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**DOCTORAL THESIS**

**INVESTIGATION OF CELL RESPONSES UNDER PROTRACTED  
EXPOSURE TO IONIZING RADIATION**

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**INVESTIGATION OF CELL RESPONSES UNDER PROTRACTED  
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## TITLE

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## ABSTRACT

Ionizing radiations (particularly X-rays) contribute to not only image diagnosis but also radiation therapy in medical practices, whilst biological effects such as cell kill and mutation after exposure to the ionizing radiations are induced in mammalian cells. Cell kill is one of cell responses caused by some mis-repaired lesions and non-reparable lesions after exposure, which is one of endpoints to discuss non-stochastic effects according to International Commission on Radiological Protection (ICRP). After the accident of Fukushima 1st Nuclear Power Plant (F1-NPP) following the earthquake and tsunami on 11th March, 2011, biological effects (radio-sensitivity) under the protracted (long-term) exposure to low-dose ionizing radiations have drawn a keen interest of the public. Generally interpreted, radio-sensitivity decreases as dose or dose-rate (fluence rate) is decreased. However, recent biological experiments show several reversal radio-sensitivity in low-dose and low-dose-rate range. Evaluation of the reversal radio-sensitivity is a crucial issue from the standpoints of radiation therapy and radiation protection. In this study, focusing on the time course of cell responses during the protracted exposure at various dose-rates, cell responses and radio-sensitivity were investigated by the combination of *in vitro* experimental study and model analysis.

To investigate the time course of cell responses and reversal radio-sensitivity, two main subjects were discussed in this study: one is cell responses and radio-sensitivity under 250 kVp X-rays (standard radiation) protracted exposure at various dose-rates, and the other is development of mathematical modelling considering targeted (energy deposition along radiation particle track) and non-targeted effects (intercellular communication) in wide dose range.

Cell-cycle study showed dose-rate dependence of cell-cycle dynamics in one of the mammalian cell lines, Chinese hamster ovary (CHO-K1), during the exposure at various dose-rates of 0.186-6.0 Gy/h. DNA damage checkpoints through cell cycle depend on the magnitude of dose-rate and the CHO-K1 cells exhibit following cell responses: (i) an cell accumulation in G<sub>2</sub> phase during exposure at lower dose-rates (e.g., 0.186 and 1.0 Gy/h), (ii) the delay of DNA synthesis and an accumulation of the cells in S/G<sub>2</sub> during the exposure at intermediate dose-rate (e.g., 3.0 Gy/h), and (iii) the blocks of cell cycle progressing in G<sub>1</sub>/M and G<sub>2</sub>/M checkpoints and the delay of DNA synthesis during the

exposure at higher dose (e.g., 6.0 Gy/h). The CHO-K1 cells exhibit reversal radio-sensitivity (inverse dose-rate effects: IDREs) with a subtly high radio-sensitivity under exposure at 1.0 Gy/h and an unexpected greater radio-resistance under the exposure at 3.0 Gy/h. The model analysis gives the theoretical explanations that this tendency is caused by the change of DNA amount per nucleus and increases in SLDR (sub-lethal damage repair) rate associated with the fraction of cells in S phase during long-term exposure. Taking account of both the higher radio-sensitivity under 1.0 Gy/h exposure and the radio-resistance after exposure to 3.0 Gy/h, the changes in cell-cycle distribution during exposure might play a key rule of modulating the cell survival curve and are possibly responsible for IDREs.

The involvement of intercellular communication between hit cells and non-hit cells (non-targeted effects) in reversal radio-sensitivity was also evaluated. To analyse the mechanism of non-targeted effects, the integrated cell-killing model considering DNA-targeted and non-targeted effects was developed. A couple of new features of this integrated model are given as follows: (i) the traditional stochastic hit theory with linear-quadratic (LQ) relation is adopted to describe the hit probability to emit cell-killing signals, (ii) the repair kinetics of signal-induced DNA lesions is incorporated, and (iii) repair efficiency for lesions induced by intercellular communication is much lower than that in DNA-targeted effects of radiation. Based on this framework, the present model provides quantitative formulae that enable us to describe a series of cell responses such as signal kinetics, DNA repair kinetics and cell survival. The model was verified by comparing with experimental data of signals, DNA damage number per nucleus and cell kill, suggesting that (i) the LQ relation has a potential to express hit mechanism in non-targeted effects and (ii) the low repair efficiency in non-hit cells is intricately related with low-dose hyper radio-sensitivity (HRS). From the model estimation, it was shown that the low-dose HRS is enhanced more as the DNA repair efficiency in non-hit cells is lower, providing new clues to understand the cell responses in non-targeted effects.

In this thesis, the investigations to estimate the contribution of cell-cycle dynamics and low-dose HRS to reversal of radio-sensitivity after long-term exposure are summarised. Through the analysis by the integrated cell-killing model, a couple of new interpretations for cell responses under low-dose or protracted exposure were presented. This study would contribute to more precise understandings of cell responses after the long-term exposure and low-dose exposure to ionizing radiations.

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## LIST OF PUBLICATIONS

### PAPER AS THE FIRST AUTHOR DURING DR. COURSE

**Modeling cell survival and change in amount of DNA during protracted irradiation.**

Yusuke Matsuya, Kaori Tsutsumi, Kohei Sasaki, Yuji Yoshii, Takaaki Kimura, Hiroyuki Date.

*Journal of Radiation Research* 58 (3): 302-312 (2017)

**Markov chain Monte Carlo analysis for the selection of a cell-killing model under high-dose-rate irradiation**

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**Integrated Modelling of Cell Responses after irradiation for DNA-Targeted Effects and Non-Targeted Effects.**

Yusuke Matsuya, Kohei Sasaki, Yuji Yoshii, Go Okuyama and Hiroyuki Date

Under submission

**Investigation of dose-rate effects and cell-cycle distribution under the protracted exposure for various dose rates.**

Yusuke Matsuya, Stephen J. McMahon, Kaori Tsutsumi, Kohei Sasaki, Go Okuyama, Yuji

Yoshii, Ryosuke Mori, Joma Oikawa, Kevin M. Prise & Hiroyuki Date

Under revision

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## LIST OF ABBREVIATION

|                     |                                                     |
|---------------------|-----------------------------------------------------|
| <b>AIC</b>          | Akaike's information criterion                      |
| <b>ATM</b>          | Ataxia telangiectasia-mutated                       |
| <b>ATP</b>          | Adenosine triphosphate                              |
| <b>BED</b>          | Biological effective dose                           |
| <b>CHO</b>          | Chinese Hamster Ovary                               |
| <b>DNA-PKcs</b>     | DNA-dependent protein kinase catalytic subunit      |
| <b>DRE</b>          | Dose-rate effect                                    |
| <b>DSB</b>          | Double-strand breaks                                |
| <b>F1-NPP</b>       | Fukushima 1st Nuclear Power Plant                   |
| <b>FBS</b>          | Fetal bovine serum                                  |
| <b>HR</b>           | Homologous recombination                            |
| <b>HRS</b>          | Hyper radio-sensitivity                             |
| <b>IC</b>           | Intercellular communication                         |
| <b>ICCM</b>         | Irradiated cell condition medium                    |
| <b>ICRP</b>         | International Commission on Radiological Protection |
| <b>ICRU</b>         | International Commission on Radiation Units         |
| <b>IDRE</b>         | Inverse dose-rate effect                            |
| <b>IL-6</b>         | Interleukin 6                                       |
| <b>IL-8</b>         | Interleukin 8                                       |
| <b>IMK model</b>    | Integrated Microdosimetric-Kinetic model            |
| <b>IndRep model</b> | Induced repair model                                |
| <b>IRR</b>          | Increased radio-resistance                          |
| <b>LET</b>          | Linear energy transfer in keV/ $\mu$ m              |
| <b>LL</b>           | Lethal Lesion                                       |
| <b>LPL model</b>    | Lethal-potentially lethal model                     |
| <b>LQ model</b>     | Linear-Quadratic model                              |
| <b>MK model</b>     | Microdosimetric-Kinetic model                       |
| <b>MTBE</b>         | Medium transfer bystander effect                    |
| <b>NHEJ</b>         | Non-homologous end joining                          |
| <b>NO</b>           | Nitric oxide                                        |

|                  |                                        |
|------------------|----------------------------------------|
| <b>NTE</b>       | Non-targeted effects                   |
| <b>PI</b>        | Propidium iodide                       |
| <b>PLL</b>       | Potentially Lethal Lesion              |
| <b>RBE</b>       | Relative biological effectiveness      |
| <b>RIBE</b>      | Radiation-induced bystander effect     |
| <b>RMR model</b> | Repair-misrepair model                 |
| <b>ROS</b>       | Reactive oxygen species                |
| <b>RPA</b>       | Replication protein A                  |
| <b>SF</b>        | Surviving fraction                     |
| <b>SLDR</b>      | Sub-lethal damage repair               |
| <b>SSB</b>       | Single-strand breaks                   |
| <b>TE</b>        | Targeted effect                        |
| <b>TEPC</b>      | Tissue equivalent proportional counter |
| <b>TGFβ1</b>     | Transforming growth factor-β1          |

## Chapter 1

# Introduction

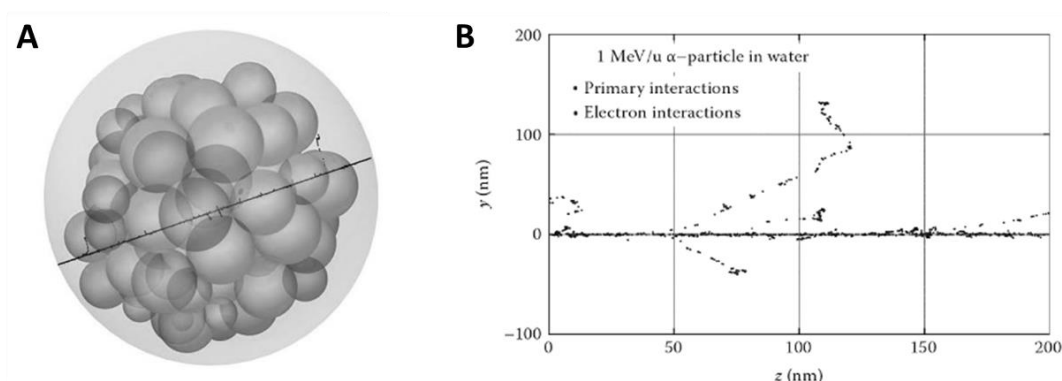
Ionizing radiations contribute to not only image diagnosis but also radiation therapy in medical practice. However, biological effects such as DNA damage with toxicity to cells are induced in human tissues.<sup>1</sup> From the standpoint of radiation protection, it is essential to make clear underlying mechanisms of cell responses after exposure by ionizing radiation. After the exposure, energy is deposited to cells along radiation particle,<sup>2</sup> causing tissue damage represented as DNA damage.<sup>2-6</sup> Most of the damage can be repaired by virtue of DNA repair function which cells potentially have.<sup>7,8</sup> Some non-reparable lesions trigger the induction of cell death.<sup>9</sup> Among the biological effects mentioned above, cell killing after irradiation is one of the important materials to discuss non-stochastic effects according to International Commission on Radiological Protection (ICRP).<sup>10-12</sup> After the accident of Fukushima 1st Nuclear Power Plant (F1-NPP) following the earthquake and tsunami on March 11, 2011, biological effects under low-dose or low-dose-rate exposure to ionizing radiation have drawn a keen interest of the public.<sup>13,14</sup> However, cell responses during long-term exposure and after low-dose exposure remain unclear. In this study, focusing on the time course of cell responses during the protracted exposure at various dose-rates, cell responses and radio-sensitivity were investigated by the combination of *in vitro* experiments and model analysis.

### 1.1. Physical Characteristics of Ionizing Radiation

#### 1.1.1. Basic Knowledge of Radiation

Radiation is the emission or transmission of energy as particles or waves through space or a material. Radiation is separated into two types: one is charged particles and the other is non-charged particles.<sup>15</sup> For example, whilst  $\alpha$ -particles,  $\beta$ -particles, protons and electrons

( $\delta$ -rays) are charged particles, photons (X-rays,  $\gamma$ -rays) and neutrons are non-charged particles.<sup>15</sup> Characteristics of each ionizing radiation are summarized in Table 1-1. The ability for radiation to pass through in materials such as tissue depends on the type of radiation. For example,  $\alpha$ -particles cannot go through even a paper, but  $\beta$ -particles and  $\gamma$ -rays are able to go through a few millimeters Aluminum. Along the particle track, ionizing radiations impart their energy to matter (cells for the case of tissue). Representative images for track structure and energy deposition are shown in Fig. 1-1. The energy spectrum of radiation is intricately related with energy deposition along radiation particle track, which is separated into two types, monoenergetic and heterogeneous radiations (Fig.1-2).<sup>16</sup> Particularly, 200 kVp, 250 kVp X-rays and  $^{60}\text{Co}$   $\gamma$ -rays are often used as standard radiation, because radiation weighting factor  $w_R$  for photons and electrons is defined as unity from the standpoint of radiation protection (Table 1-2).<sup>17</sup>

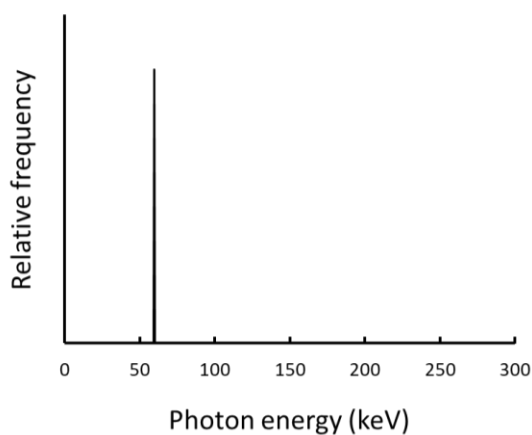


**Figure 1-1. Track structure and energy deposition in matter by charged particle of  $\alpha$ -particle.** A illustrates 1 MeV/u  $\alpha$ -particles crossing a cell nucleus as a radiation target to induce DNA damage. B shows a short segment of the 1 MeV/u  $\alpha$ -particles.<sup>15</sup>

| Name                      | Symbol         | Electric Charge (e) | Mass ( $m_e$ ) | Mean Life (s)         | Production Method            | Remarks                           |
|---------------------------|----------------|---------------------|----------------|-----------------------|------------------------------|-----------------------------------|
| $\gamma$ -Ray             | $\gamma$       | 0                   | 0              |                       | Radioisotope (RI)            | Single energy                     |
| X-ray                     | X              | 0                   | 0              |                       | Accelerator                  | Continuous energy                 |
| Neutrino                  | $\nu$          | 0                   | $\sim 0$       |                       | Radioisotope                 | $\beta$ -decay                    |
| Electron ( $\beta^-$ ray) | $e^-, \beta^-$ | -1                  | 1              |                       | Accelerator, RI              | $\beta$ -ray is continuous energy |
| Positron ( $\beta^+$ ray) | $e^+, \beta^+$ | +1                  | 1              |                       | Accelerator, RI              | Annihilation quanta               |
| Proton                    | p              | +1                  | 1,836          |                       | Accelerator                  |                                   |
| Neutron                   | n              | 0                   | 1,839          | $1.1 \times 10^3$     | Accelerator, Nuclear reactor |                                   |
| Deuteron                  | d              | +1                  | 3,670          |                       | Accelerator                  |                                   |
| Triton                    | t              | +1                  | 5,479          | $10^9$                | Accelerator                  |                                   |
| $\alpha$ -Particle        | $\alpha$       | +2                  | 7,249          |                       | Accelerator, RI              |                                   |
| Muon                      | $\mu^\pm$      | $\pm 1$             | 207            | $2.15 \times 10^{-6}$ | High energy nuclear reaction |                                   |
| Pion (charged)            | $\pi^\pm$      | $\pm 1$             | 273            | $2.65 \times 10^{-8}$ | High-energy nuclear reaction |                                   |
| Pion (neutral)            | $\pi^0$        | 0                   | 264            |                       | High energy nuclear reaction |                                   |
| Fission fragment (light)  |                | $\sim 36$           | $\sim 96 m_p$  |                       | Nuclear fission              |                                   |
| Fission fragment (heavy)  |                | $\sim 56$           | $\sim 140 m_p$ |                       | Nuclear fission              |                                   |

