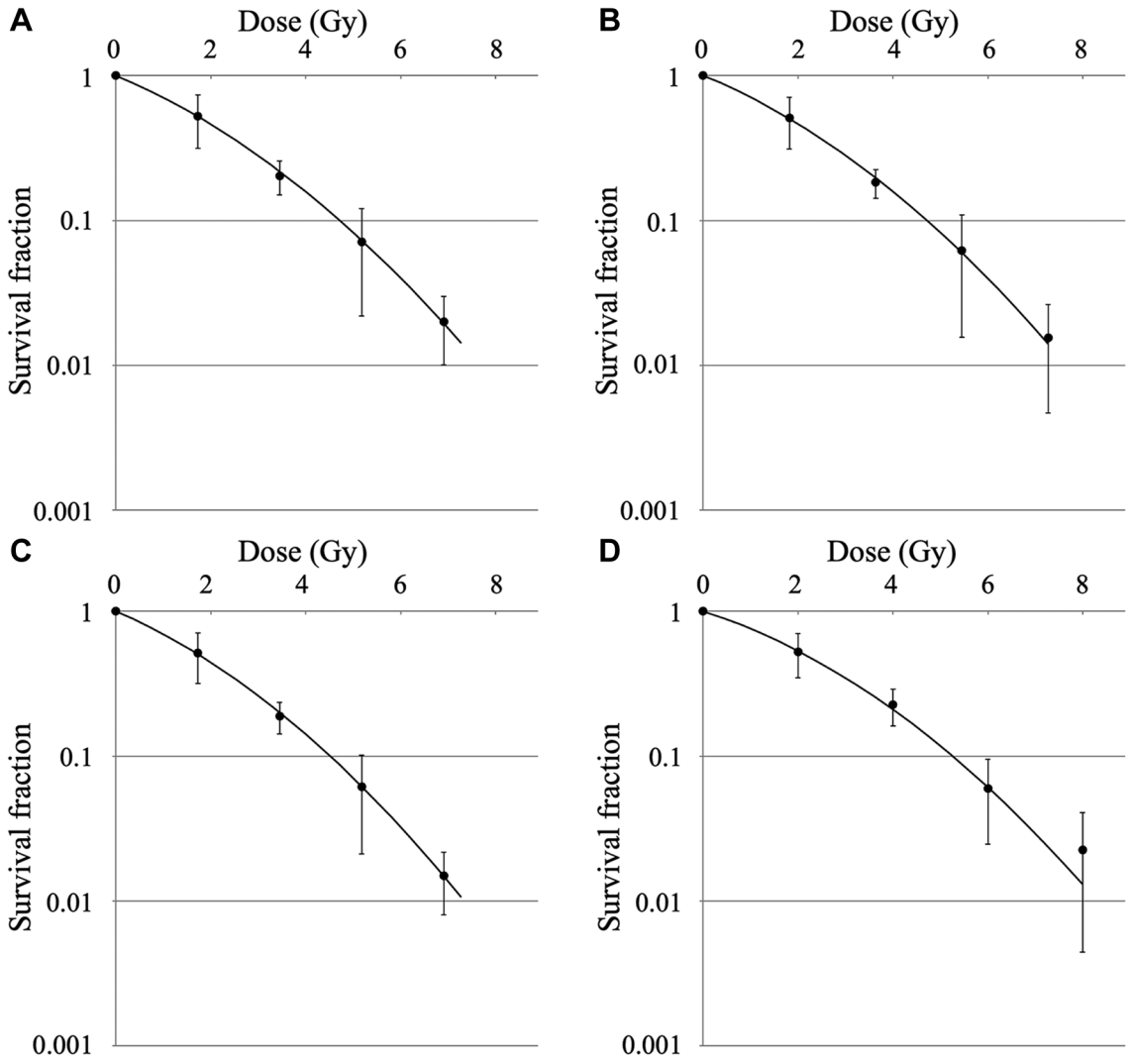

Figure 2. *Survival curves of human salivary gland cells after proton beam and X-ray irradiation. (A) Proton beam irradiation at the proximal 95% physical dose point to the spread-out Bragg peak (SOBP) center. (B) Proton beam irradiation at the center of the SOBP. (C) Proton beam irradiation at the distal 95% physical dose point to the SOBP center. (D) X-ray irradiation. Data are presented as the mean±standard deviation, fitted to the linear-quadratic model.*

