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ABSTRACT

Purpose: High-dose-rate irradiation with 6 MV linac X-rays is a wide-spread means to treat cancer tissue in radiotherapy. The treatment planning relies on a mathematical description of surviving fraction (SF), such as the linear-quadratic model (LQM) formula. However, even in the case of high-dose-rate treatment, the repair kinetics of DNA damage during dose-delivery time plays a function in predicting the dose-SF relation. This may call the SF model selection into question when considering the dose-delivery time or dose-rate effects (DREs) in radiotherapy and *in vitro* cell experiments. In this study, we demonstrate the importance of dose-delivery time at high-dose-rate irradiations used in radiotherapy by means of Bayesian estimation.

Methods: To evaluate the model selection for SF, three types of models, the LQM and two microdosimetric-kinetic models with and without DREs (MKM_{DR} and MKM) were applied to describe *in vitro* SF data (our work and references). The parameters in each model were evaluated by a Markov chain Monte Carlo (MCMC) simulation.

Results: The MCMC analysis shows that the cell survival curve by the MKM_{DR} fits the experimental data the best in terms of the deviance information criterion (DIC). In the fractionated regimen with 30 fractions to a total dose of 60 Gy, the final cell survival estimated by the MKM_{DR} was higher than that by the LQM. This suggests that additional fractions are required for attaining the total dose equivalent to yield the same effect as the conventional regimen using the LQM in fractionated radiotherapy.

Conclusions: Damage repair during dose-delivery time plays a key role in precisely estimating cell survival even at a high dose rate in radiotherapy. Consequently, it was suggested that the cell-killing model without repair factor during a short dose-delivery time may overestimate actual cell killing in fractionated radiotherapy.

Key words:

linear-quadratic model, microdosimetric-kinetic model, dose-delivery time, Markov chain Monte Carlo simulation, fractionated irradiation

I. INTRODUCTION

Photon beam (X-ray or γ -ray) irradiation is currently the main method to treat cancer in radiation therapy. For planning a fractionated irradiation scheme with X-rays, the linear-quadratic model (LQM) has been widely used as a cell-killing model.^{1, 2} The LQM can describe the relationship between dose and cell survival by using only two parameters, the coefficients of the dose and the dose squared (α [Gy^{-1}] and β [Gy^{-2}]). However, the actual relation is fraught with complications in practice, where the biological effects of radiation on cell survival depend on the radiation's energy spectrum, dose rate, tumor cell repopulation, cell cycle distribution and oxygen concentration.³⁻⁶ DNA lesions such as double-strand breaks (DSBs) can be repaired during irradiation at a certain dose rate by a DNA damage repair function.⁷ Our previous investigation via the microdosimetric-kinetic model (MKM) showed that dose-rate effects (DREs) can be attributed to sublethal damage (SLD) repair^{7, 8} during irradiation and thus the linearity of the cell survival curve is exhibited in the high dose range.⁹ At very high-dose-rate irradiation, the LQM independent of the dose-rate factor is suitable^{10, 11} because the dose-delivery time is very short.⁹ Furthermore, the hypo-fractionated irradiation scheme has recently been investigated¹², which requires a short but non-negligible period of dose-delivery time. The influence of the DREs on cell survival should therefore be evaluated even at a relatively high dose rate.

In order to determine the model parameters, both the least squares method and the maximum likelihood method have been used. Although these methods are valid to find the mean values of the parameters, they cannot evaluate the deviances (or uncertainties) of the parameter values arising from variations in the experimental data. For evaluating the probability density function (PDF) of the parameters, Bayesian estimation is useful, in which the Markov chain Monte Carlo (MCMC) technique is of advantage.^{13, 14} This technique employs the algorithm known as a hierarchical model and a set of parameters can be sampled according to Markov property, which makes a prediction of the posterior state based on the prior state.¹⁵ The MCMC technique enables us to estimate the uncertainty of each parameter and to verify the model selection from the uncertainty.¹⁵ In clinical practice of radiotherapy, fractionation schemes have been utilized under consideration of both tumor control probability (TCP) and normal tissue complication probability (NTCP).¹⁶ The scheme is largely dependent on the model parameters on the basis of 4R's concept, i.e., repair, redistribution, repopulation, and reoxygenation.¹⁷ In the application of the LQM, the ratio of α and β (α/β) has potent influence on the fractionated irradiation regimen in radiation therapy because the biological effective dose (BED) is used as an index to optimize the fractionation, which is deduced based on this ratio.¹⁸ For example, α/β value for NSCLC is around 10 Gy and that for salivary gland cancer is 4.1 Gy.^{19, 20} A few reports discuss the uncertainties of the parameters (α and β)^{21, 22}, however, the cell survival curve considering the dose-delivery time has rarely been investigated in the literature. Thus our interest was directed to the evaluation of the cell survival curve after a relatively high-dose-rate irradiation, typically used in radiotherapy, with the uncertainties of the model parameters and the model selection for surviving fractions.

In this study, we investigated the effects of parameter determination on the cell survival curve after 6 MV linac irradiation. As an example supposing radiation therapy, we used experimental cell survival data in NSCLC (H1299 and A549) with faster cell turnover²³ and human salivary gland cancer (HSG) with slower cell growing²⁴ after 6MV linac irradiation at a high dose rate. To evaluate the uncertainties of model parameters and the model selection, three types of models, the LQM and two microdosimetric-kinetic models with and without DREs (MKM_{DR} and MKM) were applied to describe *in vitro* SF data. The model parameters in both models were determined by a MCMC simulation. Through the MCMC analysis, we show the impact of the DREs on cell survival curve in the case of high-dose-rate irradiation with 6 MV linac X-rays, to demonstrate that the LQM may overestimate the actual cell damage in fractionated radiotherapy.

II. MATERIALS AND METHOD

II.A. Cell culture, irradiation condition and cell surviving fraction

To obtain cell survival data in a wide dose range, a human non-small cell lung cancer cell line, H1299, was purchased from the American Type Culture Collection (ATCC). The cells were maintained in Dulbecco's Modified Eagle Medium (DMEM, Sigma, St Louis, Mo, USA) supplemented with 10% fetal bovine serum (FBS, Equitech-Bio Inc., Kerrville, TX) at 37°C in a humidified 95% air and 5% CO₂ incubator. The cells were kept within cell culture dishes with $\phi 100$ mm (Falcon), and were grown to a logarithmic phase.

The cultured cells were irradiated with 6 MV therapeutic X-rays (Mitsubishi Electric Co., Tokyo, Japan) at a dose rate of 2.5 Gy/min. The dish containing the cells was placed on a water-equivalent phantom with 50 mm-thickness at the side of the gantry head in order to compensate for the build-up effect. The absorbed dose (D) in water was determined according to the dose protocol of Japanese standard dosimetry 12.²⁵ The irradiation was performed at room temperature.

After irradiation, the cells were trypsinized and the appropriate number of cells was reseeded in $\phi 100$ mm (Falcon). Next, the cells were cultured in a CO₂ incubator for 10-14 days, replacing the DMEM every 2 days. The cells were then fixed with methanol and stained with 2% Giemsa solution (Kanto Chemical Co. Inc., Tokyo, Japan) to count the number of colonies per dish. The surviving fraction (SF) was determined from the colony counts with the plating efficiency of the non-irradiated cells. In order to reconfirm the result in the first study, we examined the survival for two other cell lines, human non-small cell lung cancer (A549)²⁶⁻³⁰ and human salivary gland cancer (HSG).³¹ The dose rates for 6 MV X-rays were 2.0 Gy/min for A549 and 0.8 Gy/min for HSG.