Table 1-1. Type of ionizing radiation and the characteristics<sup>15</sup>

A. Monoenergetic photon



B. Heterogeneous photon

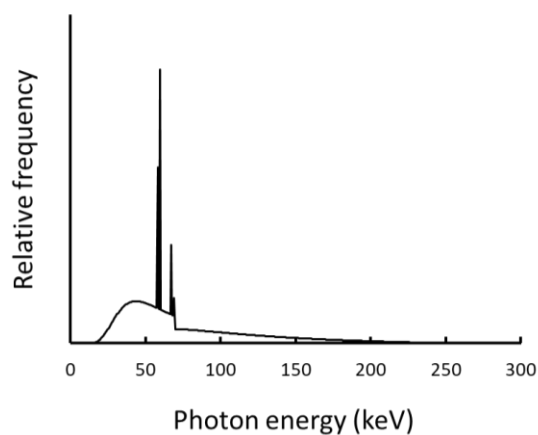
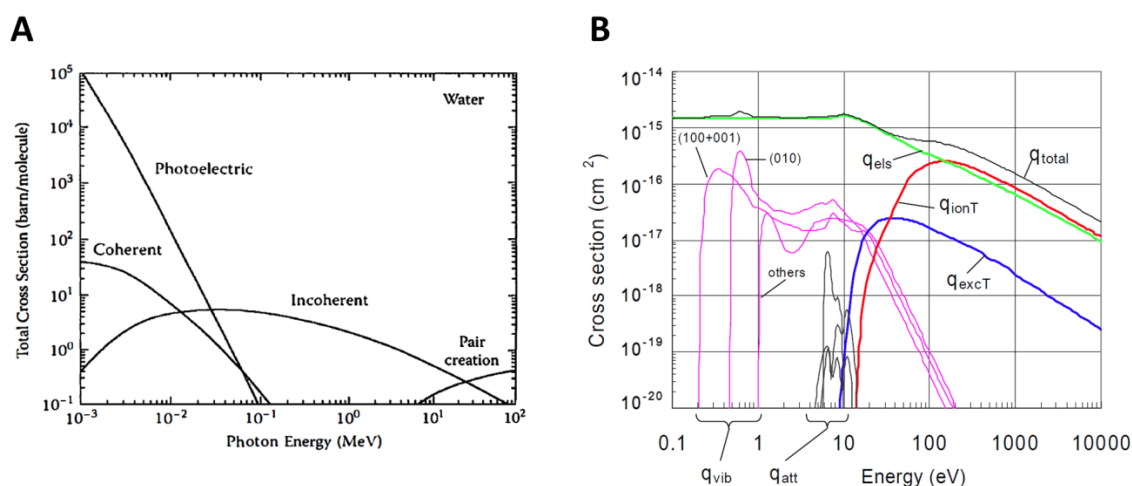


Figure 1-2. Typical spectra for photon beams: A for monoenergetic photon and B for heterogeneous photon beams.<sup>16</sup>

| Radiation type                                 | Radiation weighting factor, $w_R$       |
|------------------------------------------------|-----------------------------------------|
| Photons                                        | 1                                       |
| Electrons and muons                            | 1                                       |
| Protons and charged pions                      | 2                                       |
| Alpha particles, fission fragments, heavy ions | 20                                      |
| Neutrons                                       | A continuous function of neutron energy |

**Table 1-2. Radiation weighting factors of ionizing radiations**<sup>17</sup>

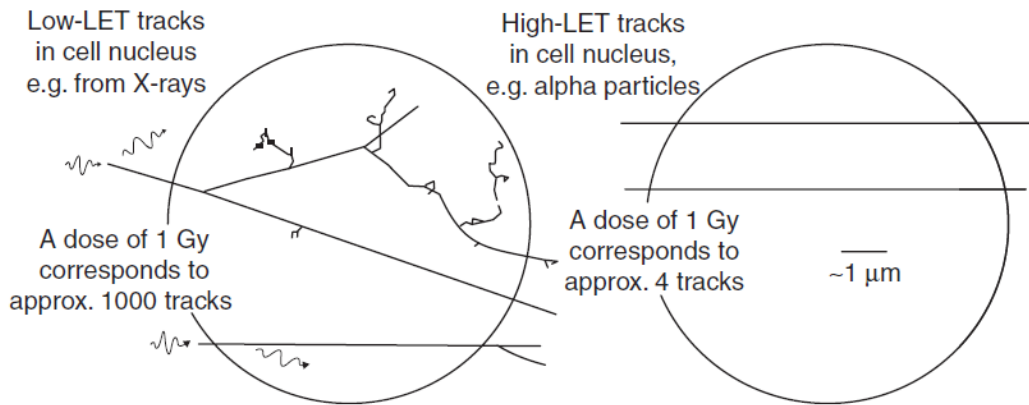
Several types of interactions occur after radiation particles such as photons, electrons and charged heavy ion particles pass through matters.<sup>18-20</sup> Referring the standard radiation of photon beams, electrons are generated via interactions with matter such as photoelectric effect, Compton scattering and pair production.<sup>18</sup> The electrons then deposit their energy to matter via several events such as ionization, excitation and so on.<sup>19</sup> There are lots of published data of the interactions probability with liquid water (equivalent to tissue), in which the cross sections are given as shown in Fig. 1-3. The interactions of electrons with matter in cell induce cell toxicity after the exposure to standard radiation.



**Figure 1-3. Cross sections for the interactions with liquid water:** A for photons (photoelastic, pair production, coherent and incoherent)<sup>18</sup> and B for electrons (ionization ( $q_{\text{ionT}}$ ), excitation ( $q_{\text{excT}}$ ), elastic ( $q_{\text{els}}$ ) and so on).<sup>19,21</sup>

### 1.1.2. Unit of Radiation Quantities

The quantities of radiation has been discussed by using units defined by International Commission on Radiation Units (ICRU) Report 33 (1980)<sup>22</sup> and Report 51 (1993).<sup>23</sup> Biological effects after exposure to ionizing radiation depend on the physical characteristics such as localization of energy deposition along particle track, quality of energy in matter and so on. In this regard, linear energy transfer (LET) in keV/μm as a track structure, absorbed dose in Gy as density of energy deposition in matter and dose rate in Gy/h as rate of energy fluence are defined.



**Figure 1-4. Illustration of low- and high-LET tracks of ionizing radiations:** left panel for low-LET track by X-rays and right for high-LET track by alpha particles. In this figure, the circles represent the typical size of mammalian cell nuclei.<sup>24</sup>

#### 1.1.2.1. Linear Energy Transfer (LET)

The linear energy transfer (LET) is defined for charged particles. The LET in matter is expressed by

$$\text{LET} = \left( \frac{dE}{dl} \right)_{\Delta}, \quad (1-1)$$

where  $dE$  is energy loss of charged particles within the length of  $dl$  in matter, and  $\Delta$  represents the energy of secondary electrons generated by collision. In general, whilst X-rays is defined

as low-LET radiation, alpha particles belong to high-LET radiation. Representative illustration of track of low- and high-LET tracks passing through cell nucleus is described in Fig. 1-4.

### 1.1.2.2. Absorbed Dose and Absorbed Dose rate

The quantity of ionizing radiation is defined as the division of mean energy deposition  $d\varepsilon$  by a given mass  $dm$  of matter. The absorbed dose (hereafter described as  $D$ ) in Gy given by

$$D = \frac{d\varepsilon}{dm}. \quad (1-2)$$

The unit of absorbed dose  $D$  is J/kg and 1 Gy equals to 1 J/kg.

Absorbed dose rate  $\dot{D}$  is defined as the increment of absorbed dose  $dD$  in the time  $dt$ . The dose rate  $\dot{D}$  is given by

$$\dot{D} = \frac{dD}{dt}. \quad (1-3)$$

The general unit according to SI unit is J/kg/s = Gy/s. The unit of dose rate in Gy/h is used in this study.

### 1.1.2.3. Specific Energy and Lineal Energy

In the recent decades, absorbed dose in a site (micro-order volume) is getting lots of attention for discussing the impact of ionizing radiation to tissue cells. The dosimetry for localization of dose at the level of micro-meter is called microdosimetry. The definitions and the units of microdosimetric quantities are described in ICRU Report 36.<sup>25</sup>

The energy deposition in a site is discussed by using specific energy  $z$  in J/kg (Gy), lineal energy  $y$  in keV/ $\mu$ m. The specific energy and lineal energy are defined by

$$z = \frac{\varepsilon}{m}, \quad (1-4)$$

$$y = \frac{\varepsilon}{l}, \quad (1-5)$$

where  $\varepsilon$  is the energy deposited in a site;  $m$  is the mass of a site;  $l$  is the mean chord length expressed as 2/3 times the site diameter.

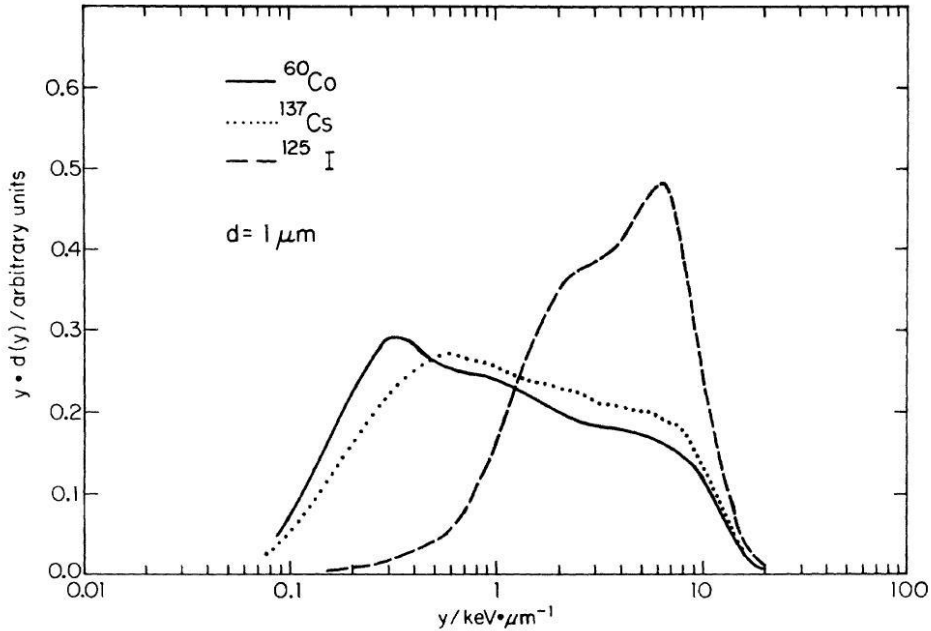
Considering the probability density of lineal energy  $f(y)$ , the frequency-mean lineal energy  $y_F$  [keV/ $\mu\text{m}$ ] is given by

$$y_F = \int_0^{\infty} y f(y) dy. \quad (1-6)$$

It is also useful to consider the dose probability density of the lineal energy  $d(y)$ . The  $d(y)$  is independent of the absorbed dose and dose rate. By using the  $d(y)$ , the expected value defined as dose-mean lineal energy  $y_D$  [keV/ $\mu\text{m}$ ] is given by

$$y_D = \int_0^{\infty} y d(y) dy = \frac{\int_0^{\infty} y^2 f(y) dy}{\int_0^{\infty} y f(y) dy}. \quad (1-7)$$

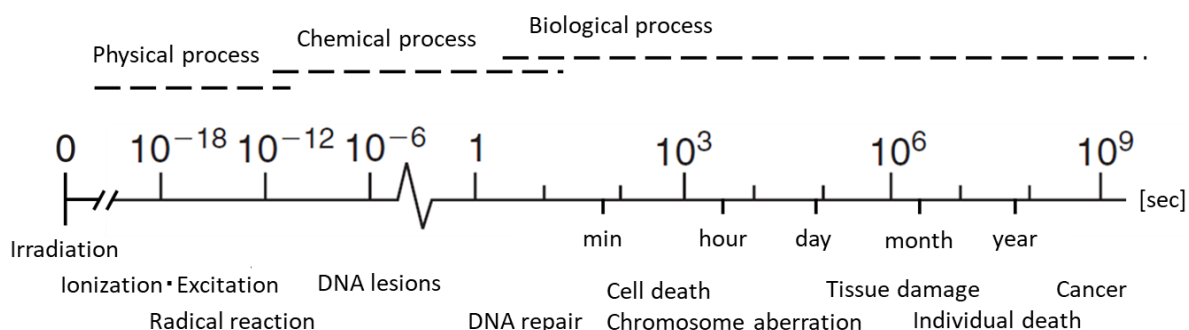
The  $y_D$  value for the case of site size with 1-2  $\mu\text{m}$  diameter has been well discussed in the references.<sup>26,27</sup> The examples of dose probability density of lineal energy are shown in Fig. 1-5.



**Figure 1-5. Dose probability density of lineal energy ( $y$ - $y_d(y)$  distribution).** The site size is set to be sphere with 1  $\mu\text{m}$  diameter. The  $y$ -distribution for  $^{60}\text{Co}$ ,  $^{137}\text{Cs}$  and  $^{125}\text{I}$  are shown in this figure.

## 1.2. Biological Response after Exposure to Ionizing Radiation

Energy depositions by events (ionization and excitation) and radiation-induced radical reactions (OH radicals etc.) occur in liquid water (tissue equivalent component) as physical and chemical processes under the exposure to ionizing radiation.<sup>28</sup> After that, DNA lesions are induced in cell nuclei as earlier biological response. In this regard, cell nuclei are interpreted as sensitive targets to induce cell killing after the exposure.<sup>1,3-6</sup> Most of DNA lesions can be repaired by virtue of DNA repair function<sup>7,8</sup>, however miss-repaired DNA lesions induce later responses such as loss of cell viability (cell death), chromosome aberration, tissue damage individual death and cancer induction. Figure 1-6 represents the time-scale of the biological effects of exposure to ionizing radiation.<sup>28,29</sup> Biological effects under exposure to ionizing radiation depend on cell (tissue) type, absorbed dose in Gy, radiation quality, dose rate, cell-cycle distribution, cell proliferation, non-targeted effects, oxygen effect (radical reaction) and so on.