Results

The survival curves under different irradiation schemes in HSG, SAS, and MG-63 cell lines are shown in Figure 2, Figure 3, and Figure 4, respectively. D_{10} of proton beams at the proximal, center, and distal sides and X-rays in HSG were 4.71, 4.71, 4.51, and 5.25 Gy, respectively; those in SAS were 5.08, 5.04, 5.01, and 5.59 Gy, respectively; and those in MG-63 were 5.36, 5.42, 5.12, and 6.06 Gy, respectively. The RBE_{10} values at the proximal, center, and distal sides in HSG were 1.11, 1.11, and 1.16, respectively; those in SAS were 1.10, 1.11, and 1.12, respectively; and those in MG-63 were 1.13, 1.12, and 1.18, respectively. The D_{10}, D_{37}, SF_2, RBE_{10}, and RBE_{37} for each scheme and cell lines are shown in Table I. These results showed that RBE_{10} values ranged from 1.10 to 1.18 in all cell lines, with little change in RBE at different depths of SOBP.

Discussion

We demonstrated the confirmation of RBE values in the cell lines HSG, SAS, and MG-63. RBE_{10} and RBE_{37} values in HSG, SAS, and MG-63 ranged from 1.10 to 1.18 and 1.00 to 1.21, respectively, at three different depths of SOBP. *In vitro* experiments, including those in HSG cells, have previously shown RBE values of 0.9-2.1, and the present study showed