II.B. Linear-Quadratic model

For expressing the relationship between dose [Gy] and cell surviving fraction (hereafter called the cell survival curve), the linear-quadratic model (LQM) is widely used,¹ which is given by the following formula as

$$-\ln S = \alpha D + \beta D^2 \quad (1)$$

where S is the surviving fraction of cells, and α and β are the proportionality factors of the absorbed dose (D) [Gy^{-1}] and the dose squared (D^2) [Gy^{-2}], respectively. In many cases, the LQM fits fairly well to the survival data by using only these two coefficients.

In this study, the tumor repopulation was not considered because we adopted experimental surviving fraction data in a single-dose irradiation *in vitro*.

II.C. Microdosimetric-Kinetic model

The microdosimetric-kinetic model (MKM) is another cell-killing model, which can consider both the energy deposition in micro-order territories (called domains) and the DNA repair during irradiation. In this model, a cell nucleus is divided into a few hundreds of spherical domains with diameter from 1.0 to 2.0 μm .³²⁻³⁴ After exposing the bio-cells to ionizing radiation, potentially lethal lesions (PLLs) may be induced in a domain. It is postulated that PLLs undergo one of three transformations until the PLLs vanish: (i) a PLL may transform into a lethal lesion (LL) via a first-order process at a constant rate a , (ii) two PLLs may transform into a LL via a second-order process at a constant rate b_d , (iii) a PLL may be repaired via a first-order process at a constant rate c .³³

Here, we assume that the radiation energy is deposited into the domain instantaneously. Let z be a dose to one of the domains at a specific energy in Gy, and the PLLs are created in proportion to the specific energy and the DNA amount in the domain. If the mass of DNA per domain is denoted by g that varies from domain to domain according to the cell phase,^{35, 37} the number of PLLs in the domain as a function of time after irradiation, $x_d(t)$, is described by

$$\begin{aligned} \frac{d}{dt} x_d(t) &= -(a+c)x_d(t) - 2b_d x_d(t)^2 \\ &\cong -(a+c)x_d(t). \quad Q(a+c)x_d(t) \gg 2b_d x_d(t)^2 \end{aligned} \quad (2)$$

This equation can be solved as

$$x_d(t) = k_d g z e^{-(a+c)t}, \quad (3)$$

where $k_d g z$ is the average number of PLLs per domain and k_d is the DSB induction yield. To achieve the prescribed dose, a certain period of irradiation time is required depending on the dose-rate. During the irradiation, the energy to a domain is discontinuously absorbed. We can thus describe the energy deposition into domains in a short period of time ΔT by dividing the irradiation time T into N sections as $T = N\Delta T$.^{38, 39} Let $z_1, z_2, \dots, z_N$ and $g_1, g_2, \dots, g_N$ be the specific energy and the DNA amount per domain at every period, $0 \sim \Delta T, \Delta T \sim 2\Delta T, \dots, (N-1)\Delta T \sim N\Delta T$, respectively. The kinetic equation of PLLs per domain is described as

$$\begin{aligned}
x_d(t) &= k_d g_1 z_1 e^{-(a+c)t} \\
&\quad [0 \leq t < \Delta T] \\
x_d(t) &= \sum_{n=1}^2 k_d g_n z_n e^{-(a+c)[t-(n-1)\Delta T]} \\
&\quad [\Delta T \leq t < 2\Delta T] \\
&\quad \text{M} \\
x_d(t) &= \sum_{n=1}^{N-1} k_d g_n z_n e^{-(a+c)[t-(n-1)\Delta T]} \\
&\quad [(N-2)\Delta T \leq t < (N-1)\Delta T] \\
x_d(t) &= \sum_{n=1}^N k_d g_n z_n e^{-(a+c)[t-(n-1)\Delta T]} \\
&\quad [(N-1)\Delta T \leq t]
\end{aligned} \tag{4}$$

The number of LLs per domain, w_d , can be expressed by the next equation,

$$\frac{d}{dt} w_d = a x_d(t) + b_d x_d(t)^2. \tag{5}$$

Substituting Eq. (4) into Eq. (5) for each period of time, the accumulated number of LLs per domain is given by

$$\begin{aligned}
w_d &= A \sum_{n=1}^N (g_n z_n) + B \sum_{n=1}^N (g_n^2 z_n^2) \\
&\quad + B \left\{ 2 \sum_{n=1}^{N-1} \sum_{m=n+1}^N [e^{-(m-n)(a+c)\Delta T}] g_n g_m z_n z_m \right\},
\end{aligned} \tag{6}$$

$$\text{where } A = \frac{a k_d}{(a+c)} \text{ and } B = \frac{b_d k_d^2}{2(a+c)}.$$

Let $\langle w \rangle_T$ be the average number of LLs per cell nucleus and $\langle w_d \rangle$ the average number of w_d per domain, then $\langle w \rangle_T$ can be stochastically linked to $\langle w_d \rangle$ as

$$\begin{aligned}
\langle w \rangle_T &= \sum_{i=1}^p \langle w_d \rangle \\
&= \sum_{n=1}^N \left[\left(\alpha_n + \frac{y_D}{\rho \pi_d^2} \beta_n \right) D_n + \beta_n D_n^2 \right] \\
&\quad + 2 \sum_{n=1}^{N-1} \sum_{m=n+1}^N \{ \beta_{nm} [e^{-(m-n)(a+c)\Delta T}] D_n D_m \},
\end{aligned} \tag{7}$$

where

$$D_n = \langle z_n \rangle = \int_0^\infty z_n f_z(z_n) dz_n, \tag{8a}$$

$$D_n^2 + \gamma D_n = \langle z_n \rangle^2 + \frac{y_D}{\rho \pi_d^2} \langle z_n \rangle = \int_0^\infty z_n^2 f_z(z_n) dz_n, \tag{8b}$$

$$\alpha_n = A \langle G_n \rangle = A p \int_0^\infty g_n f_g(g_n) dg_n \text{ and} \tag{8c}$$

$$\beta_n = B' \langle G_n \rangle^2 \Phi_n = Bp \int_0^\infty g_n^2 f_g(g_n) dg_n. \quad (8d)$$

Here, p is the average number of domains per cell nucleus; $f_z(z_n)$ is the probability density of the specific energy for each period; γ ($=y_D/\rho\pi r_d^2$) represents the radiation quality including the microdosimetric parameter y_D [keV/ μ m] (dose-mean lineal energy); r_d and ρ represent the radius (0.5 μ m) and the density (1.0 g/cm³) of the domain, respectively; $f_g(g_n)$ is the probability density of the domain having a DNA amount g_n per domain, and $B' \equiv B/p$ and $\Phi_n = \langle g_n^2 \rangle / \langle g_n \rangle^2$ (dimensionless). Assuming that the DNA amount per nucleus does not change within a short irradiation period of time (i.e., $\alpha_n = \alpha_0 = \text{constant}$ and $\beta_n = \beta_{nm} = \beta_0 = \text{constant}$) in the constant absorbed dose rate $\dot{D}$ during irradiation time T [h] ($\langle z_1 \rangle = \langle z_2 \rangle = \dots = \langle z_N \rangle = D_n = \dot{D} \Delta T$), we have the next equation

$$\begin{aligned} \langle w \rangle_T = \sum_{n=1}^N & \left[\left(\alpha_0 + \frac{y_D}{\rho\pi r_d^2} \beta_0 \right) \mathcal{B} \Delta T + \beta_0 (\mathcal{B} \Delta T)^2 \right] \\ & + 2\beta_0 \sum_{n=1}^{N-1} \sum_{m=n+1}^N \left\{ \left[e^{-(m-n)(a+c)\Delta T} \right] \right\} (\mathcal{B} \Delta T)^2. \end{aligned} \quad (9)$$