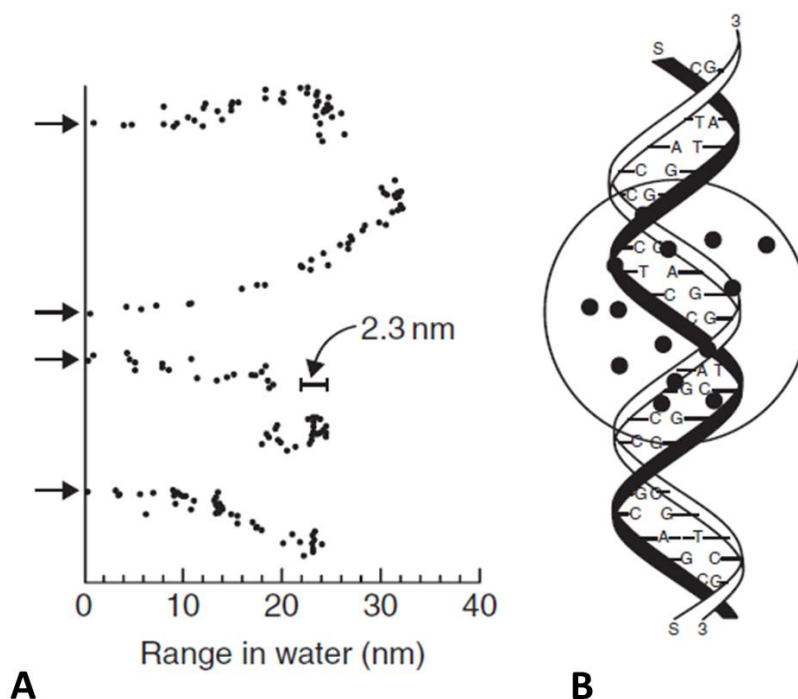


**Figure 1-6. Time-scale of the radiation effects on biological systems.** The biological effects can be divided into three phases of physical, chemical and biological processes.<sup>28,29</sup>

### 1.2.1. DNA damage induction and DNA repair after exposure

The DNA lesions are induced when the location of energy deposition corresponds to the DNA structure. Figure 1-7 represents the illustrations of 1 keV electrons track structure with energy deposition (Fig.1-7A) and concept of a cluster of ionizations impinging on DNA (Fig.1-7B).

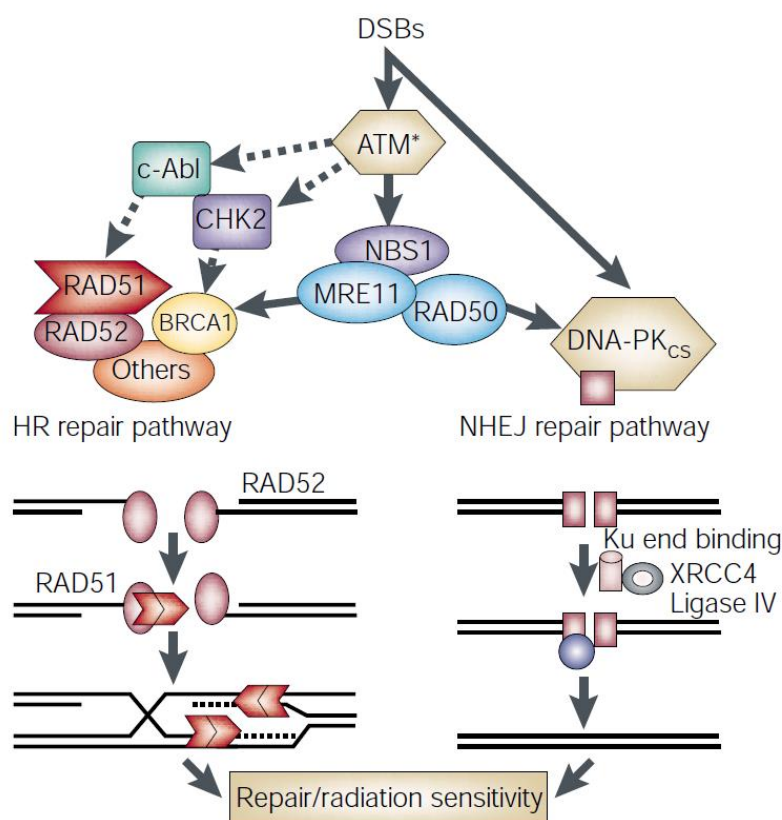
In Fig. 1-7B, the electrons have a Bragg-peak like clustered energy deposition at the end of the track, which is intricately related with DNA damage induction (Fig. 1-7B).<sup>2</sup>



**Figure 1-7. Illustration of clustered energy deposition and DNA damage induction:** A for the computer-simulated tracks of 1 keV electrons and B for the concept of a local multiply-damaged site produced by a cluster of ionizations impinging on DNA.<sup>2,30</sup>

There are several types of DNA damages such as single-strand breaks (SSBs), double-strand breaks (DSBs), base damage etc.<sup>2-6</sup> Among them, DSBs are interpreted as a main cause leading to cell death.<sup>31-34</sup> DSBs can be repaired by two major pathways: one is non-homologous end joining (NHEJ) and the other is homologous recombination (HR).<sup>34</sup> For the former, the NHEJ cascade is initiated by recruitment of DNA-dependent protein kinase catalytic subunit (DNA-PKcs) and Ku to the DNA ends, which is followed by recruitment of the XRCC4–ligase IV complex.<sup>36</sup> For the latter, Rad51 and replication protein A (RPA) are essential factors for the HR pathway in vertebrate cells.<sup>37</sup> The damage recognition by NHEJ system precedes that by HR.<sup>38</sup> NHEJ factor is recruited to DSBs more rapidly than HR factors,

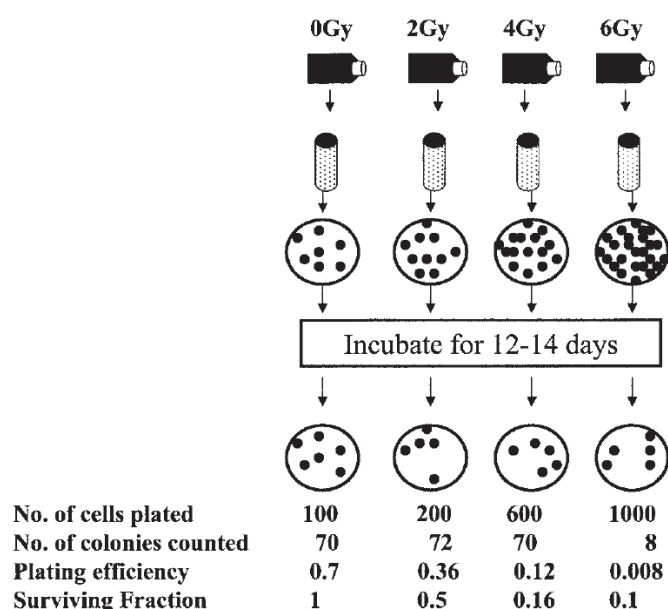
there being a significantly different period of time until both the factors are present at damage sites.<sup>38</sup> Whereas the damage site retention by NHEJ factors is transient, that by HR factor persists at unrepaired lesions. In this regards, there are unique roles of the two DNA repair pathways in mammalian cells. The quicker repair response of NHEJ pathway during exposure to ionizing radiation must be important for discussing dose-rate effects on radio-sensitivity.



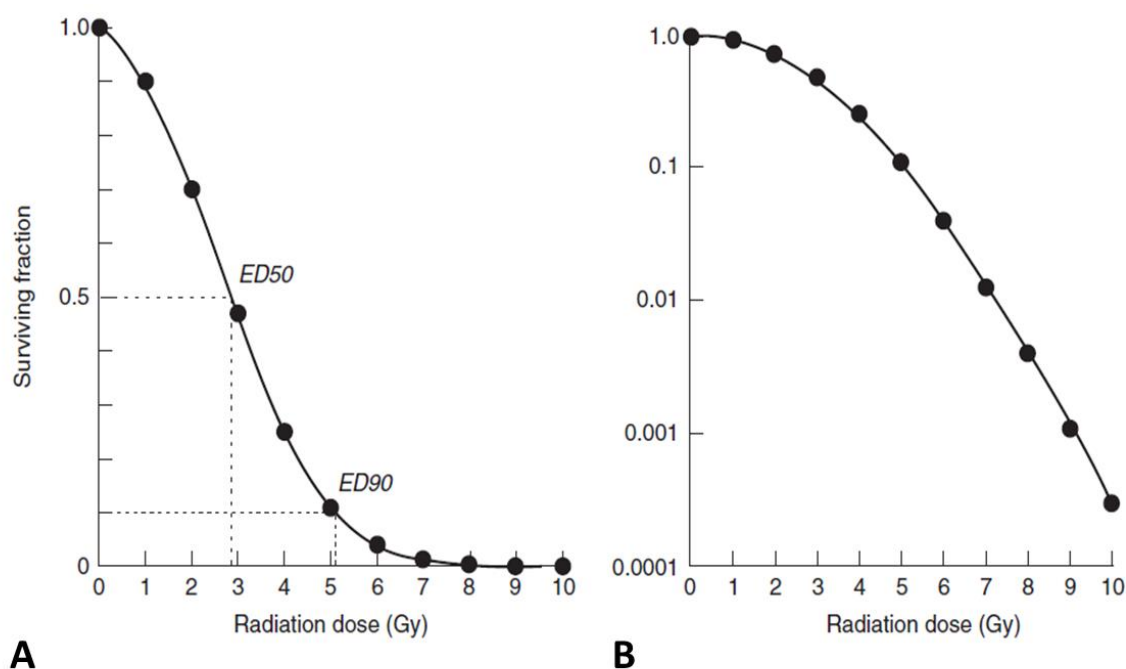
**Figure 1-8. Conceptual illustration of repair pathways for DNA double-strand breaks (DSBs).** Right pathway is non-homologous end joining (NHEJ), whereas left pathway is homologous recombination (HR).<sup>34</sup>

### 1.2.2. Cell Killing after Radiation Exposure

Cell death (called as cell killing in radiotherapy) is one of the important endpoint to discuss non-stochastic effects according to ICRP.<sup>10-12</sup> Famous technique to quantify biological effects following radiation is colony formation assay (clonogenic survival assay) reported by Puck *et al* in 1955 and 1956.<sup>45,46</sup> Figure 1-9 shows the illustration of colony formation assay.<sup>47</sup> The steps of colony formation assay are simple as follows: (i) irradiated or non-irradiated cells are prepared in a suspension. (ii) The number of cells per milliliter in the suspension is then counted by using a hemocytometer. (iii) The optimal number of cells are seeded onto a culture dish and incubated for approximately 10-14 days. Following these steps, each cell proliferates many times and forms single colony easily visible by eye. Counting the number of colony stained with Giemza or Crystal violet solution, cell surviving fractions for irradiated cell population are measured in comparison with control cells. By means of colony formation assay, we can obtain quantitative relation between absorbed dose and cell survival as described in Fig. 1-10.



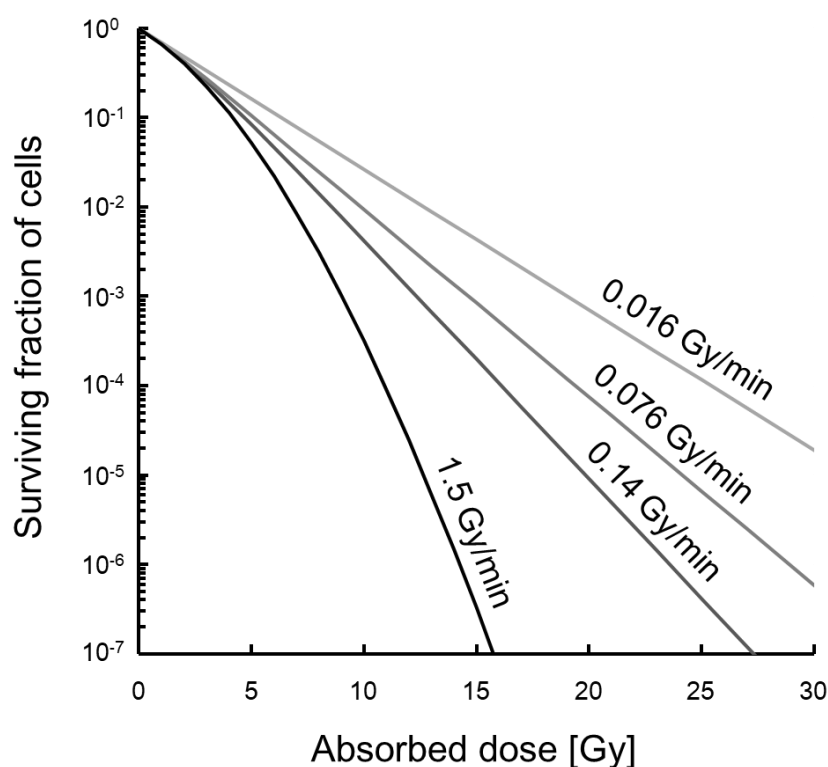
**Figure 1-9. Conceptual illustration of colony formation assay.** Cell survivals after irradiation are measured following several simple steps as: (i) irradiated or non-irradiated cells are prepared in a suspension. (ii) The number of cells per milliliter in the suspension is counted. (iii) The optimal number of cells are seeded onto culture dish and incubated for approximately 10-14 days.



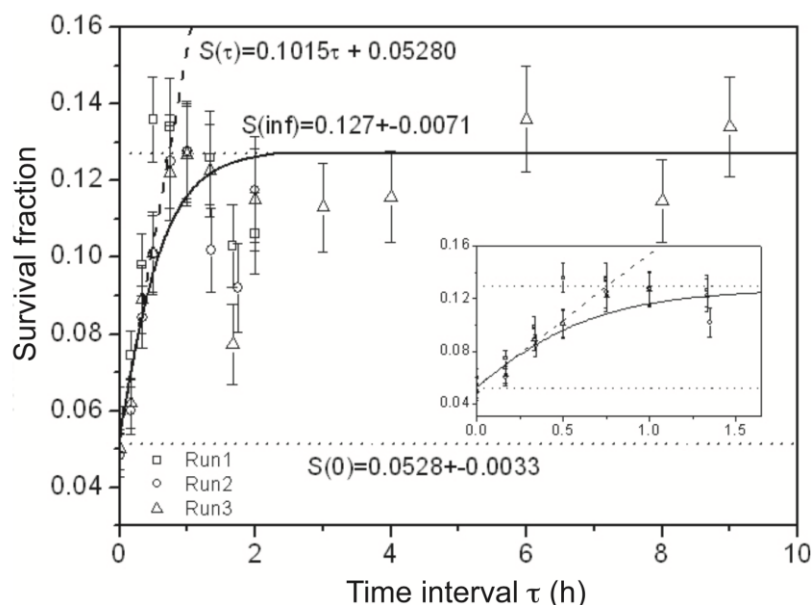
**Figure 1-10.** A typical cell survival curve for cells irradiated in tissue culture, plotted (A) on a linear survival scale. (B) The same data plotted on a logarithmic scale.

### 1.2.3. Dose-Rate Effects on Cell Survival

Under exposure at lower dose-rate, DNA damage repair competes against DNA damage induction during the exposure, which reduces radio-sensitivity even at the same dose as higher dose-rate. This phenomenon is called dose-rate effects<sup>49</sup> as shown in Fig. 1-11. In the relation between absorbed dose and log of cell survival, we can see that dose-response curve under lower dose-rate exposure is linear relation whilst the curve under higher dose-rate exposure exhibits linear-quadratic relation. At the endpoint of cell survival, reduction of radio-sensitivity during continuous irradiation can be explained by sub-lethal damage repair (SLDR)<sup>50,51</sup>, which can be quantified by cell recovery curve for a split-dose experiment<sup>53</sup> (Fig. 1-11). The cell recovery curve is potentially affected by re-distribution and proliferation of cells.