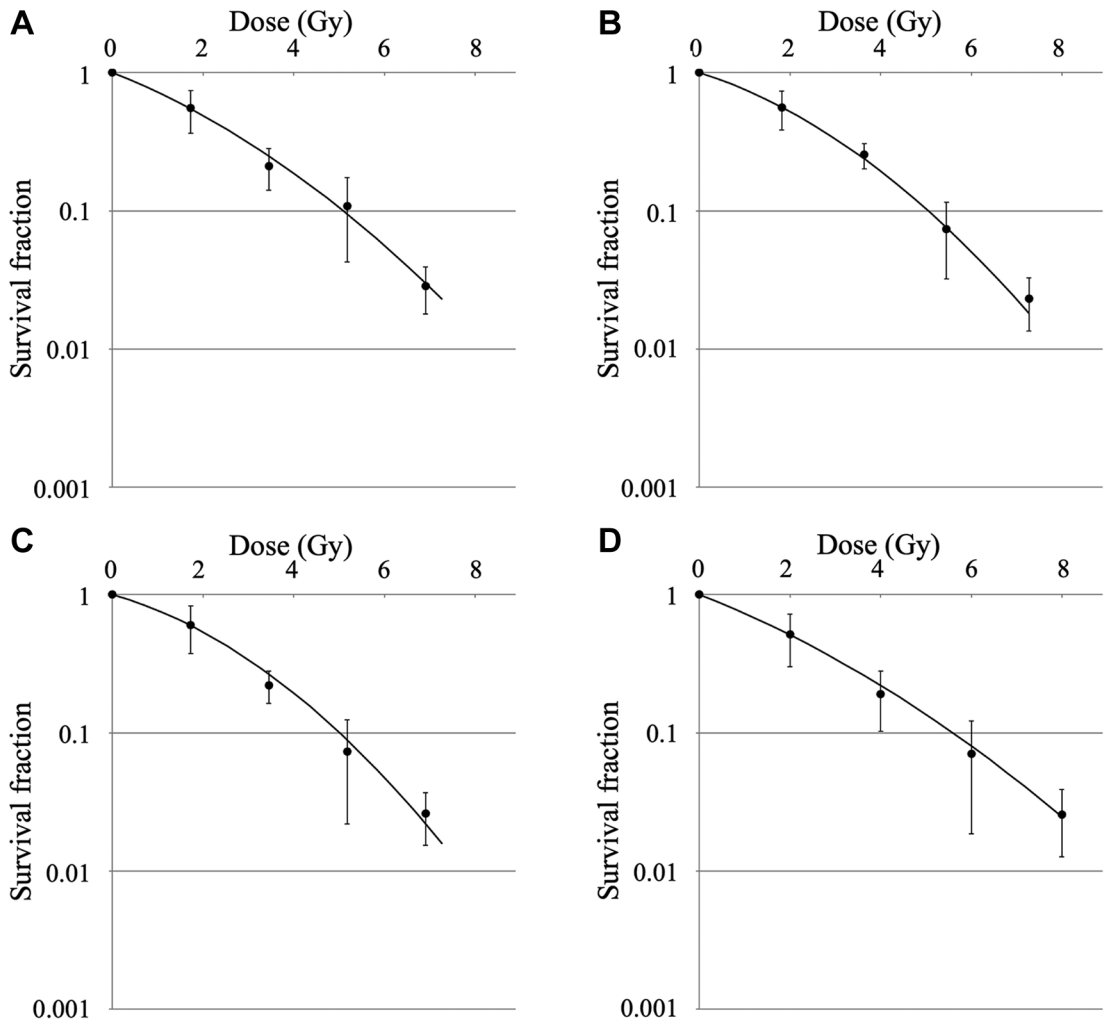

Figure 3. *Survival curves of human tongue squamous-cell carcinoma cells after proton beam and X-ray irradiation. (A) Proton beam irradiation at the proximal 95% physical dose point to the spread-out Bragg peak (SOBP center). (B) Proton beam irradiation at the center of the SOBP. (C) Proton beam irradiation at the distal 95% physical dose point to the SOBP center. (D) X-ray irradiation. Data are presented as the mean±standard deviation, fitted to the linear-quadratic model.*

similar results to those previously reported, confirming that the proton beam irradiation system at our facility is expected to be as effective as irradiation systems at other facilities (9, 12, 13). Therefore, our results suggest that the clinical efficacy of PBT is comparable to other prior facilities.

In the present study, there was little variation in RBE, and a previous study showed similar results of RBE value within the SOBP (12). In contrast, previous reports showed RBE values of 1.35 at the distal edge and 1.7 at the distal fall-off (14). The distal edge or distal fall-off might have higher RBE value than the isocenter or proximal side (15). Therefore, in clinical practice, RBE is calculated uniformly at 1.1 at the beamline, so we should be careful that the dose calculation may be underestimated at the distal edge or distal fall-off area. Further studies are required to confirm the point of higher RBE value in the beam line and optimize the dose distribution using RBEs for different points in the beam line, rather than uniformly using RBE value of 1.1.

The RBE values were similar in the three cell lines used in this experiment; however, SF_2 and D_{10} were slightly higher in MG-63 than in SAS and HSG. Komatsu *et al*. reported that SF_2 might be the most useful indicator of radiation sensitivity (16). Therefore, our results suggest that MG-63 is more radioresistant than HSG and SAS. The *in vitro* experiment using osteosarcoma cells irradiated by high-linear energy transfer (LET) beams of carbon ions has shown high cell killing effect with low D_{10} and high RBE values (17). Therefore, when treating such radioresistant tumors (*e.g*., osteosarcoma), high-LET RTs, such