Taking the limit N to infinity, Eq. (9) can be approximately expressed as

$$\begin{aligned} \lim_{N \rightarrow \infty} \langle w \rangle_T &= \lim_{N \rightarrow \infty} \sum_{n=1}^N \left[\left(\alpha_0 + \frac{y_D}{\rho\pi r_d^2} \beta_0 \right) \mathcal{B} \Delta T + \beta_0 (\mathcal{B} \Delta T)^2 \right] \\ &+ 2 \lim_{N \rightarrow \infty} \sum_{n=1}^{N-1} \sum_{m=n+1}^N \left\{ \beta_0 \left[e^{-(m-n)(a+c)\Delta T} \right] \right\} (\mathcal{B} \Delta T)^2 \\ &\equiv \left(\alpha_0 + \frac{y_D}{\rho\pi r_d^2} \beta_0 \right) D + \beta_0 \left\{ \frac{2}{(a+c)^2 T^2} \left[(a+c)T + e^{-(a+c)T} - 1 \right] \right\} D^2. \end{aligned}$$

Next assuming that the LLS' number per nucleus follows the Poisson distribution, the relation between dose ($D = \dot{D}T$) and surviving fraction (S) including dose-delivery time (T) can be described by

$$-\ln S = \left(\alpha_0 + \frac{y_D}{\rho\pi r_d^2} \beta_0 \right) D + \frac{2}{(a+c)^2 T^2} \left[(a+c)T + e^{-(a+c)T} - 1 \right] \beta_0 D^2 \quad (10)$$

where

$$\alpha = \alpha_0 + \frac{y_D}{\rho\pi r_d^2} \beta_0 \quad (11a)$$

$$\beta = \frac{2}{(a+c)^2 T^2} \left[(a+c)T + e^{-(a+c)T} - 1 \right] \beta_0 = F \beta_0. \quad (11b)$$

Equation (10) is the formula of the cell survival curve considering SLD repair during irradiation. If the dose-delivery time T [h] is negligibly short as a special case of high-dose-rate irradiation (instantaneous single-dose irradiation), Eq. (10) can be approximated as a linear-quadratic relation.

$$\begin{aligned} -\ln S &= (\alpha_0 + \gamma \beta_0) D + \beta_0 D^2 \\ &= \alpha D + \beta D^2 \end{aligned} \quad (12)$$

This formula of the MKM takes the same form as that of the LQ model in Eq. (1). However, it should be noted that the coefficient (α) of the dose (D) includes a microdosimetric parameter (γ). Hereafter,

we will use Eq. (12) and Eq. (10) for the formulae of MKM and MKM_{DR}, respectively.

II.D. Markov chain Monte Carlo method to determine the model parameters and DIC-value

To determine the model parameters in the LQ model or MK model, we adopted a Bayesian Markov chain Monte Carlo (MCMC) simulation. In the MCMC simulation, the sets of parameters (θ) for the LQM, MKM and MKM_{DR} consist of $\theta(\alpha, \beta, 1/\sigma)$, $\theta(\alpha_0, \beta_0, 1/\sigma)$ and $\theta(\alpha_0, \beta_0, a+c, 1/\sigma)$, respectively. σ is the standard deviation of $-\ln S$. The algorithm of the MCMC simulation is summarized in figure 1. According to this algorithm, we sampled the parameters in each model. Here, we assumed that the uncertainty for $-\ln S$ follows the normal distribution and the prior distributions of α, β, α_0 and β_0 are uniform because there is no pre-information about the parameters for each model. On the other hand, the prior distribution of SLD repair ($a+c$) for tumor cells was assumed to be normally-distributed as 2.187 ± 0.395 according to the report by Inaniwa et al.³⁸ Thus, the relation between the likelihood function $P(d_i|\theta)$ and the posterior probability $P(\theta|d)$ is given by

$$P(d|\theta) = \prod_{i=1}^N [P(d_i|\theta)]$$

$$= \prod_{i=1}^N \left\{ \frac{1}{\sqrt{2\pi}\sigma} \exp \left[-\frac{(-\ln S_{\text{exp}_i} + \ln S_{\text{model}})^2}{2\sigma^2} \right] \right\} \quad (13)$$

where d_i ($i=1 \sim N$) are the set of experimental cell survival data represented by the vector ($D_i, -\ln S_{\text{exp}_i}$) ($i=1 \sim N$), S_{exp_i} is the measured survival value, S_{model} is the surviving fraction calculated by each model (LQM, MKM or MKM_{DR}). The ratio of posterior probability for the parameter's candidate (at timing of $t+1$) $P(\theta^{\text{candidate}}|d)$ and that for the previous condition (at timing of t) $P(\theta^{(t)}|d)$ is given by

$$\alpha_p = \frac{P(\theta^{\text{candidate}}|d)}{P(\theta^{(t)}|d)}, \quad (14)$$

where α_p is the ratio of posterior probability to determine the next set of parameters, which corresponds to the transition probability.⁴⁰

The model fidelity was also evaluated by using an index, the deviance information criterion (DIC). This index is useful in the Bayesian model selection problem, where the posterior distributions of the models have been obtained by the MCMC method.¹⁵ The DIC is defined as

$$\text{DIC} = D_{\text{hat}} + 2p_D \quad (15)$$

where

$$p_D = D_{\text{bar}} - D_{\text{hat}} \quad (16)$$

and D_{bar} is the posterior mean of the deviance ($D_{\text{bar}} = L^{-1} \sum_{l=1}^L [-2\log P(d|\theta^{(l)})]$), D_{hat} is a point estimate of the deviance (thus $D_{\text{hat}} = -2\log P(d_i|\bar{\theta})$). We calculated the DIC values for the LQM, MKM and MKM_{DR} to evaluate the adequacy of the model for cell survival curve taking DREs into account.