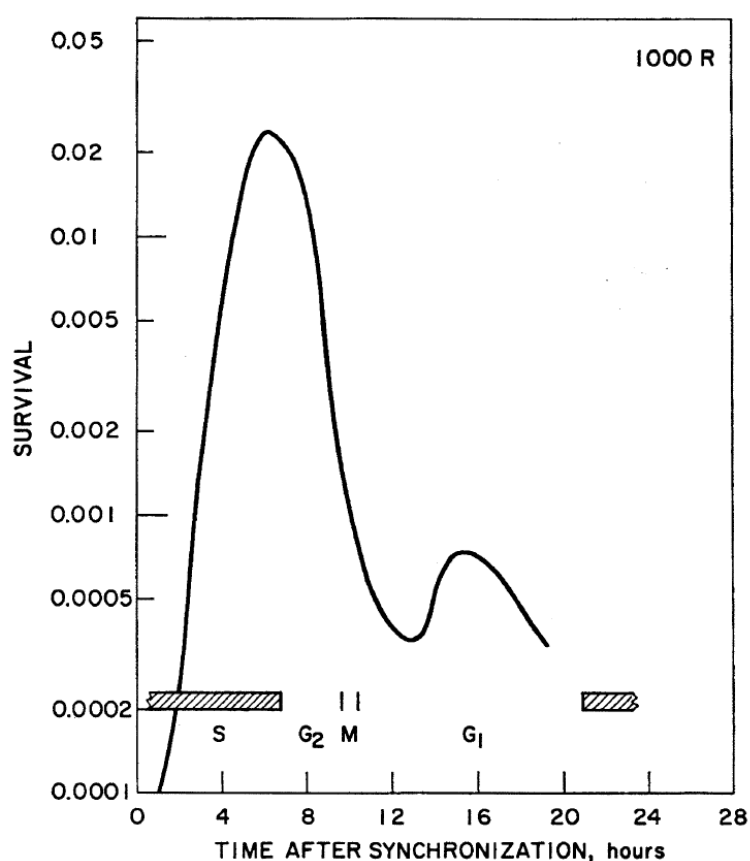
**Figure 1.11. Dose-rate effects on cell survival curve.** Under lower dose-rate exposure, DNA repair competes against damage induction of DNA during exposure. In the relation between absorbed dose and log of cell survival, the contribution of DNA repair function during exposure reduces cell killing after exposure to lower dose-rate.



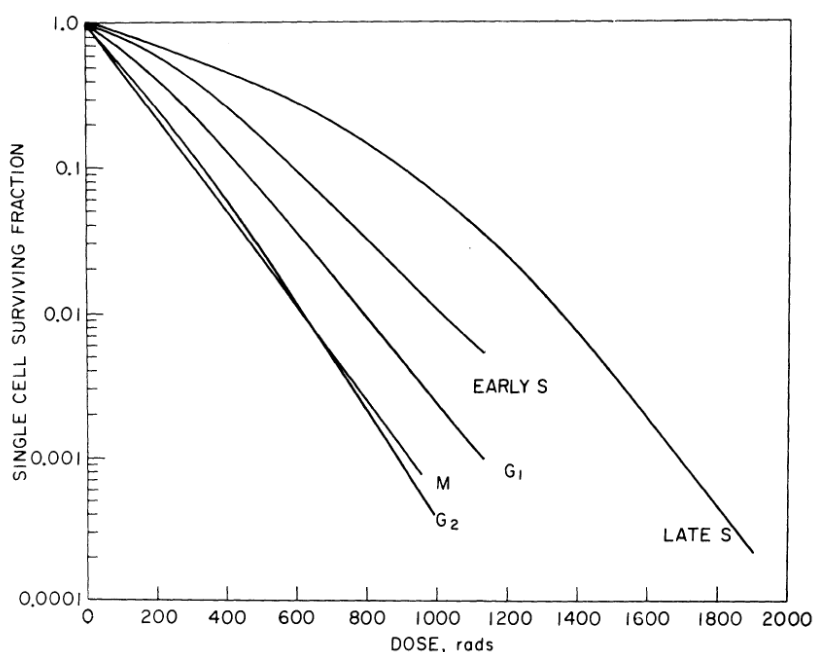
**Figure 1-12. Example of cell survival recovery between dose fractionation.** HSG tumor cells are irradiated with two equal doses of carbon beams of 2.5 Gy separated by time intervals from 0 to 9 h.<sup>53</sup> From this relation, DNA repair during interfractionation of dose and continuous exposure (so-called SLDR) can be deduced.

#### 1.2.4. Cell-Cycle Distribution and Radio-Sensitivity

Radio-sensitivity after exposure to ionizing radiation also depends on phase of the cell in growth cycle.<sup>54-58</sup> Initial studies to examine the dependence of the radiation exposure on cell-cycle phase were conducted by using synchronized Chinese hamster cells.<sup>54,55</sup> In general, cells exhibit most sensitive to radiation exposure during mitosis and in  $G_2$  phase, less sensitive in  $G_1$  phase, and least sensitive during the latter part of the S phase.<sup>59,60</sup> Figure 1-13 shows the example of dependence of phase of cell growth phase on cell survival after 10 Gy irradiation in human kidney cells.<sup>59</sup> The characteristics of phase-dependence on dose-response curve are described in Fig. 1-14.



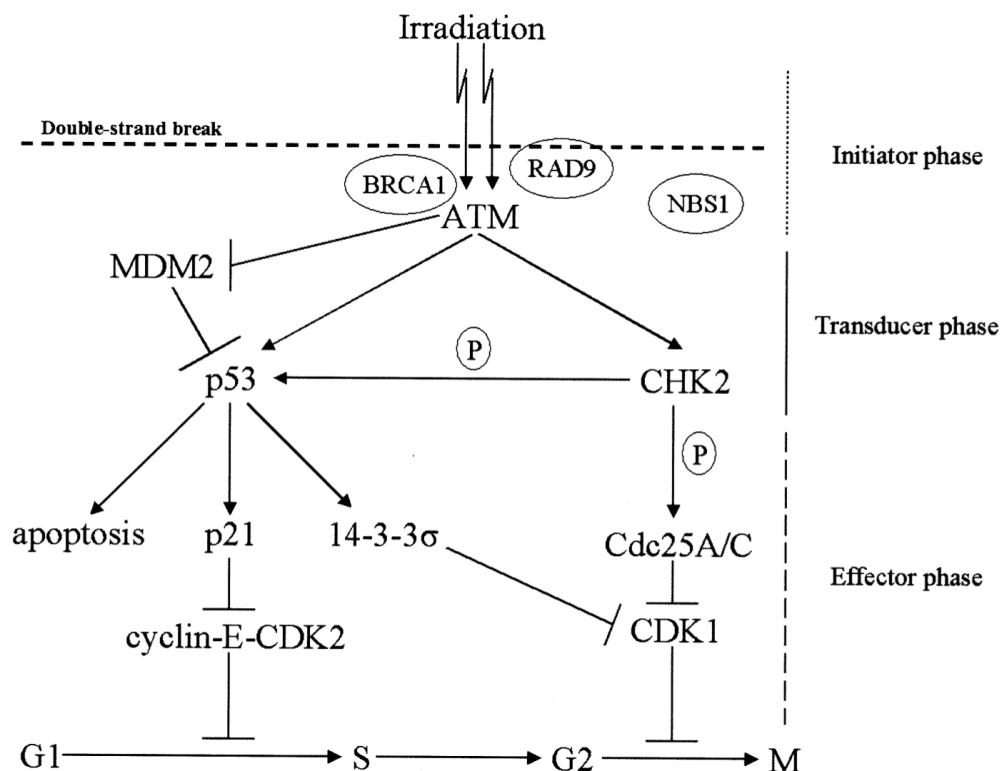
**Figure 1-13. Dependence of cell phase on survival after 10 Gy irradiation in human kidney cells.** The cells exhibit most sensitive to radiation exposure during mitosis and in  $G_2$  phase, less sensitive in  $G_1$  phase, and least sensitive during the latter part of the S phase.<sup>61</sup>



**Figure 1-14. A single-cell survival for various cell-cycle stages.** The relationships between absorbed dose in rad (100 rads = 1.0 Gy) and cell survival for five subdivided phases of G<sub>1</sub>, early S, late S, G<sub>2</sub> and M are shown in this figure.<sup>59</sup>

Whilst radio-sensitivity can be modified by phase of cells through cell cycle (Figs. 1-13 and 1-14), cells commonly respond to radiation-induced DNA damage by activating cell-cycle checkpoints to maintain genomic integrity after exposure to ionizing radiation in multiple pathways.<sup>62</sup> These checkpoints control temporary arrest at specific stages such as G<sub>1</sub>/S checkpoint, S checkpoint, early G<sub>2</sub> and G<sub>2</sub>/M checkpoint.<sup>62,63</sup> The activity of ATM protein kinase rapidly induced by ionizing radiation interacts with a broad network to block cell-cycle progression as shown in Fig. 1-15. And the ATM then activates two pathways of p53 and CHK2, which lead to activation of cell-cycle checkpoint at either a G<sub>1</sub>/S or G<sub>2</sub>/M stage.<sup>61</sup>

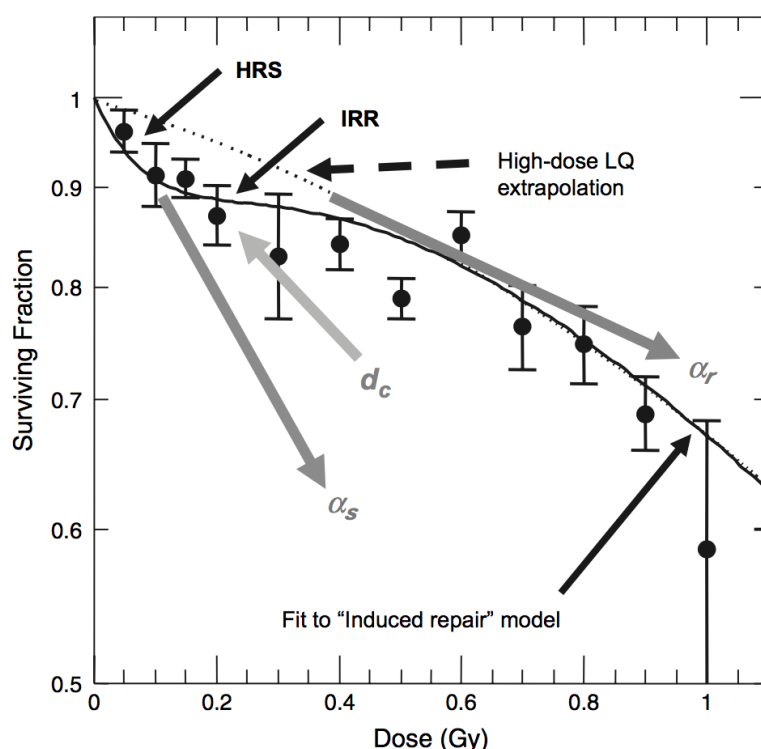
Mentioned above, phase of cells and DNA damage checkpoints play key role of determining the radio-sensitivity and cell fate.



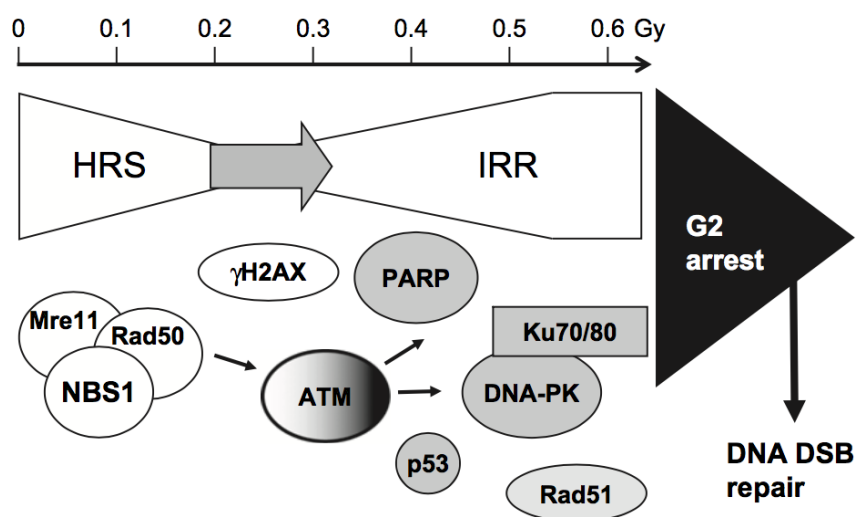
**Figure 1-15. Broad network of protein activation to block progression of cell cycle.** The activity of ATM protein kinase induced after radiation exposure interacts with two pathways of p53 and CHK2, leading to activation of cell-cycle checkpoint at either a G<sub>1</sub>/S or G<sub>2</sub>/M stage.<sup>61</sup>

### 1.2.5. Non-Targeted Effects and Low-dose Hyper Radio-Sensitivity

Radio-sensitivity of cells after exposure to ionizing radiation is affected by not only targeted effects (TEs)<sup>1</sup> mentioned above but also non-targeted effects (NTEs).<sup>64-66</sup> In NTEs, cells without direct hits by radiation also exhibit the same behavior as TEs, at the endpoints of abnormal chromosome damage, mutations, clonogenicity and so on. These are called NTEs or radiation-induced bystander effects (RIBEs), or in some cases low-dose hyper radio-sensitivity (HRS) (Fig. 1-16).<sup>67,68</sup> Low-dose HRS to doses with < 30 cGy is interpreted as a result from failure to arrest in G<sub>2</sub> (Fig. 1-17),<sup>67-69</sup> whilst intercellular signalling has also been reported to have the potential capacity to enhance cell-killing.<sup>70-72</sup> However, these effects remain to be elucidated in detail.



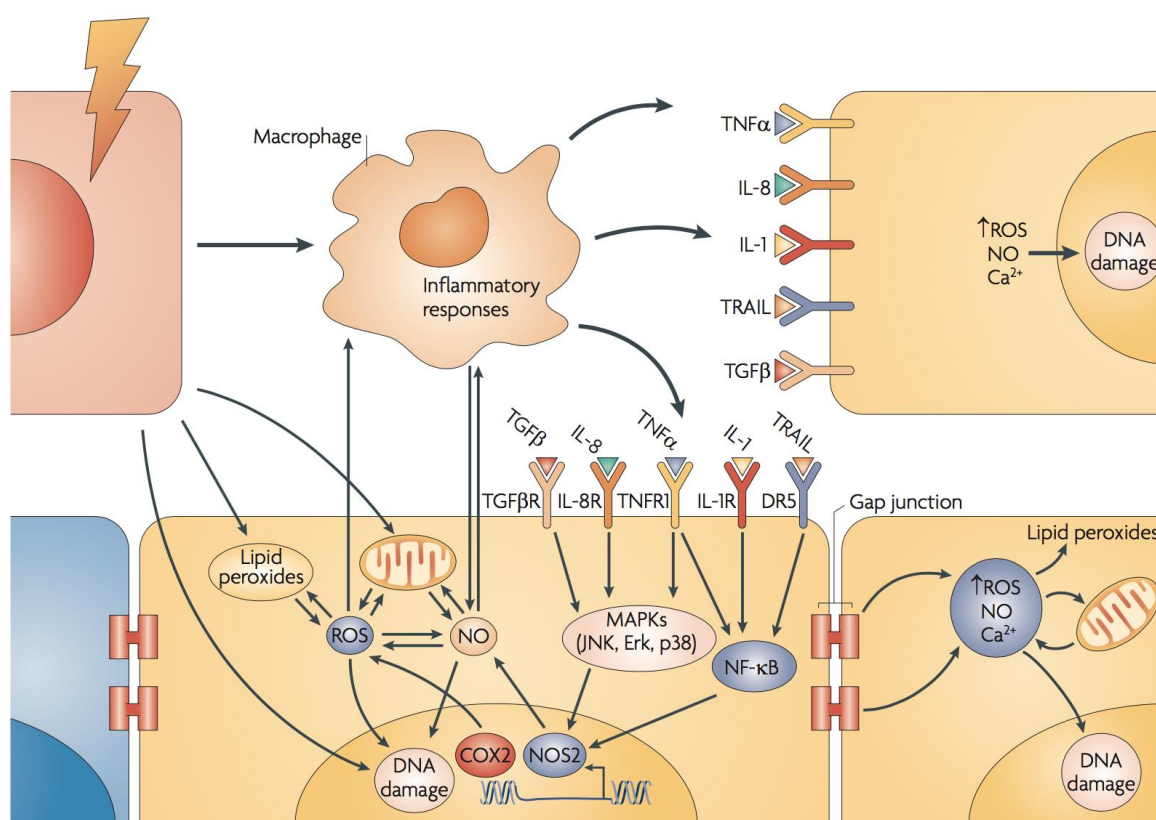
**Figure 1-16. Typical cell survival with hyper radio-sensitivity and increased radio-resistance at low-dose range.** Unexpected higher radio-sensitivity is described compared with cell survival estimated from high dose data by using the linear-quadratic model which is generally used for quantifying dose-survival relation.<sup>69</sup>



**Figure 1-17. Interpretation of protein activation linked with the HRS/ IRR).** Open and filled circles represent proteins which are interpreted as key role for HRS/IRR. In this interpretation, the ataxia telangiectasia-mutated (ATM) is designed to reflect dose-dependent activation and transition from HRS to IRR.<sup>69</sup>

There are several types of signals, such as cytokines including interleukin 6 (IL-6)<sup>73</sup> and interleukin 8 (IL-8)<sup>74</sup>, transforming growth factor- $\beta$ 1 (TGF $\beta$ 1)<sup>75</sup>, TNF $\alpha$ <sup>76</sup>, calcium<sup>77</sup>, reactive oxygen species (ROS)<sup>78</sup>, nitric oxide (NO)<sup>79</sup>, adenosine triphosphate (ATP)<sup>80</sup> and so on. These signals are assumed to transfer to the non-hit cells from hit cells via gap junction or culture medium.<sup>71</sup> Figure 1-18 shows the key pathways to induce cell killing by the signals.