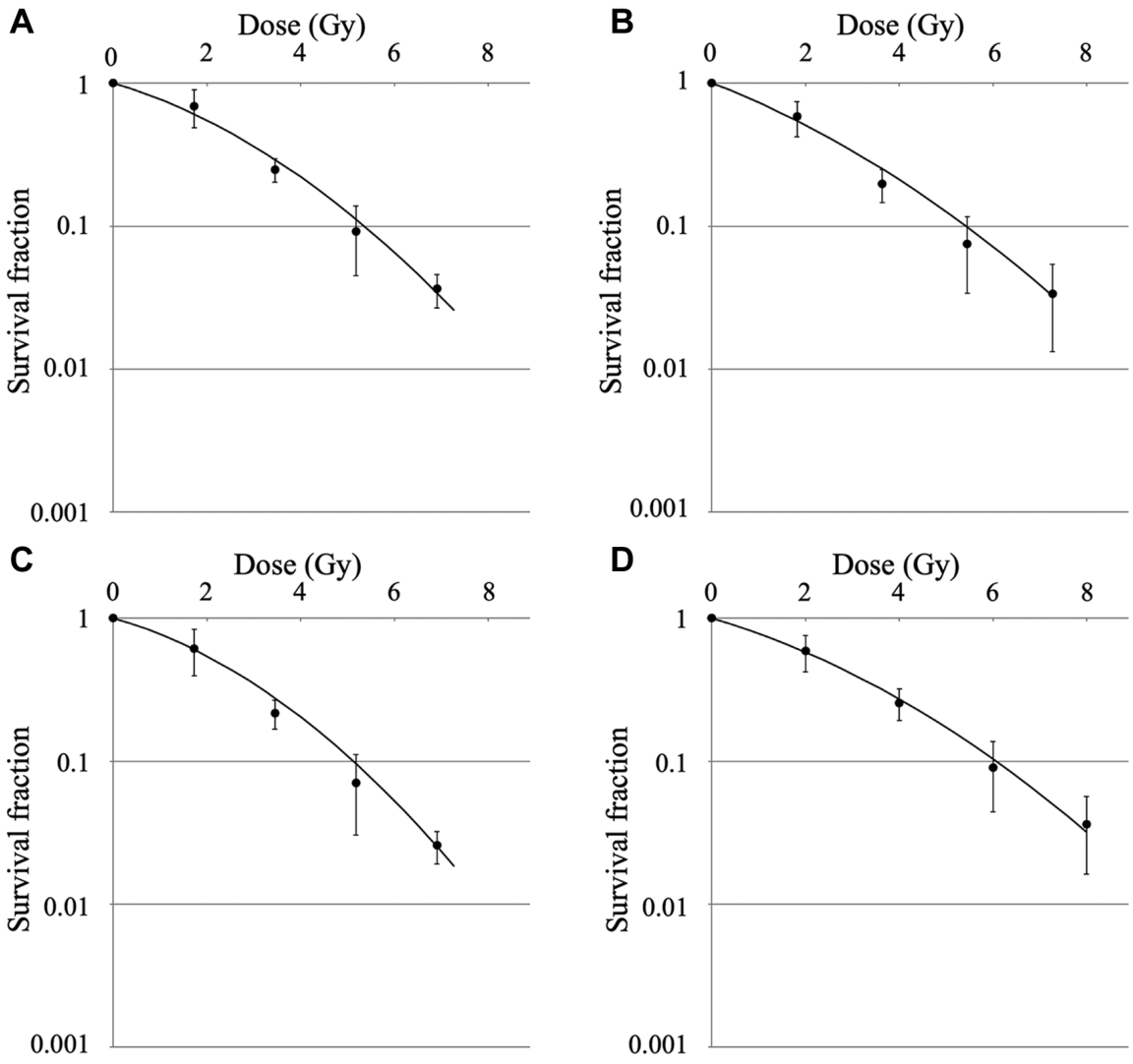

Figure 4. *Survival curves of human osteosarcoma cells after proton beam and X-ray irradiation. (A) Proton beam irradiation at the proximal 95% physical dose point to the spread-out Bragg peak (SOBP) center. (B) Proton beam irradiation at the center of the SOBP. (C) Proton beam irradiation at the distal 95% physical dose point to the SOBP center. (D) X-ray irradiation. Data are presented as the mean±standard deviation, fitted to the linear-quadratic model.*

as carbon-ion RT or boron neutron capture therapy, may have a better therapeutic effect than low-LET RT (18, 19).

Our study has a limitation. The irradiation was performed at three different depths in the SOBP. Further experiments confirming the RBE value in the beam line, especially within the range of distal fall-off where RBE value might be high, are required.

Conclusion

We confirmed RBE values of 1.10-1.18 by *in vitro* experiments using our PBT system, which is comparable to the results of previous studies. These results were considered acceptable for clinical practice in terms of therapeutic efficacy and safety.

Conflicts of Interest

The Authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors' Contributions

Conceptualization: S.Shiba; methodology: S.Shiba; formal analysis: S.Shiba; investigation: S.Shiba, M.Y., K.M., A.Y., T.S., S.Suzuki, T.Y., K.N.; resources: S.Shiba; data curation: S.Shiba; writing–original draft preparation: S.Shiba; writing–review and editing: S.Shiba, T.O., K.T., M.O.; visualization: S.Shiba; supervision: T.O., T.O., K.T., M.O.; project administration: M.O.

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