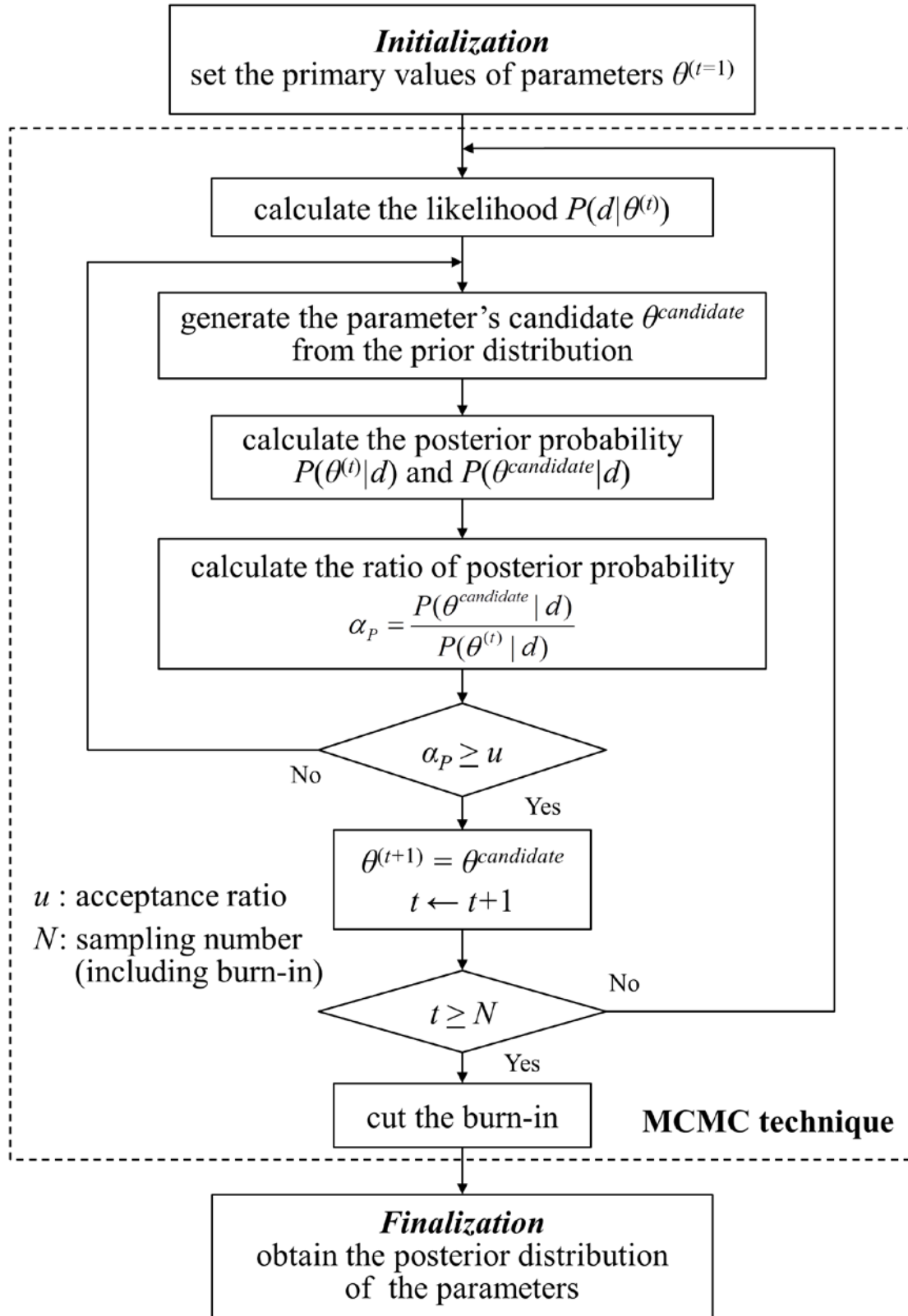


Fig. 1. Algorithm of the Markov chain Monte Carlo (MCMC) method used in this study. Here, we assume that the sampling number is equal to 11000 including a burn-in of 1000.

II.E. Calculation of the uncertainties of α/β and BED

The ratio of α and β (α/β) in the LQM is of importance to evaluate the curative effects in radiotherapy. For example, this ratio is widely used for calculating the biological effective dose (BED).⁴¹ While the parameter α is characterized by a microdosimetric quantity (y_D) in the MKM, β is given as a function of the dose-delivery time T in the MKM_{DR}.³⁶ By considering the change of β with T , the ratio α/β in the MKM_{DR} is given by

$$\frac{\alpha}{\beta} = \frac{\alpha_0}{F\beta_0} + \frac{y_D}{F\rho\pi_d^2} \quad (17)$$

In addition, the BED for each model was also evaluated in the same manner as α/β . BED is defined as

$$\text{BED} = nD_n \left(1 + \frac{D_n}{\alpha/\beta} \right) \quad (18)$$

where D_n is the absorbed dose per fraction in an n -fractionated regimen.

In this study, focusing on the case of human non-small cell lung cancer, H1299, we evaluated the uncertainty of α/β and the relation between the dose and BED. The uncertainties of α/β in the LQM and MKM_{DR} were evaluated by using the set of parameters of (α, β) sampled via MCMC. Subsequently, the BED in each model was deduced by Eq. (18) with the α/β value obtained.

II.F. Comparison with another cell-killing model (USC)

The MKM_{DR} exhibits a linearity of dose-SF relation in a higher dose range.⁹ In recent decades, a couple of cell-killing models, i.e., universal survival curve (USC)⁴² and LQ-Linear (LQ-L) model⁴³, have been proposed to describe properly cell killing in the high dose range. These models are composed of LQ model at dose range of $D < D_T$ and multitarget (MT) model at dose range of $D \geq D_T$. D_T is the transition dose at which the LQ model smoothly connects to the terminal asymptote of the MT model. In addition to the comparison among the three models mentioned earlier, we compared the MKM_{DR} with the USC (essentially the same as the LQ-L model) and examined the model selection.

The dose-SF relation in the USC is given by

$$\begin{aligned} -\ln S &= \alpha D + \beta D^2 & \text{if } D < D_T \\ -\ln S &= (\alpha + 2\beta D_T)D - \beta D_T^2 & \text{if } D \geq D_T \end{aligned} \quad (19)$$

where α and β are the LQ parameters in Eq.(1). As the same manner, the MCMC analysis for the cell-killing model of USC in Eq. (19) was further performed.

III. RESULTS AND DISCUSSION

III.A. Cell survival curves in consideration of dose-rate effects

To evaluate the cell survival curves and the model parameters in the LQM, MKM and MKM_{DR}, we used cell survival data after irradiation with therapeutic 6 MV linac X-rays at a relatively high dose rate (0.8-2.5 Gy/min) in human non-small cell lung cancer (H1299, A549) and human salivary gland cancer (HSG). Among them, we experimentally obtained the cell survival data for H1299 in a wide

range of dose ≤ 18 Gy by means of clonogenic survival assay. The parameters in each model were determined with their uncertainties by a Markov chain Monte Carlo (MCMC) simulation. The information associated with the parameters in this study is summarized in Table 1. In this table, the uncertainties of the parameters are represented by standard deviation (SD). The microdosimetric radiation quality γ is deduced from the y_D value reported so far.³¹ The γ depends on radiation energy and type, but it has less depth-dependence on y_D value in water for a fixed $10 \times 10 \text{ cm}^2$ field.⁴⁴ Thus, the approximated y_D value as in Table 1 is used in the present study. By using the mean values of the parameters in Table 1, we described the cell survival curves for the LQM, MKM and MKM_{DR} (using Eqs. (1), (10) and (12)) as shown in Fig. 2. In Fig. 2, the shape of the curves by the MKM_{DR} coincided with that by the LQM and the MKM. In contrast, in the low dose range (≤ 6 Gy) and in a high dose range (17.5-25 Gy), the curve in the MKM_{DR} is slightly higher than that by the LQM (or the MKM) as shown in Fig. 3 for the H1299 cell line. The MKM_{DR} has the advantage that enables us to consider DNA repair during irradiation (DREs). Inferring from the fitting result in Fig. 3, the DREs have an influence on not only the linearity of SF in the high dose range but also the convexity upward in the low dose range as illustrated in Fig. 4. Although the difference is minor, the result here suggests that the cell survival is affected by DNA repair during irradiation even at high dose rates in radiotherapy.