The signals induce DNA damage such as DNA double-strand breaks in cell nuclei.<sup>80,81</sup> According to the report by Hu et al,<sup>81</sup> the number of DSBs in irradiated and in bystander (non-hit) cells reaches peak at 30 min after radiation, which suggested that the induction of DNA damage in bystander cells is rapid. The repair kinetics of DNA damage in NTEs is different from that in TEs.<sup>82-86</sup> DNA damage in bystander cells persists for a prolonged time,<sup>83</sup> suggesting that the disorder of DNA repair efficiency may have occurred in non-hit cells.<sup>84-86</sup>



**Figure 1-18. Key pathways to induce cell killing by intercellular communication.** There are several types of signals, such as IL-8, TGF $\beta$  and so on.<sup>71</sup>

### 1.2.6. Inverse Dose-Rate Effects

In recent decades, inverse radio-sensitivity of dose-rate effects has been reported from *in vitro* experiments, so called inverse dose-rate effects (IDREs). The dose-rate effects mentioned above mean the general rule that cellular sensitivity decreases as dose-rate is lower. IDREs were observed not only in the form of mutations or chromosomal aberrations<sup>87,88</sup> but also in cell viability (clonogenic survival).<sup>89</sup> Figure 1-18 shows the example of IDRE on clonogens per flask after multi-fractioned exposure (lower dose rate on average).<sup>89</sup>

In early studies of dose-rate effects, Mitchell and Bedford investigated the involvement of phase of cells through cell cycle by using synchronized cell population, suggesting a slight inversion of radio-sensitivity which is attributed to cell-cycle progression into more sensitive phases (greater damage).<sup>42,49,90,91</sup> In 2002, Mitchel again reported that the fraction of cells in sensitive phase of G<sub>2</sub>/M gradually increases during lower dose-rate exposure, i.e., at 60 cGy/h in human glioblastoma multiforma and human glioblastoma astrocytoma.<sup>89</sup> Meanwhile after the discovery of low-dose HRS,<sup>67-69</sup> the debates of underlying mechanisms for IDREs have been thrust into the spotlight. Short et al and Terashima et al reported the cumulative low-dose HRS during fractioned low-dose irradiation in 2001 and in 2017, respectively.<sup>92,93</sup>

Mentioned above, the dose rate effects have been investigated by many investigators. However, the underlying mechanisms of IDREs still remain unclear, much less mechanistic modelling.

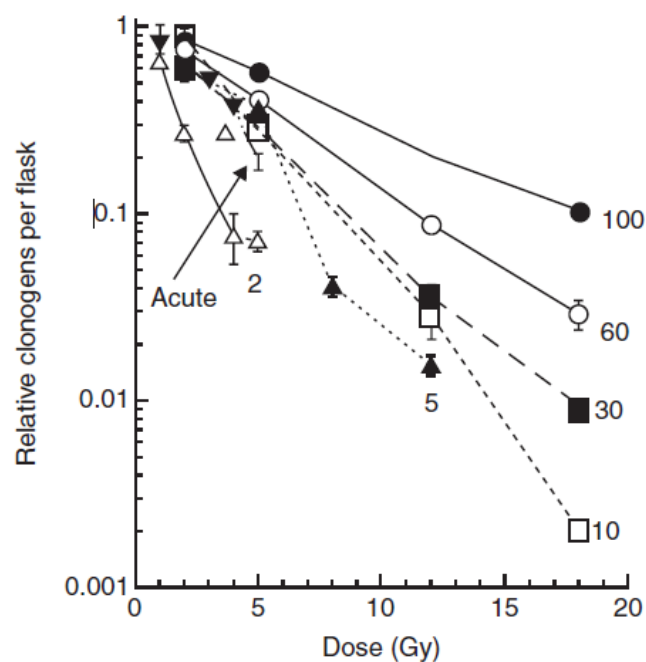


Figure 1-19. Example of the inverse dose rate effect of clonogens per flask at dose-rate range from 2-100 cGy/h. Number in this figure indicate the dose-rate of radiation exposure in cGy/h.<sup>89</sup>

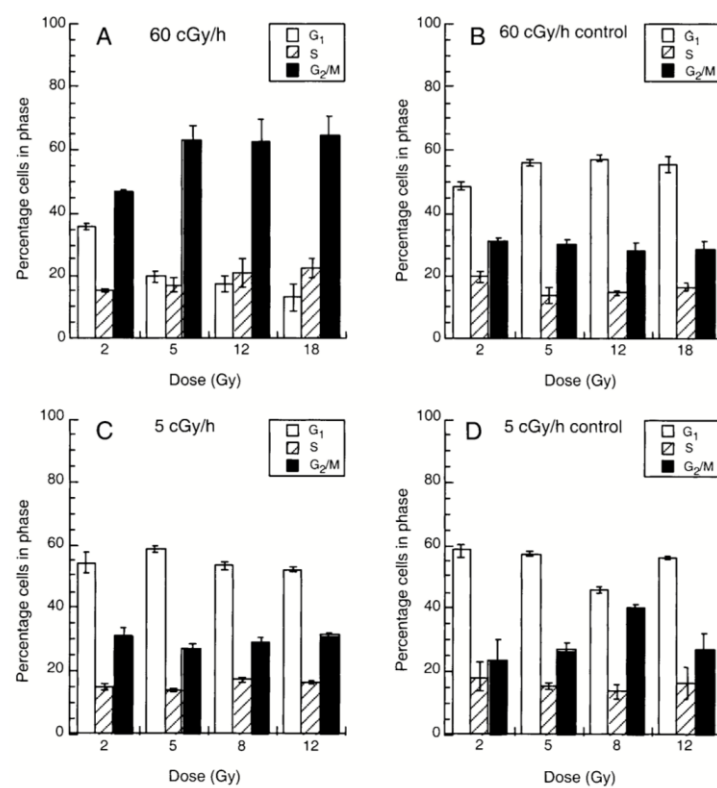


Figure 1-20. Cell-cycle distribution of asynchronous T98G cells after irradiation with  $^{60}\text{Co}$   $\gamma$ -rays: A and C for 60 cGy/h and 5 cGy/h, respectively, and B and D for control group.<sup>89</sup>

### 1.3. Quantification of Cell Killing after Exposure to Ionizing Radiation

#### 1.3.1. Target Theory and Linear-Quadratic Model

Target theory stands on an idea that hits by radiation make sensitive targets in DNA inactivated and lead to the reduction of cell viability<sup>2</sup>, which may be explained by the number of DNA lesions induced along ionizing radiation particle.<sup>1,2</sup> The first model of this theory is based on just one hit by radiation on a single sensitive target, called single-target single-hit inactivation,<sup>48</sup> which exhibits straight curve in a semi-logarithmic plot of cell survival against dose (see Fig. 1-21). The equation of single-target single-hit inactivation is expressed based on Poisson statistics as

$$S(\text{survival}) = p(0 \text{ hits}) = \exp(-D/D_0) \quad (1-8)$$

where  $p$  is the probability of the next hit occurring in a given cell,  $D_0$  is dose that gives an average of one hit per target and  $D$  is absorbed dose in Gy. Log of cell survival  $-\ln S$  is the equivalent number of lethal lesions per cell. Whilst the single-target single-hit inactivation is just straight curve as shown in Fig. 1-21A, general dose-response curve in mammalian cells has shouldered survival curves. To consider the characteristics, a more general model of target theory, multitarget single-hit inactivation was proposed<sup>48</sup>, in which it is assumed that one hit by radiation to each of  $n$  sensitive targets contained in cells is needed to induce cell killing. Using Poisson statistics, cell survival  $S$  is given by

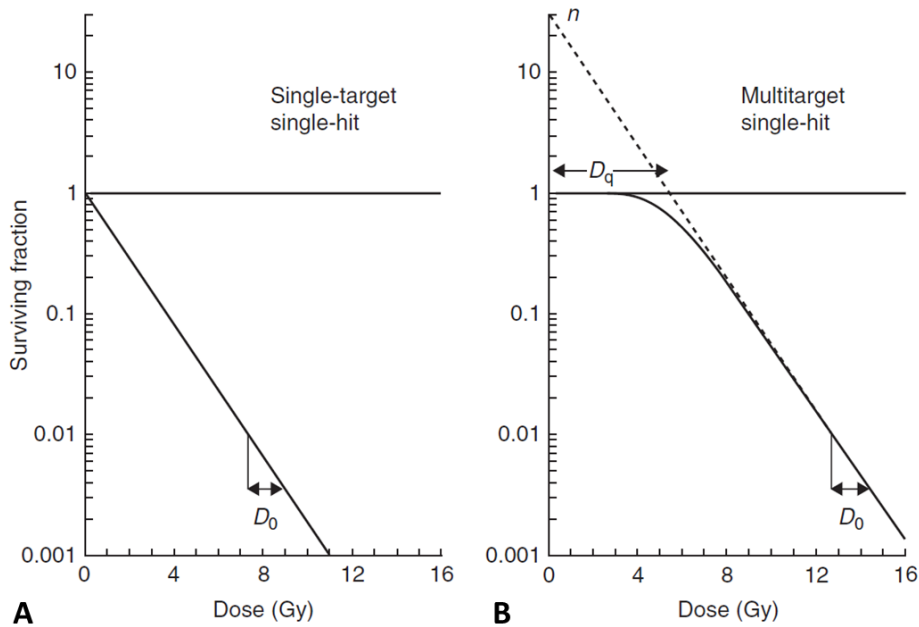
$$\begin{aligned} S(\text{survival}) &= p(\text{not all targets inactivated}) \\ &= 1 - (1 - \exp(-D/D_0))^n \end{aligned} \quad (1-9)$$

Figure 1-21B is the example of multitarget single-hit survival curves with a shoulder in low-dose range.

The description of the cell survival for simplicity, linear-quadratic (LQ) model is widely used in radiation biology and radiotherapy.<sup>48,94</sup> In many cases, the LQ curve fits fairly well to the survival data, which exhibits the linear-quadratic relation in a semi logarithmic survival against dose. The cell surviving fraction (SF) in the LQ model is expressed by simple formula as follow,

$$-\ln S = \alpha D + \beta D^2 \quad (1-10)$$

where  $\alpha$  and  $\beta$  are the proportionality factors to dose  $D$  [ $\text{Gy}^{-1}$ ] and the dose squared  $D^2$  [ $\text{Gy}^{-2}$ ], respectively.<sup>48</sup> And these coefficients are determined empirically from clinical data or simply by fitting the LQ formula to the experimental cell data of SF. In this model, the dose at which the linear and quadratic components are equal to the ratio  $\alpha/\beta$ . The linear-quadratic curve bends continuously but is a good fit to experimental data for the first few decades of survival.<sup>48</sup>



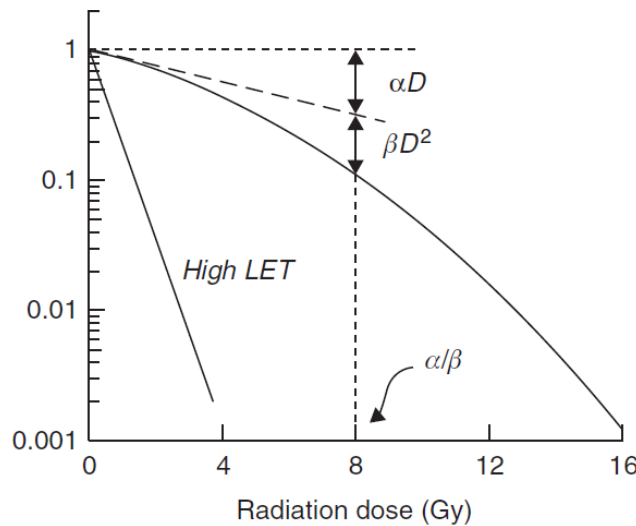
**Figure 1-21. Model based on target theory.** A is single-target single hit model and B is multitarget single-hit model in a semi-logarithmic plot of cell survival against dose.<sup>48</sup>

By using two coefficients of  $\alpha$  and  $\beta$  in the LQ model, it enables us to discuss relative biological effectiveness (RBE) and biological effective dose (BED) in radiotherapy. For example biological effects relative to standard radiation of 200 kVp X-rays and BED are given by

$$\begin{aligned} \text{RBE} &= \left[ \frac{D_{200\text{kVp}}}{D} \right]_{\text{same biological effect}} \\ &= \left( \frac{\sqrt{\alpha_{200\text{kVp}}^2 + 4\beta_{200\text{kVp}}S^*} - \alpha_{200\text{kVp}}}{2\beta_{200\text{kVp}}} \right) \cdot \left( \frac{\sqrt{\alpha^2 + 4\beta S^*} - \alpha}{2\beta} \right)^{-1} \end{aligned} \quad (1-11)$$

$$\text{BED} = nd \left( 1 + \frac{d}{\alpha/\beta} \right) \quad (1-12)$$

where  $D_{200\text{kV}}$ ,  $\alpha_{200\text{kV}}$  and  $\beta_{200\text{kV}}$  represent the absorbed dose,  $\alpha$  and  $\beta$  of standard radiation (200 kVp X-rays),  $S^*$  is the endpoint of cell survival for discussing the biological effects after exposure to ionizing radiation (usually defined as 37 % or 10% survival level),  $n$  is the number of dose fractionation in fractionated radiotherapy and  $d$  is dose per fraction.