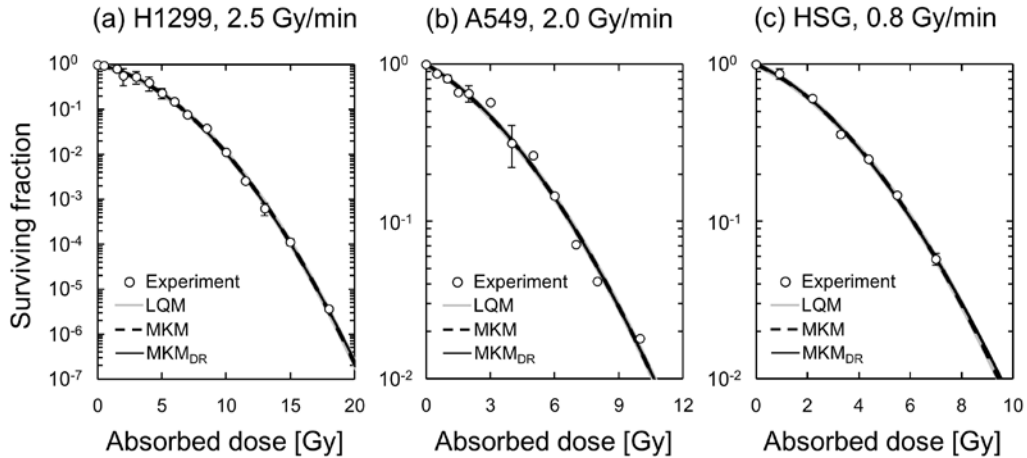


Fig. 2. Cell survival curves given by the LQM, the MKM and the MKM_{DR}: the gray solid line is for the LQM, the black dotted line is for the MKM and the black solid line is for the MKM_{DR}. The symbols represent the data points from the cell biological experiment, in which the error bar is the deviance in the data. The averaged cell survival in H1299 cell line is derived from three experimental points, and the data in A549 and HSG cell lines were taken from references.²⁶⁻³¹

Table 1. Model parameters determined by MCMC simulation			
Cell line type	Model type	Parameters	mean $\pm$ sd
H1299	LQM	α [Gy ⁻¹]	0.138 $\pm$ 0.027
		β [Gy ⁻²]	0.031 $\pm$ 0.002
	MKM	α_0 [Gy ⁻¹]	0.122 $\pm$ 0.028
		β_0 [Gy ⁻²]	0.031 $\pm$ 0.002
		γ [Gy]	0.480 *
	MKM _{DR}	α_0 [Gy ⁻¹]	0.100 $\pm$ 0.027
		β_0 [Gy ⁻²]	0.035 $\pm$ 0.002
		γ [Gy]	0.480 *
		$(a+c)$ [h ⁻¹]	2.218 $\pm$ 0.401
A549	LQM	α [Gy ⁻¹]	0.185 $\pm$ 0.051
		β [Gy ⁻²]	0.023 $\pm$ 0.007
	MKM	α_0 [Gy ⁻¹]	0.174 $\pm$ 0.053
		β_0 [Gy ⁻²]	0.023 $\pm$ 0.006
		γ [Gy]	0.480 *
	MKM _{DR}	α_0 [Gy ⁻¹]	0.166 $\pm$ 0.057
		β_0 [Gy ⁻²]	0.025 $\pm$ 0.007
		γ [Gy]	0.480 *
		$(a+c)$ [h ⁻¹]	2.202 $\pm$ 0.393
HSG	LQM	α [Gy ⁻¹]	0.174 $\pm$ 0.095
		β [Gy ⁻²]	0.033 $\pm$ 0.016
	MKM	α_0 [Gy ⁻¹]	0.157 $\pm$ 0.099
		β_0 [Gy ⁻²]	0.033 $\pm$ 0.016
		γ [Gy]	0.480 *
	MKM _{DR}	α_0 [Gy ⁻¹]	0.144 $\pm$ 0.106
		β_0 [Gy ⁻²]	0.039 $\pm$ 0.018
		γ [Gy]	0.480 *
		$(a+c)$ [h ⁻¹]	2.202 $\pm$ 0.392

* The γ -value for linac-6MV X-rays was obtained from other reports²⁴

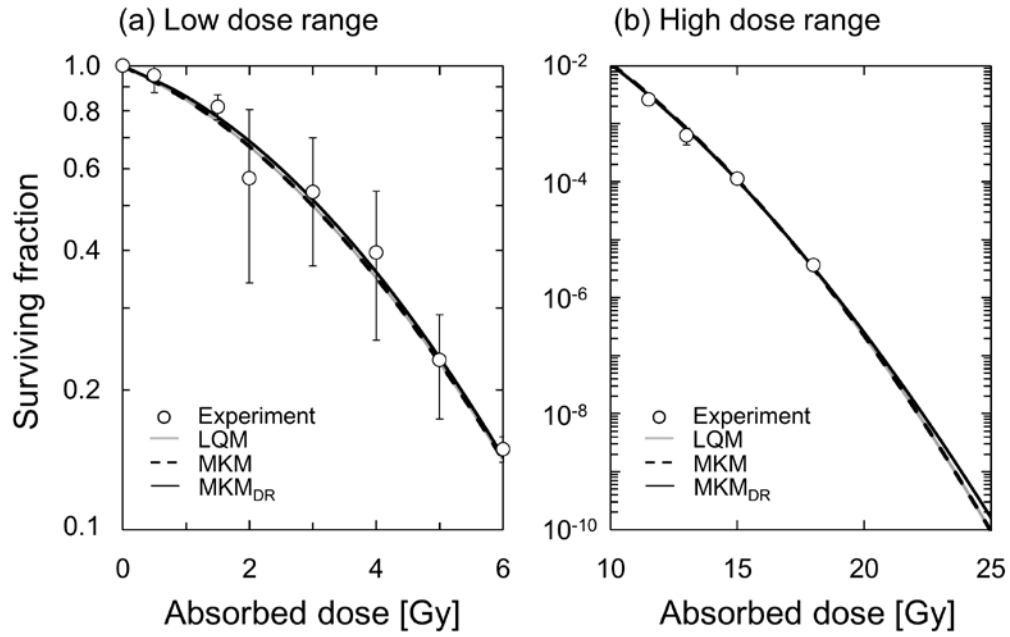


Fig. 3. Cell survival curves described by the models in comparison with *in vitro* SF data in H1299 cell line (described in Fig. 2a): (a) for the low dose range up to 6 Gy and (b) for the high dose range 10-35 Gy. The SF estimated by the MKM_{DR} is slightly higher than that by the LQM or the MKM.

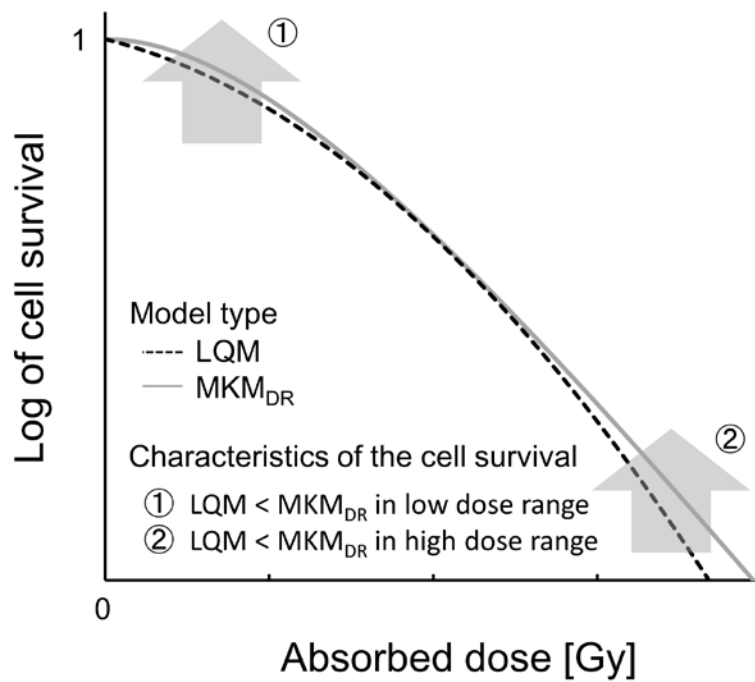


Fig. 4. Schematic image for the characteristics of the cell-killing model including the DREs (the MKM_{DR}). At the fitting procedure, the DREs have an influence on the curve shape in the low dose range as well as on the linearity of SF in the high dose range.

III.B. Uncertainties of the model parameters and the model selection

The determination of the parameters in the surviving fraction model is of importance because it has a direct effect on treatment planning in radiation therapy. The set of parameters (α , β) in each model (LQM, MKM, or MKM_{DR}) were sampled via MCMC technique in this study. Figure 5 shows two-dimensional contour plots in each model ((a) for LQM, (b) for MKM and (c) for MKM_{DR}). The uncertainties represented by SD are summarized in Table 1. The data number-dependent uncertainties for the parameters (α and β) are observed in Fig. 5, where the uncertainties in the H1299 cell line are much smaller than those in the other cell lines because of the largest number of experimental data among them. It is also recognized that the set of parameters in the MKM_{DR} has a different characteristics in that α has a smaller value while β has a larger value than those in the LQM and MKM. This feature explains the higher SF in the low-dose range in Fig. 4.

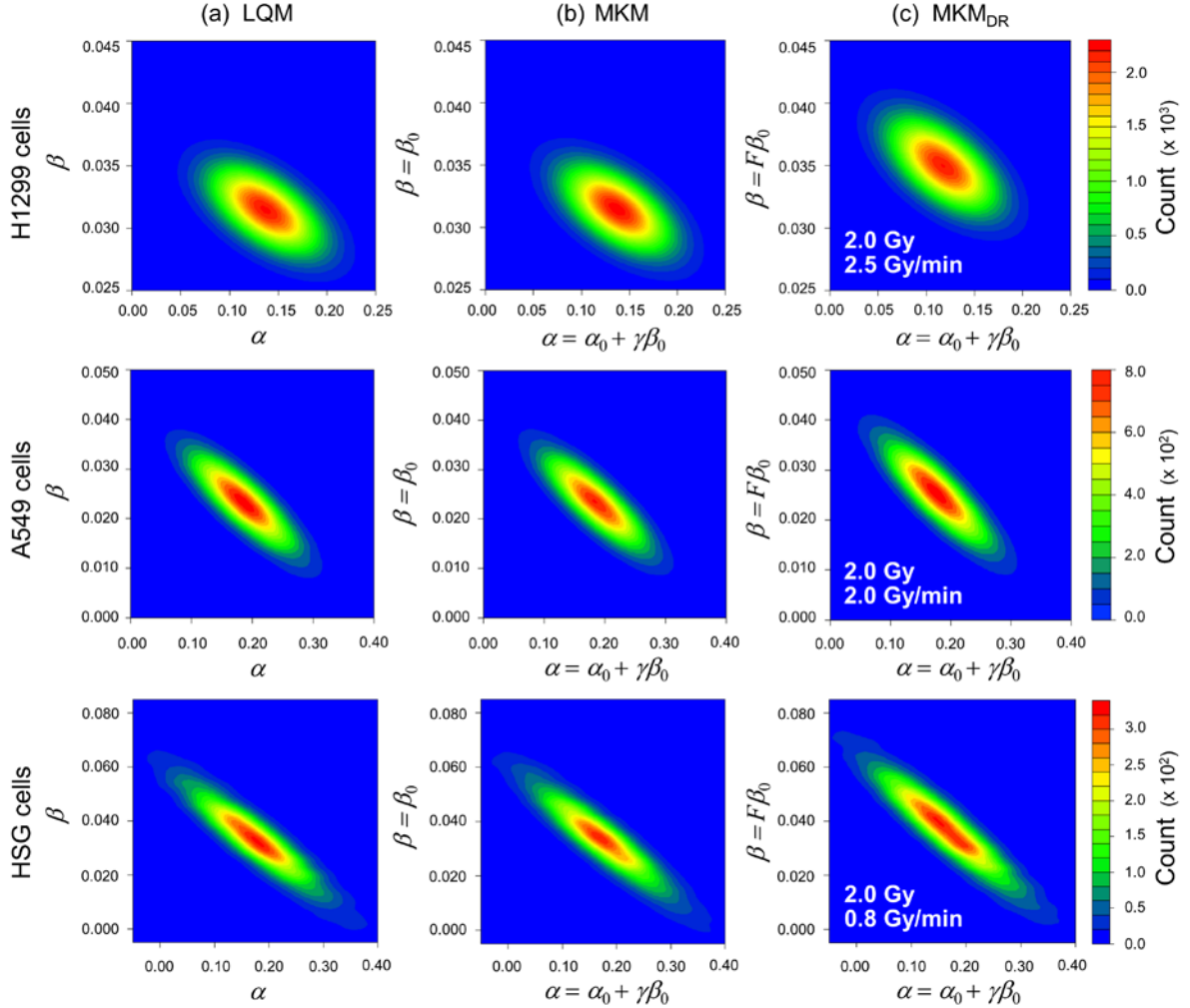


Fig. 5. Uncertainties of the model parameters (α and β) in a 2-dimensional contour map: (a) is for the LQM, (b) is for the MKM, and (c) is for the MKM_{DR} including the DREs.

For evaluating the model selection, we compared the values of the deviance information criterion (DIC) among the LQM, MKM and MKM_{DR}. The DIC is deduced from the uncertainty and the number of parameters. The calculated DIC values are listed in Table 2. As to the comparison between the LQM and MKM, the DIC value in the MKM is worse than that in the LQM. This is because the formula of MKM (Eq. (10)) includes more parameters than that of the LQM. In contrast, the DIC value of the MKM_{DR} considering the DREs is the smallest among the models. This suggests that the fit quality is improved by using the MKM_{DR} despite the fact that the model has the largest parameter number. The result therefore suggests that the cell-killing model including the DREs is better suited for predicting the surviving fraction even in the case of high-dose-rate irradiation.