**Figure 1-22. Dose-response curve described by Linear-Quadratic (LQ) model.** There are two coefficients to dose and dose square: One is proportional to dose ( $\alpha D$ ); the other is proportional to the square of dose ( $\beta D^2$ ).<sup>48</sup>

### 1.3.2. Cell-Killing Model for Dose-Rate Effects

Dose-rate effects on cell survival can be explained by reduction of lesion interaction by virtue of the repair of sub-lesions during irradiation.<sup>42</sup> Whilst the standard LQ mode is described in Eq. (1-10), the LQ formalism with time-dose relation designed by Lea-Catcheside time factor

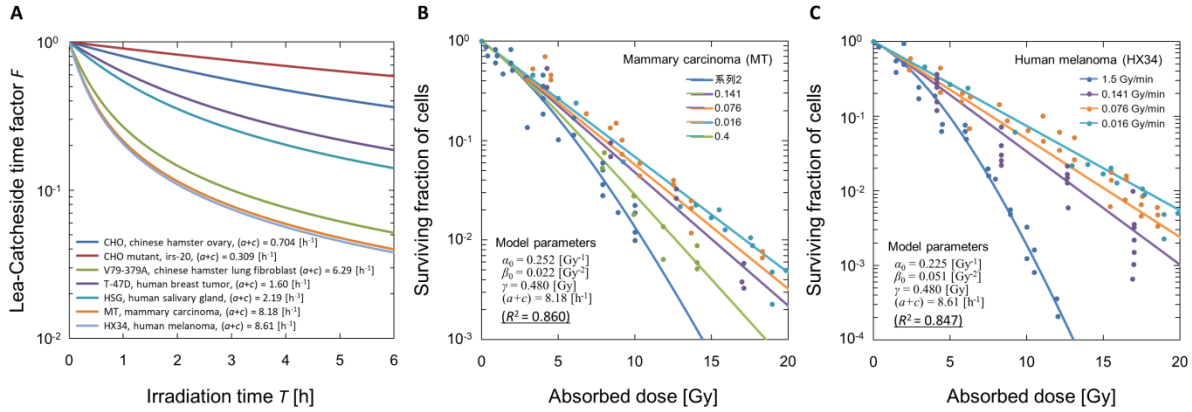
$G$  is also proposed, which takes into account dose protraction or fractionation.<sup>95</sup> Cell survival in the LQ model with  $G$  factor is given by

$$-\ln S = \alpha D + G\beta D^2 \quad (1-13)$$

$$G = \frac{2}{D^2} \int_{-\infty}^{\infty} \dot{D}(t) dt \int_{-\infty}^t e^{-\lambda(t-t')} \dot{D}(t') dt'. \quad (1-14)$$

Here  $\dot{D}(t)$  is the variation in dose rate, and  $\lambda$  is a rate of damage repair. According to Brenner et al,  $G$  value is from 0 to 1 and  $G=1.0$  for the case of acute-dose irradiation such as radiotherapy.<sup>95</sup>

Meanwhile, in 1986 Curtis proposed the lethal-potentially lethal (LPL) model considering two different types of DNA lesions of repairable lesion (potentially lethal lesion) and non-repairable lesion (lethal lesion).<sup>96</sup> In this model, repairable lesions are assumed to be repaired or binary misrepair.<sup>96</sup> In 1996, Hawkins proposed the microdosimetric-kinetic (MK) model<sup>52</sup> considering microdosimetric quantities<sup>25</sup> based on two repair models of the LPL model<sup>96</sup> and repair-misrepair (RMR) model.<sup>100</sup> In recent decades, it has been verified that the MK model has potentials to reproduce cell surviving fractions for various radiation types<sup>98,99</sup> and dose-rate effects.<sup>53,100</sup> So far, the MK model for continuous irradiation has time factor corresponding to Lea-Catcheside time factor, however the model has some room for being developed so as to consider cell condition such as DNA amount<sup>101</sup> and cell growth<sup>102</sup> as biological characteristics and decrease of dose-rate<sup>103</sup> as physical characteristics.



**Figure 1-23. Model performance of MK model for dose-rate effects:** (A) for the relationship between irradiation time  $T$  [h] and the Lea-Catcheside time factor in various cells of CHO, irs-20, V79-379A, T-47D, HSG, MT and HX3; (B) and (C) for dose-response curve for mammary carcinoma and human melanoma for various dose rates, respectively. The SLDR rate were taken from the previous reports<sup>52,53,100</sup> and fitting experimental data. In Figs. B and C, the MK model<sup>53</sup> was compared with the experimental data reported by Trevor et al.<sup>104</sup> The lines and symbols represent the results predicted by the model and experimental data, respectively.

### 1.3.3. Cell-Killing Model for Non-Targeted Effects

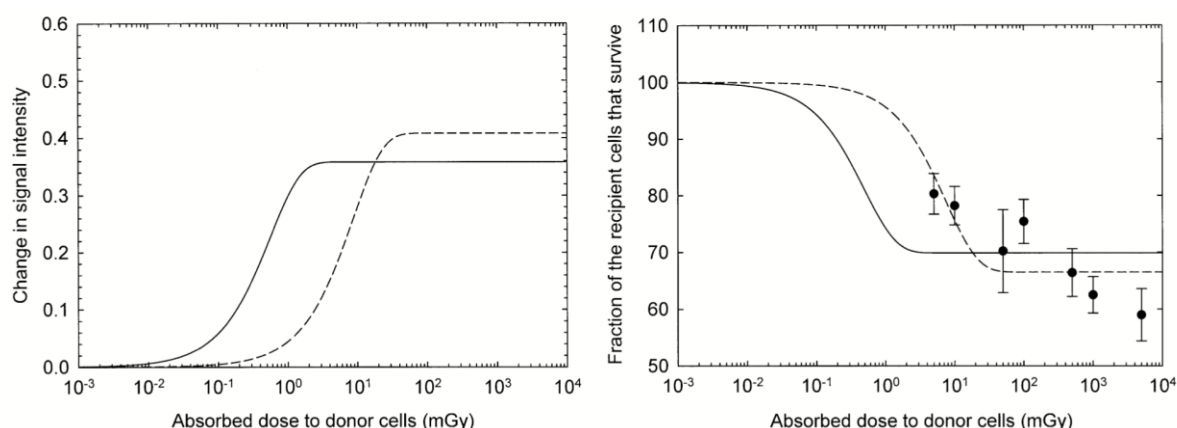
There are several formulations to quantify the low-dose HRS, non-targeted effects and intercellular communication.<sup>105-112</sup> The first modelling of cell survival with low-dose HRS/IRR is designed by incorporating the induced repair due to the failure to arrest in  $G_2$  checkpoint (Fig. 1-17). The model is called induced repair (IndRep) model<sup>113</sup> given by

$$-\ln S = \alpha_r D (1 + (\alpha_s / \alpha_r - 1) \exp(-D/D_c)) + \beta D^2 \quad (1-15)$$

where  $\alpha_r$  is low-dose  $\alpha$  value,  $\alpha_s$  is high-dose  $\alpha$  value, and  $d_c$  represents the dose at which 63% ( $1 - e^{-1}$ ) of induction has occurred. The survival curve by IndRep model is described in Fig. 1-16.<sup>69</sup> The reports by Stewart et al in 2006<sup>114</sup> used microdosimetric model for the induction of cell killing through medium-borne signals, in which the size of the target for non-targeted effects is micrometer order. The survival model after exposure to medium-borne signals is simply expressed by

$$-\ln S = \omega \left( 1 - e^{-\frac{D}{\bar{z}_F}} \right) \quad (1-16)$$

where  $\bar{z}_F$  frequency-mean specific energy per event,  $\omega$  is cell specific parameter in the microdosimetric model.

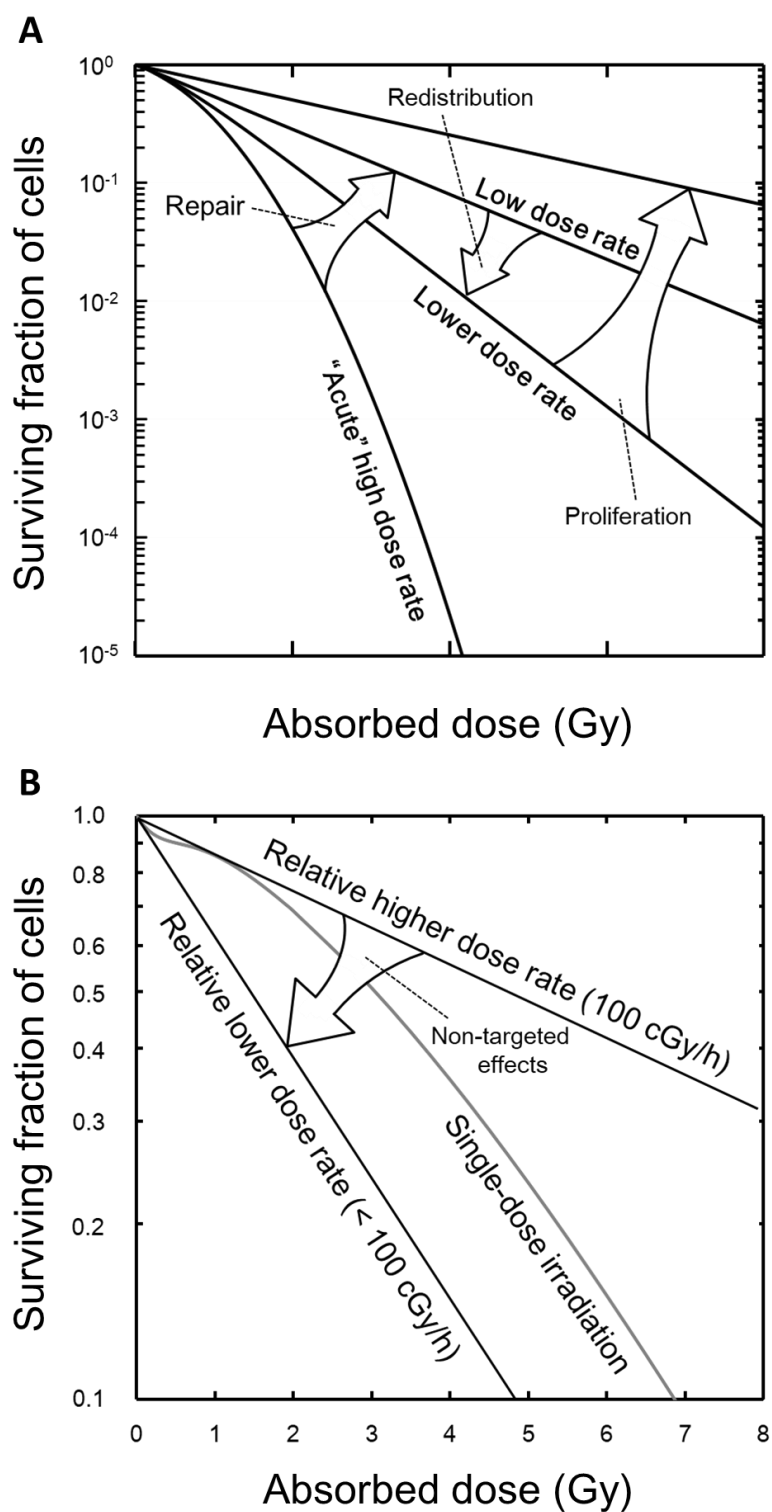


**Figure 1-24. Analysis by microdosimetric model for the survival after exposure to medium transfer bystander signal:** left panel shows the change in the signal intensity and right panel is comparison between the experimental survival data in HPV-G cells exposed to irradiated cell condition medium (ICCM).<sup>114</sup>

Whilst temporal characteristic of cell-killing signals has been modeled by using rate equation according to the report by Kunderát et al,<sup>110</sup> stochastic model of signal-induced mutation and cell death was proposed by McMahon et al.<sup>109</sup> Focusing on various types of ionizing radiation and both targeted and non-targeted effects, Sato et al presented the cell surviving fraction model based on microdosimetric probability densities.<sup>115</sup> There are many partial models to discuss cell responses for NTEs, however there is no single-framework to express the cell survival based on DNA damage kinetics so far.

#### 1.4. Meaning for Investigating Biological Effects after Protracted Exposure

After the accident of Fukushima 1st Nuclear Power Plant (F1-NPP) following the earthquake and tsunami on March 11, 2011, biological effects under the protracted exposure to low-dose or low-dose-rate ionizing radiation have drawn a keen interest of the public.<sup>13,14</sup> However mechanisms of cell responses and induced hyper radio-sensitivity including inverse dose-rate effects remain unclear from the standpoint of radiation biology and quantification by cell-killing model.



**Figure 1-25. Potential factors to modify radio-sensitivity under protracted exposure:** A for change of dose-response curve by repair, redistribution, proliferation (cell growth and phase of cells), B for the stacking of low-dose HRS during multi-dose fractionation.

During the protracted exposure to ionizing radiation, several radio-sensitive factors have possibility for modifying biological effects. Specifically, two potential factors seem to be involved in reversal of radio-sensitivity: one is cell-cycle dynamics and cell proliferation during exposure (Fig. 1-25A), the other is accumulative low-dose HRS during low-dose fractionation (Fig. 1-25B). As for the former factor, it has been reported that the fraction of cells in sensitive phase of G<sub>2</sub>/M gradually increases during lower dose-rate exposure,<sup>89</sup> however there is no cell-cycle data for various dose-rates and theoretical explanation of the dependence of cell phase change on cell survival curve. As for the low-dose HRS, mechanistic modelling and interpretation is not enough from the standpoint of model development. Aiming to the estimating time course of cell survival and cell responses under low-dose/ low-dose-rate exposure, it is necessary to obtain (i) the relationship between cell-cycle change during protracted exposure and radio-sensitivity (cell killing) in mammalian cell line, and (ii) integrated modelling considering targeted and non-targeted effects based on signal and damage kinetics.

### 1.5. Motivation and Purpose in this Study

Reversal radio-sensitivities such as low-dose hyper radio-sensitivity (HRS) and inverse dose-rate effects (IDREs) are crucial problems to discuss the biological effects after long-term exposure to ionizing radiation. Several *in vitro* experimental data of reversal radio-sensitivity are being obtained from the reports by many investigators from the standpoint of radiation biology, whilst mechanistic explanations for time course of cell responses during long-term exposure in wide dose-rate range is not enough from the standpoint of model estimation. More precise understandings of cell responses associated with cell-cycle dynamics, repair kinetics, fluence rate of radiation particle and intercellular signalling during long-term exposure is necessary to predict biological effects after the exposure.

Two main topics associated with cell responses under protracted exposure were

investigated in this study. The earlier study described in chapter 3 is focused on the cell-cycle change and radio-sensitivity under X-rays protracted exposure at various dose-rates by the combination of *in vitro* experiments and model analysis. The latter study in chapter 4 is mainly for development and verification of integrated cell-killing model considering targeted and non-targeted effects. Both investigations were conducted in the aim of estimating contribution degree of cell-cycle dynamics and non-targeted effects to reversal of radio-sensitivity.

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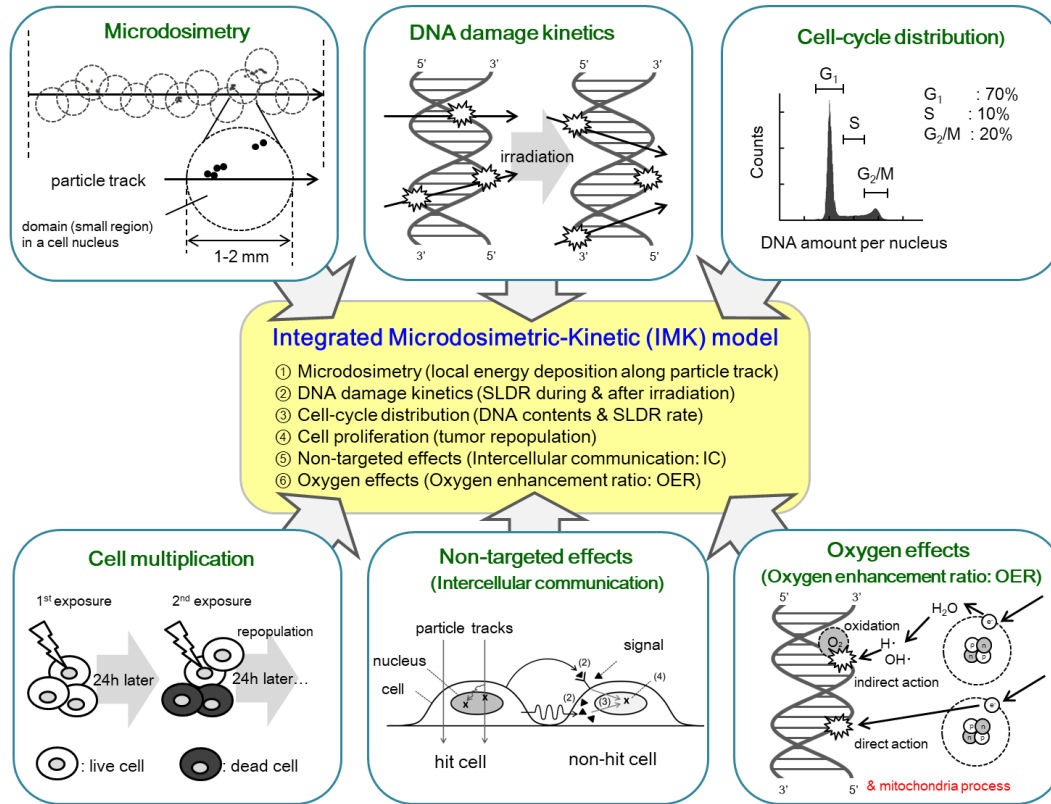
## Chapter 2

# Model Overview

## -Integrated Microdosimetric-Kinetic Model-

For analysing the mechanism of dose-rate effects and radiation-induced biological effects on surviving fraction of cells after irradiation, the conventional microdosimetric-kinetic (MK) model<sup>1</sup> is being developed so as to incorporate local energy deposition along ionizing radiation particle represented by microdosimetry<sup>2</sup> and time course of DNA damage response under inter-fractionation or continuous irradiation with dose rate.<sup>3</sup> The model has been upgraded as one of the cell-killing models including some factors which modify radio-sensitivity<sup>4-9</sup> into the original MK model. Hereafter, the author calls it an “*integrated microdosimetric-kinetic model*” (IMK model).