Table 2. DIC value in each model (LQM, MKM or MKM_{DR}) to evaluate model selection

Model type	Cell line type		
	H1299	A549	HSG
LQM	-1.29	-2.58	-1.32
MKM	-1.25	-2.57	-1.31
MKM _{DR}	-3.34	-2.83	-1.49

III.C. Uncertainties of α/β in radiation therapy

For evaluating the accuracy of the ratio of α and β (α/β) in indices such as BED, the uncertainty of the value was estimated in both the LQM and MKM_{DR}. Because the α/β value depends on the dose-delivery time T in the MKM_{DR}, we adopted a popular fractionated scheme where the dose per fraction is 2.0 Gy/fraction and the fractionation number is 30 (i.e., 60 Gy in total dose). Figure 6 shows the comparison between the results for α/β in the LQM and MKM_{DR} as a function of dose per fraction. The values of α/β in the LQM and in MKM_{DR} at 2.0 Gy/fraction were 4.45 ± 1.14 and 3.41 ± 0.96 , respectively. The mean α/β in the MKM_{DR} is smaller than that in the LQM although it is within the same degree of uncertainty. This result suggests that the traditional α/β for cancer cells in the LQM is higher because the model doesn't consider the dose-rate effect. In addition, the value of α/β in the LQM with uncertainty is 4.45 ± 1.14 , indicating that the irradiation scheme is affected by 25.6% uncertainty of α/β in radiation therapy.

From the view point of DIC, the fidelity of the MKM_{DR} is better than that of the LQM. However, because the α/β value in the MKM_{DR} varies depending on the dose rate, we tried to calculate the α/β value for another dose rate, 0.5 Gy/min. Figure 6 represents the dose-dependent α/β in the MKM_{DR} in comparison with the constant α/β in the LQM. In an effort to obtain a certain level of tumor control, the hypo-fractionated radiation scheme¹² or real-time tumor-tracking radiation therapy (RTRT)⁴⁵ has been conducted to eradicate cancer cells. In such treatment planning, a protraction of the dose-delivery time to 1-10 min is usually required. Thus, if we carry through the hypo-fractionated

regimen, it should be noted that the α/β value increases as the dose per fraction increases. The increasing tendency of the α/β value is similar to that reported in a previous report.⁴⁶ Our MCMC result provides additionally the uncertainty of α/β depending on dose rate.

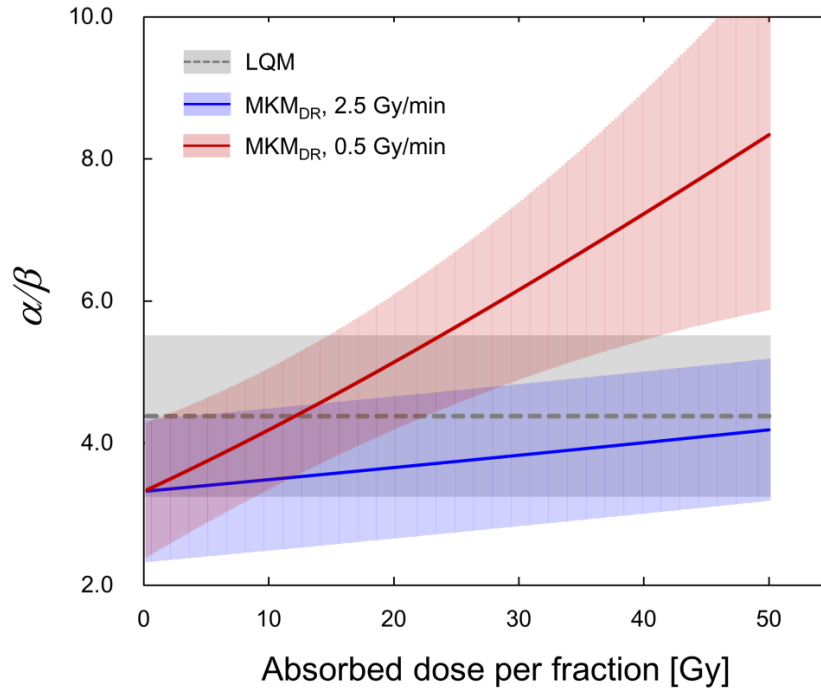


Fig. 6. Comparison between the α/β values in the LQM and MKM_{DR}. The values with uncertainties for 2.5 and 0.5 Gy/min were deduced by using Eq. (17) in the MKM_{DR} model. At low dose region below 10 Gy, the conventional α/β value in the LQM for cancer cells has a tendency to be higher than that in the MKM_{DR}.

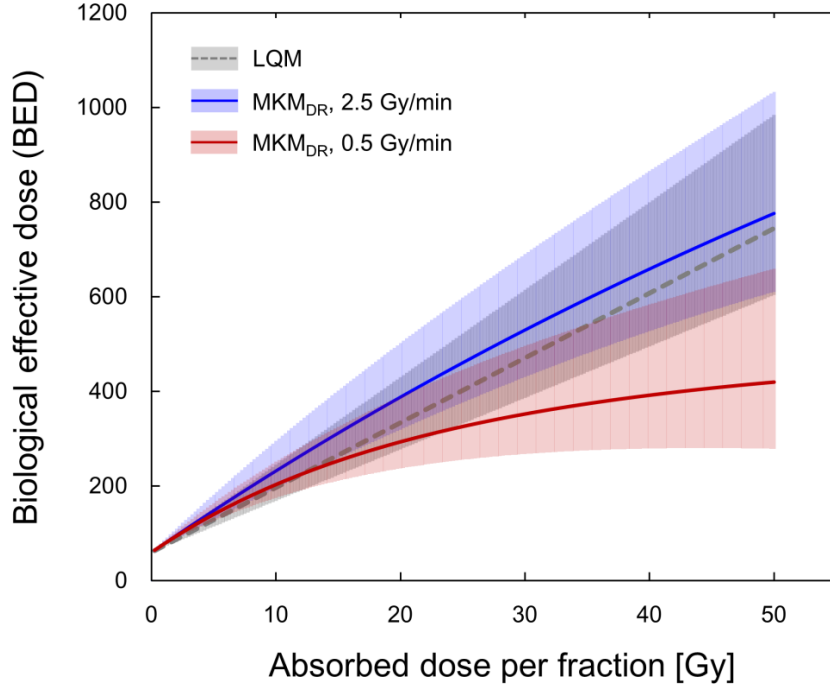


Fig. 7. Dose-dependent α/β value calculated in the MKM_{DR} in comparison with the LQM. It should be noted that the α/β value increases as the dose per fraction increases, which is important in hypo-fractionated regimens.

The BED value with its uncertainty was also estimated according to Eq. (18). Figure 7 shows the relationship between dose per fraction and BED by the MKM in comparison with that by the LQM. The probability distribution of the BED value is not a normal distribution because the formula of the BED is defined by Eq. (18). As shown in Fig. 7, when performing a fractionated regimen with 2 Gy/fraction, the BED values in the MKM_{DR} for 0.5 and 2.5 Gy/min are 95.8 (87.9-109.9: 68% credible interval) Gy and 94.4 (87.2-106.6: 68% credible interval) Gy, respectively, while the BED value in the LQM is 87.4 (81.7-97.0: 68% credible interval) Gy independent of the dose rate. It should be noted that the BED has a tendency to saturate as the dose rate increases.

III.D. Estimation of curative effects during irradiation (dose-rate effect)

Because SLD repair during irradiation has been recognized from the cell survival curve even in relatively high-dose-rate irradiation, we estimated the curative effect in the fractionated regimen. As a typical fractionation scheme, we adopted 2.0 Gy/fraction times 30 fractions and 20 Gy/fraction times 3 fractions (total dose 60 Gy)⁴⁷ as a trial examination. Figure 8 shows the dose-survival relations under these regimens with 2.0 Gy/fraction and with 20 Gy/fraction (a relatively higher dose per fraction). Especially in a multi-fractionated regimen with 2 Gy/fraction, the final SF estimated by the MKM_{DR} is much higher than that by the LQM, which suggests that additional fractions (3 times) are needed in the MKM_{DR} to attain the equivalent effect in the use of the LQM. Contrary to this, in a hypo-fractionated

dose of 20 Gy/fr, the difference of cell killing between the LQM and the MKM_{DR} is less than that in multi-fractionation. According to a clinical outcome of NSCLC,⁴⁷ stereotactic radiotherapy (SRT) with the fractionation regimen (3×20 Gy, 5×12 Gy or 8×7.5 Gy) is well tolerated at high local control rates with minimal toxicity. However, the cellular repopulation⁴⁸ between time-interval of irradiations cannot be ignored, and thus further investigation about additional source of uncertainties to affect fractionation regimen is necessary.

Previously, it has been pointed out that the LQM may overestimate cell killing by radiation in a higher dose range because the dose-response is inclined to be linear above 12 Gy.⁴⁹ For this reason, the universal survival curve (USC)⁴² and the linear-quadratic-linear (LQ-L) model⁴³ are taken into consideration for better predictions of the dose-response at doses above about 6 Gy. The DIC values of USC model for H1299 and A549 cells are summarized in Table. 3. The uncertainties of USC-specific parameter (D_T) for the both cells are very large and the DIC values of the USC are larger than those of the MKM_{DR} in Table.2. This result suggests that it is better to make a treatment planning based on the MKM_{DR} considering the dose rate and experiment-based SLD repair. In this study, the same tendency to overestimate the killing effect was exhibited in the LQM in the high dose range. The LQM also overestimated cell killing at a low dose below 7 Gy, which contributes to the additional fractions mentioned above.

Table 3. Results of MCMC analysis for USC (LQ-L model)

Cell type	Parameters	Maximum likelihood value and uncertainty	DIC
H1299	α [Gy ⁻¹]	0.118 (68% CI: 0.105-0.164)	-1.42
	β [Gy ⁻²]	0.034 (68% CI: 0.029-0.034)	
	D_T [Gy]	13.72 (min-max: 8.44- ∞)	
A549	α [Gy ⁻¹]	0.135 (68% CI: 0.109-0.241)	-1.40
	β [Gy ⁻²]	0.033 (68% CI: 0.014-0.037)	
	D_T [Gy]	5.926 (min-max: 0.136- ∞)	

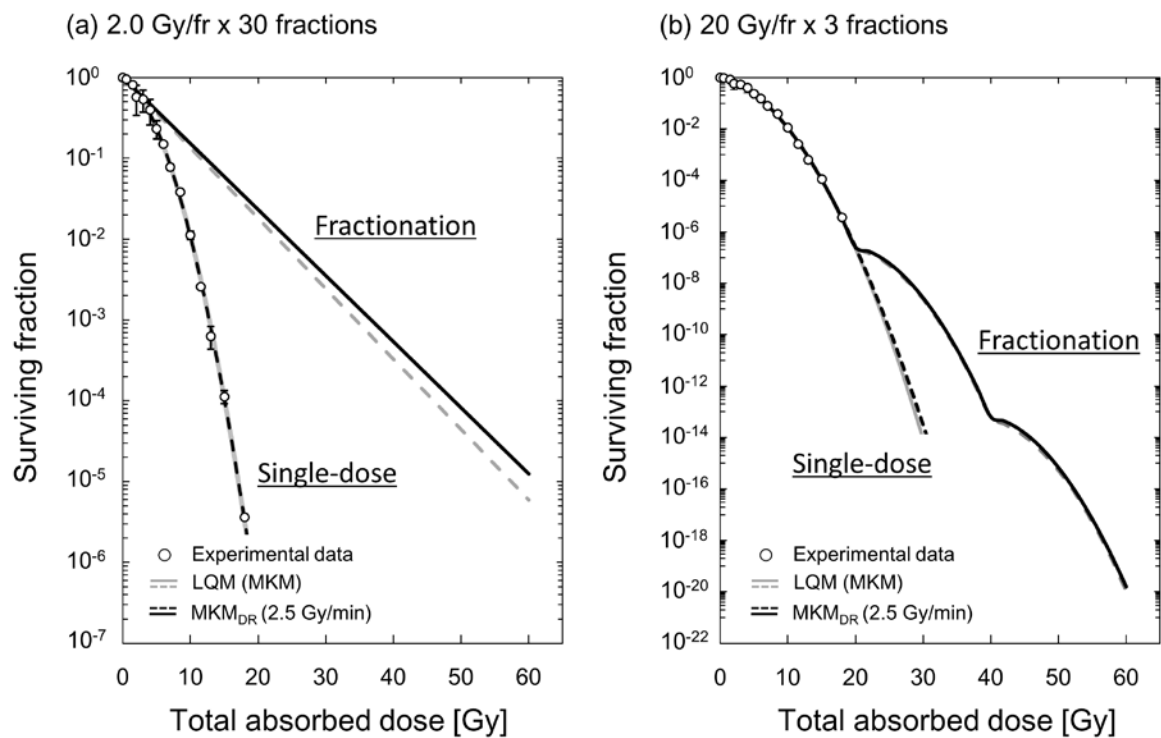


Fig. 8. Dose-survival relations under regimens with (a) 2.0 Gy/fraction and with (b) 20 Gy/fraction. In the multi-fractionated regimen with 2.0 Gy/fraction as shown in figure (a), the final SF estimated by the MKM_{DR} was higher than that by the LQM. This means that additional fractions (3 times) are needed to attain the equivalent effect to that in the LQM.

IV. CONCLUSION

In this study, we investigated model selection for cell surviving fraction under high-dose-rate irradiation. Three types of cell-killing models, the linear-quadratic model (LQM), and two microdosimetric-kinetic models with and without dose-rate effects (MKM_{DR} and MKM) were applied to describe *in vitro* SF data in human non-small cell lung cancer (H1299, A549) and human salivary gland cancer (HSG) exposed to 6 MV linac X-rays at relatively high dose rates. By means of Bayesian analysis (MCMC simulation), the uncertainties of model parameters in each model were deduced. The model selection for the cell surviving fraction was also evaluated with the deviance information criterion (DIC). The results suggest that damage repair during dose delivery plays a key function to precisely estimate cell survival even at a high dose rate typically used in radiotherapy. Consequently, it was shown that the LQM without repair factor during a short dose-delivery time may overestimate actual cell killing in fractionated radiotherapy.

CONFLICTS OF INTERESTS

The authors declare that they have no conflict of interest.

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