As shown in Fig. 2-1, the IMK model includes 6 factors such as (i) microdosimetry, (ii) DNA damage kinetics (sub-lethal damage repair: SLDR) during and after irradiation, (iii) cell-cycle distribution (DNA contents and sub-lethal damage repair), (iv) proliferation of cells, (v) oxygen effects and (vi) non-targeted effects (intercellular signalling). The parts of (i)-(iii) and (vi) in the IMK model was applied for conducting model analysis of cell responses after low-dose/ low-dose-rate long-term continuous irradiation. In this chapter, I summarize the modelling of cell responses in the parts of (i)-(iii) and (vi) of the IMK model.



**Figure 2-1. Integrated cell-killing model calling it an “*integrated microdosimetric-kinetic (IMK) model*”.** Based on biological evidences, this model considers 6 factors such as (i) microdosimetry, (ii) dose-rate effects on DNA damage kinetics, (iii) cell-cycle distribution (DNA contents and sub-lethal damage repair), (iv) proliferation of cells, (v) oxygen effects and (vi) non-targeted effects (intercellular signalling).

## 2.1. DNA-Targeted Effects

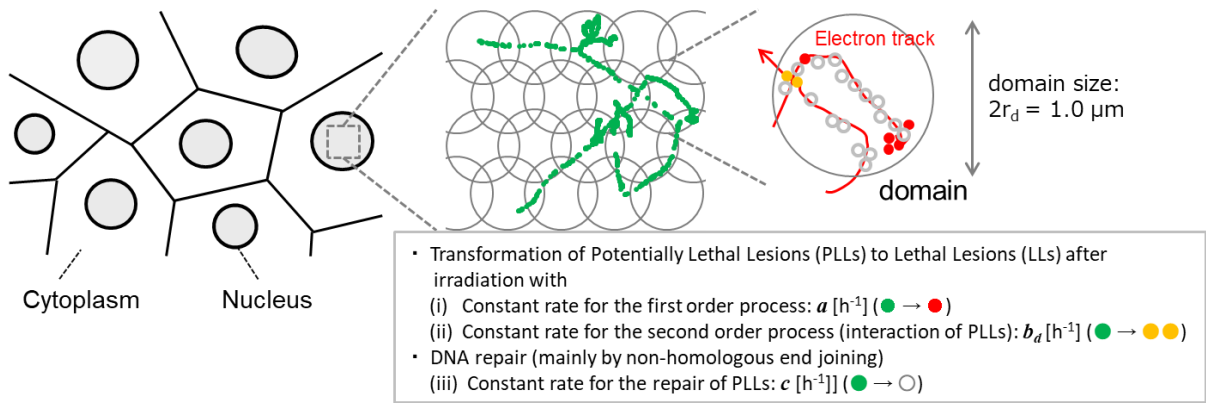
### 2.1.1. Assumptions for DNA-Targeted Effects

Basically, the MK model<sup>1</sup> considers a lot of micro-order territories (so called domains) which are parts of the cell nucleus so as to incorporate microdosimetry.<sup>2</sup> The shape of the domains is for simplicity defined as a sphere with radius from 0.5 to 1.0  $\mu\text{m}$ <sup>10,11</sup> and the energy deposition in domains has been evaluated in terms of tissue equivalent proportional counter (TEPC) measurements<sup>12</sup> and Monte Carlo simulations<sup>12,13</sup>. DNA damage that may be toxic to the cell is represented as a potentially lethal lesion (PLL) induced in a domain packaging DNA amount of  $g$  [kg]<sup>14</sup> along ionizing radiation particle track with local energy deposition

per domain  $z$  [Gy]. The DNA lesion (PLL) can gradually transform into lethal lesion (LL) or be repaired until the PLLs become non-existent:

- (i) A PLL transforms into a lethal lesion (LL) via a first-order process at a constant rate  $a$  [ $\text{h}^{-1}$ ]
- (ii) Two PLLs interact and transform into a lethal lesion (LL) via a second-order process at a constant rate  $b_d$  [ $\text{h}^{-1}$ ]
- (iii) A PLL is repaired via a first-order process at constant rate  $c$  [ $\text{h}^{-1}$ ].

Based on the three assumptions of (i) to (iii), the DNA damage kinetics are modelled by using rate equations for number of PLLs and LLs per domain.



**Figure 2-2. Assumption of the conventional MK model.** In this model, the probability density of dose in site so-called specific energy  $z$  in Gy is considered from the viewpoint of microdosimetry and DNA damage kinetics is incorporated as a biological response.

### 2.1.2. Rate Equations for DNA Damage Kinetics

The rate equation for the number of PLLs per domain after acute exposure is given by

$$\begin{aligned} \frac{d}{dt}x_d(t) &= -(a + c)x_d(t) - 2b_dx_d(t)^2, \\ &\cong -(a + c)x_d(t). \quad \because (a + c)x_d(t) \gg 2b_dx_d(t)^2 \end{aligned} \quad (2-1)$$

This can be solved as exponential function expressed by

$$x_d(t) = k_d g z e^{-(a+c)t}, \quad (2-2)$$

where  $k_d$  is the PLL induction coefficient per domain [/Gy/kg].

Let us consider continuous exposure to a cell population with dose-delivery time  $T$  [h] and dose-rate [Gy/h]. During dose-delivery, dose ( $z_1, z_2, \dots, z_N$ ) is discontinuously deposited in a domain with amount of DNA ( $g_1, g_2, \dots, g_N$ ) at each sub-section of dose-delivery time ( $0 \sim \Delta T, \Delta T \sim 2\Delta T, \dots, (N-1) \Delta T \sim N\Delta T$ ), thus we obtain the relation of  $T = N\Delta T$  where  $N$  is the number of sub sections in total dose-delivery time  $T$ .<sup>15,10</sup> In this study, we additionally assumed that the rate of SLDR ( $c_1, c_2, \dots, c_N$ ) might change at each sub-section of  $T$  ( $0 \sim \Delta T, \Delta T \sim 2\Delta T, \dots, (N-1) \Delta T \sim N\Delta T$ ). Thus, the number of PLLs per domain  $x_d(t)$  can be expressed based on Eq. (2-2) as

$$\begin{aligned}
 x_d(t) &= k_d g_1 z_1 e^{-(a+c_1)t} \\
 &\quad [0 \leq t < \Delta T] \\
 x_d(t) &= \sum_{n=1}^2 k_d g_n z_n e^{-(a+c_n)[t-(n-1)\Delta T]} \\
 &\quad [\Delta T \leq t < 2\Delta T] \\
 &\vdots \\
 x_d(t) &= \sum_{n=1}^{N-1} k_d g_n z_n e^{-(a+c_n)[t-(n-1)\Delta T]} \\
 &\quad [(N-2)\Delta T \leq t < (N-1)\Delta T] \\
 x_d(t) &= \sum_{n=1}^N k_d g_n z_n e^{-(a+c_n)[t-(n-1)\Delta T]}. \\
 &\quad [(N-1)\Delta T \leq t]
 \end{aligned} \tag{2-3}$$

Whilst PLLs induction competes with SLDR during dose-delivery, LLs gradually increase according to the next rate equation as

$$\frac{d}{dt} w_d = a x_d(t) + b_d x_d(t)^2, \tag{2-4}$$

where  $w_d$  is the number of LLs per domain. Solving Eq. (2-4) for the time dependent repair in Eq (2-3), we obtained the accumulated number of LLs at the level of domain as,

$$\begin{aligned}
 w_d &= \sum_{n=1}^N (A_n g_n z_n) + \sum_{n=1}^N (B_n g_n^2 z_n^2) \\
 &\quad + 2 \sum_{n=1}^{N-1} \sum_{m=n+1}^N [B_{nm} g_n g_m z_n z_m e^{-(m-n)(a+c_n)\Delta T}],
 \end{aligned} \tag{2-5}$$

where

$$A_n = \frac{ak_d}{(a + c_n)} \cong \frac{ak_d}{c_n} \quad (2-6a)$$

$$B_n = \frac{b_d k_d^2}{2(a + c_n)} \cong \frac{b_d k_d^2}{2c_n} \quad (2-6b)$$

$$B_{nm} = \frac{b_d k_d^2}{(a + c_n) + (a + c_m)} = \frac{2B_n(a + c_n)}{(a + c_n) + (a + c_m)} \cong \frac{2B_n c_n}{c_n + c_m} \quad (2-6c)$$

In Eqs. (2-6),  $a$  value is a few percentage of  $c_n$ ,<sup>3,11</sup> thus  $(a + c_n)$  can be simply expressed by  $c_n$ . Let  $\langle w_d \rangle$  and  $\langle w \rangle_T$  be the average number of lethal lesions per domain and the average number of LLs per nucleus, respectively. Considering the mean dose per nucleus  $\langle z_n \rangle$  and the mean amount of DNA per nucleus  $\langle G_n \rangle$  for the sub-section of exposure time  $\Delta T$  [h] and assuming that the absorbed dose rate is constant ( $\langle z_1 \rangle = \langle z_2 \rangle = \dots = \langle z_n \rangle = \dot{D}\Delta T$ ),  $\langle w \rangle_T$  is expressed as

$$\begin{aligned} \langle w \rangle_T &= p \langle w_d \rangle \\ &= \sum_{n=1}^N \left( A_n p \int_0^\infty g_n f_g(g_n) dg_n \int_0^\infty z_n f_z(z_n) dz_n \right) \\ &\quad + \sum_{n=1}^N \left( B_n p \int_0^\infty g_n^2 f_g(g_n) dg_n \int_0^\infty z_n^2 f_z(z_n) dz_n \right) \\ &\quad + 2 \sum_{n=1}^{N-1} \sum_{m=n+1}^N \left[ B_{nm} p \int_0^\infty g_n f_g(g_n) dg_n \int_0^\infty z_n f_z(z_n) dz_n \right. \\ &\quad \quad \times \left. \int_0^\infty g_m f_g(g_m) dg_m \int_0^\infty z_m f_z(z_m) dz_m e^{-(m-n)(a+c_n)\Delta T} \right], \\ \langle w \rangle_T &= \sum_{n=1}^N \left[ \left( A_n \langle G_n \rangle + \gamma \frac{B_n}{p} \langle G_n^2 \rangle \right) \dot{D}\Delta T + \frac{B_n}{p} \langle G_n^2 \rangle (\dot{D}\Delta T)^2 \right] \\ &\quad + 2 \sum_{n=1}^{N-1} \sum_{m=n+1}^N \left[ \frac{B_{nm}}{p} \langle G_n \rangle \langle G_m \rangle e^{-(m-n)(a+c_n)\Delta T} \right] (\dot{D}\Delta T)^2, \end{aligned} \quad (2-7)$$

where

$$\dot{D}\Delta T = \langle z_n \rangle = \int_0^\infty z_n f_z(z_n) dz_n, \quad (2-8a)$$

$$(\dot{D}\Delta T)^2 + \gamma \dot{D}\Delta T = \langle z_n \rangle^2 + \frac{\gamma_D}{\rho \pi r_d^2} \langle z_n \rangle = \int_0^\infty z_n^2 f_z(z_n) dz_n, \quad (2-8b)$$

$$\langle G_n \rangle = p \int_0^\infty g_n f_g(g_n) dg_n, \quad (2-8c)$$

$$\langle G_n \rangle^2 \Phi_n = \langle G_n^2 \rangle = p^2 \int_0^\infty g_n^2 f_g(g_n) dg_n, \quad (2-8d)$$

$$\langle G_n \rangle \langle G_m \rangle = p^2 \int_0^\infty g_n f_g(g_n) dg_n \int_0^\infty g_m f_g(g_m) dg_m, \quad (2-8e)$$

$p$  is the mean number of domains contained in a cell nucleus,  $f_z(z_n)$  is the probability density of the specific energy for each period  $(n-1) \Delta T \sim n \Delta T$ ,  $f_g(g_n)$  is the probability density of the DNA amount per domain for each period  $(n-1) \Delta T \sim n \Delta T$ ,  $\langle G_n \rangle = p \langle g_n \rangle$  is the amount of DNA contained in a cell nucleus. For simplicity, we define:

$$\alpha_n = A_n \langle G_n \rangle, \quad (2-9a)$$

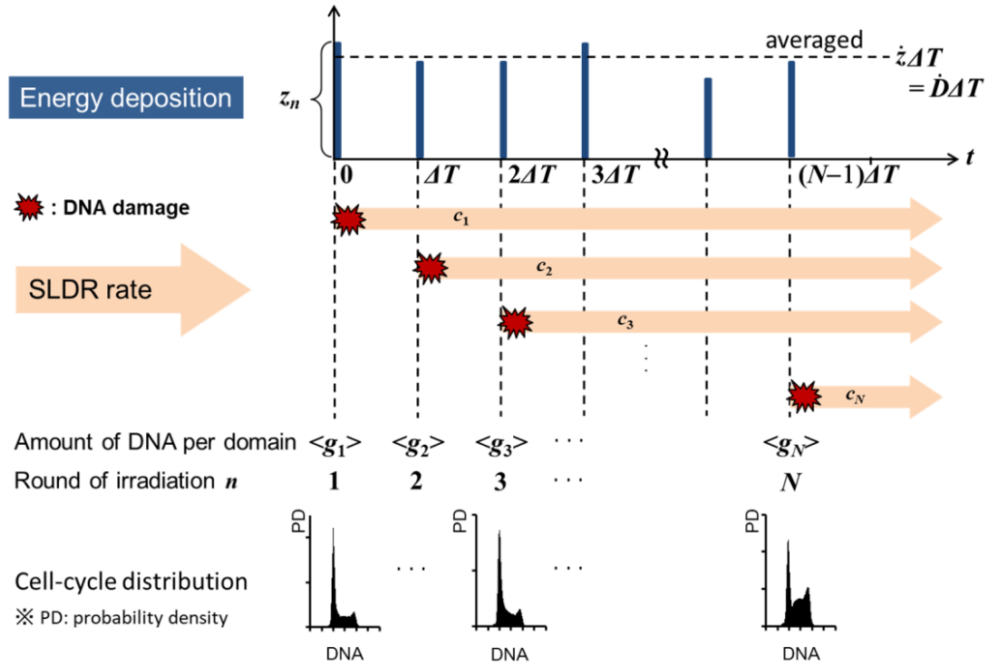
$$\beta_n = \frac{B_n}{p} \langle G_n^2 \rangle, \quad (2-9b)$$

$$\beta_{nm} = \frac{B_{nm}}{p} \langle G_n \rangle \langle G_m \rangle = \frac{2B_n}{p} \frac{c_n}{c_n + c_m} \langle G_n \rangle \langle G_m \rangle. \quad (2-9c)$$

It is assumed that the cells with no LLs have clonogenic ability. Assuming the number of LLs per nucleus follows the Poisson distribution, clonogenic cell survival ( $S$ ) can be expressed by

$$\begin{aligned} \langle w \rangle_T &= \sum_{n=1}^N \left[ (\alpha_n + \gamma \beta_n) \dot{D} \Delta T + \beta_n (\dot{D} \Delta T)^2 \right] \\ &\quad + 2 \sum_{n=1}^{N-1} \sum_{m=n+1}^N [\beta_{nm} e^{-(m-n)(a+c_n)\Delta T}] (\dot{D} \Delta T)^2 \\ &= -\ln S, \end{aligned} \quad (2-10)$$

Noted that Eq. (2-10) represents the relationship between accumulated absorbed dose and surviving fraction of cells (dose-response curve) in consideration of cell cycle (the changes in amount of DNA,  $\langle G_1 \rangle$ ,  $\langle G_2 \rangle$ , ...,  $\langle G_n \rangle$ , and in the rate of SLDR,  $c_1$ ,  $c_2$ , ...,  $c_N$ ) (see Fig. 2-3).



**Figure 2-3. Schematic image of the present modelling of cell-killing.** Dose-delivery time  $T$  [h] is divided into  $N$  sub- sections. During the dose-delivery, dose ( $z_1, z_2, \dots, z_N$ ) is discontinuously deposited in a domain with amount of DNA ( $g_1, g_2, \dots, g_N$ ) at each sub-section of dose-delivery time ( $0 \sim \Delta T, \Delta T \sim 2\Delta T, \dots, (N-1)\Delta T \sim N\Delta T$ ). In this study, to incorporate cell-cycle dependent repair ability, it is newly assumed that the rate of SLDR ( $c_1, c_2, \dots, c_N$ ) changes at each sub-section of  $T$  ( $0 \sim \Delta T, \Delta T \sim 2\Delta T, \dots, (N-1)\Delta T \sim N\Delta T$ ).

### 2.2.3. Link to the LQ model with or without Lea-Catcheside time factor

The present model can be linked to the LQ model with the classic Lea-Catcheside time factor.<sup>15</sup> Here, we assume a special case that cell condition (amount of DNA and SLDR rate) does not change during irradiation, i.e.,  $\langle G_1 \rangle = \langle G_2 \rangle = \dots = \langle G_N \rangle = \langle G \rangle = \text{constant}$ ,  $c_1 = c_2 = \dots = c_N = c = \text{constant}$ ,  $\alpha_n = \alpha_0 = \text{constant}$  and  $\beta_n = \beta_{nm} = \beta_0 = \text{constant}$ . Eq. (2-10) can be expressed by

$$\begin{aligned}
 -\ln S = & \sum_{n=1}^N \left[ (\alpha_0 + \gamma\beta_0)\dot{D}\Delta T + \beta_0(\dot{D}\Delta T)^2 \right] \\
 & + 2 \sum_{n=1}^{N-1} \sum_{m=n+1}^N \left[ \beta_0 \langle e^{-(m-n)(a+c_n)\Delta T} \rangle \right] (\dot{D}\Delta T)^2.
 \end{aligned} \tag{2-11}$$

Taking the limit  $N$  to infinity, Eq. (2-11) is approximately expressed by

$$\begin{aligned} \lim_{N \rightarrow \infty} (-\ln S) &= \lim_{N \rightarrow \infty} \sum_{n=1}^N \left[ (\alpha_0 + \gamma\beta_0) \dot{D} \Delta T + \beta_0 (\dot{D} \Delta T)^2 \right] \\ &+ 2 \lim_{N \rightarrow \infty} \sum_{n=1}^N \sum_{m=n+1}^N \left[ \beta_0 e^{-(m-n)(a+c)\Delta T} \right] (\dot{D} \Delta T)^2. \end{aligned} \quad (2-12)$$

Thus we can obtain the simple SF formula considering constant dose rate as

$$\begin{aligned} -\ln S &= (\alpha_0 + \gamma\beta_0) \dot{D} T + \frac{2\beta_0}{(a+c)^2 T^2} [(a+c)T + e^{-(a+c)T} - 1] (\dot{D} T)^2 \\ &= \alpha D + \beta D^2 \end{aligned} \quad (2-13)$$

where

$$\alpha = \alpha_0 + \gamma\beta_0, \quad (2-14a)$$

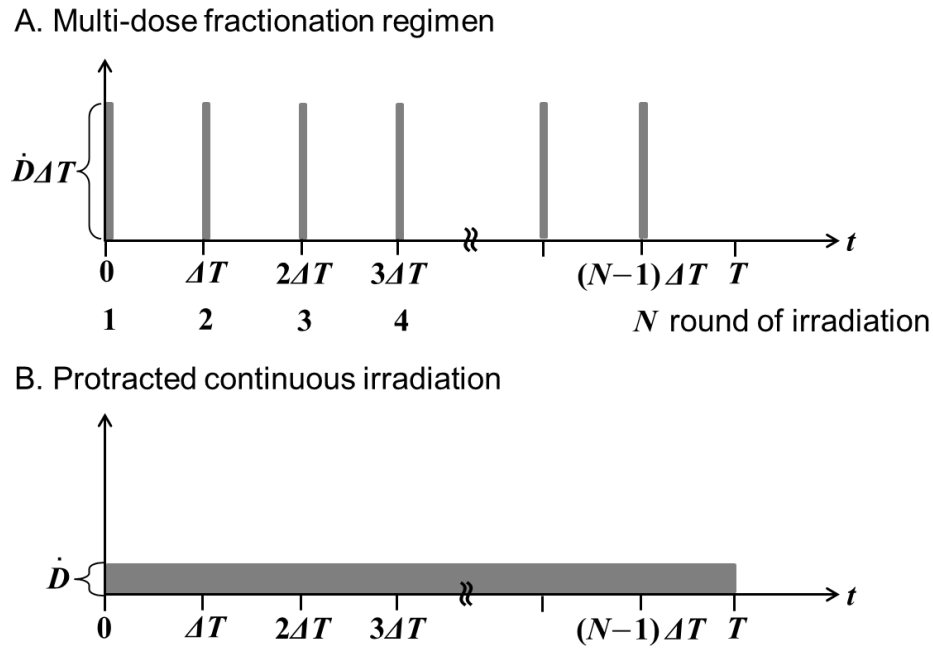
$$\beta = \frac{2\beta_0}{(a+c)^2 T^2} [(a+c)T + e^{-(a+c)T} - 1] = F\beta_0, \quad (2-14b)$$

$$D = \dot{D} T. \quad (2-14c)$$

Eq. (2-13) is cell-killing model including Lea-Catcheside time factor represented as SLDR. Further focusing on an acute exposure ( $T \rightarrow 0$ ), we obtained the following formula of surviving fraction as

$$\begin{aligned} -\ln S &= (\alpha_0 + \gamma\beta_0) D + \beta_0 D^2 \\ &= \alpha D + \beta D^2. \end{aligned} \quad (2-15)$$

Thus, linear-quadratic (LQ) model is approximate formula of surviving fraction in case of acute exposure without considering dose-delivery.<sup>15</sup>



**Figure 2-4. Schematic of irradiation regimen:** (a) for fractionated irradiation with dose  $\dot{D}\Delta T$  [Gy] at interval  $\Delta T$  [h] and (b) for continuous irradiation at dose rate [Gy/h]. The protracted continuous irradiation for various dose-rates is made according to this multi-dose fractionation.<sup>16</sup>

In this study, in comparison between dose-response curve described by Eq. (2-11) and that by Eq. (2-13), we planned a fractionated regimen equivalent to the continuous exposure with a constant dose rate (Fig. 2-4).

## 2.2. Non-Targeted Effects

### 2.2.1. Assumptions for Non-Targeted Effects

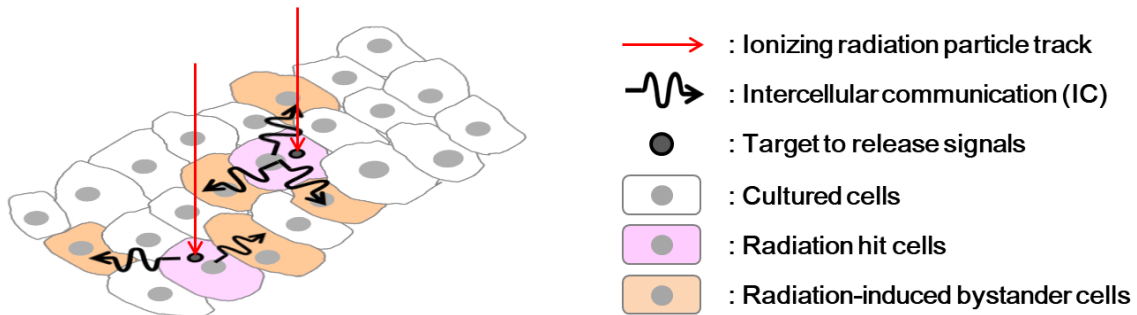
Radiosensitivity of cells is affected by not only targeted effects (TEs)<sup>17</sup> but also non-targeted effects (NTEs)<sup>18-20</sup> shown in Fig. 2-5. In the present NTE model, the scenario where the intercellular communication (IC) leads to cell death assumes:

- (i) Targets that emit the initial signals (stimulating substance) are regions (somewhere in the cell) of micro-order size as large as Mitochondria.<sup>21-23</sup> The number of hits to the region follows a linear-quadratic function of specific energy. Noted that the “hits” in

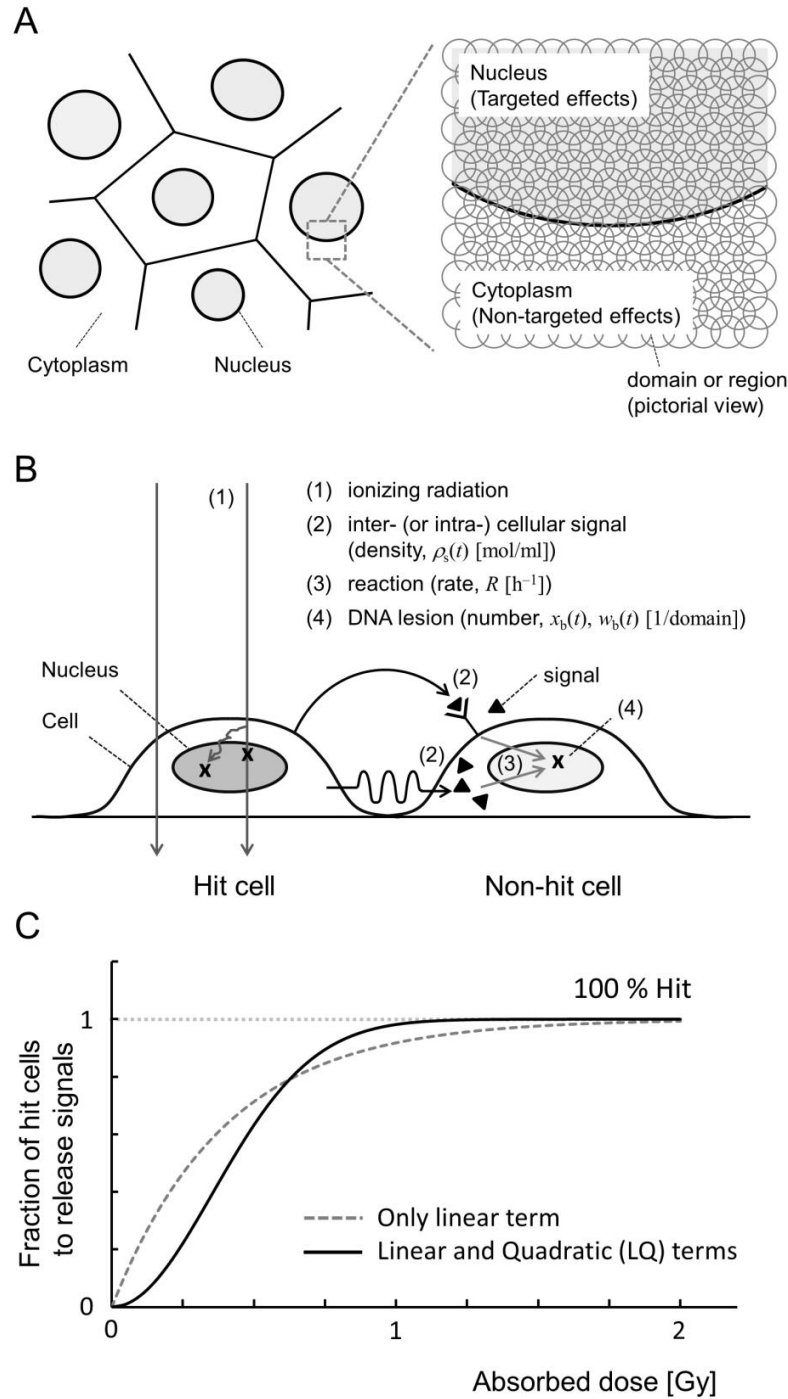
this NTEs model do not mean the events such as ionizations and excitations but the target activation to release the signals after irradiation.

- (ii) Initial signals originate and spread out in an area  $r$   $\mu\text{m}$  away from the hit cells. Cell-killing signals are increased by signal cascade but are decreased by the decay of the signals and reaction to cells.<sup>24</sup>
- (iii) In the non-hit cells reacted by cell-killing signals, PLLs are induced<sup>25</sup> in proportion to the signal concentration. According to the same constant rate of  $a$  [ $\text{h}^{-1}$ ] as the TEs<sup>3</sup> and the repair rate in the non-hit cells as  $c_b$  [ $\text{h}^{-1}$ ],<sup>26</sup> the signal-induced PLLs are transformed into LLs.
- (iv) The number of LLs per nucleus in NTEs follows the Poisson distribution in the same manner as that of the TEs. Cell death is induced if the LLs remain in the cell nucleus.

The conceptual illustration of the scenario that cell-killing signals induce DNA lesions which lead to cell killing is summarized in Fig. 2-6.



**Figure 2-5. Schematic representation of non-targeted effects (NTEs).** After the hit to some micro-order targets in cells, the cell-killing signals such as  $\text{Ca}^{2+}$ , NO and ROS are released and transferred from hit cells to non-hit cells. The signal-induced DNA damage might induce cell death in non-hit (bystander) cells.



**Figure 2-6. Conceptual illustration of the IMK model:** (A) for micrometer-order targets (domains) in a cell population, (B) for processes that induce NTEs and (C) for the LQ relation to demonstrate the number of hits to targets to release signals in NTEs. The scenario of non-hit effects in Fig. 2-5(B) is as follows: (i) when a cell population is exposed to ionizing radiation, DNA lesions are generated along the track of ionizing radiation; (ii) hit cells emit initial signals which spread out and increase by cascade reactions as cell-killing signals ( $\blacktriangle$ ); (iii) the signals that reach to the neighboring cells (non-hit cells) induce potentially lethal lesions (PLLs) in proportion to the signal density; (iv) the PLLs may transform into lethal lesions (LLs) or be repaired.

### 2.2.2. Target Activation Probability to Emit Cell-Killing Signals

In the present model, we assumed that the number of hits to the regions follows a linear-quadratic (LQ) shape as a function of specific energy. The LQ relation is mathematically useful for considering the hits by a single track and by two tracks (Fig. 2-6(C)). The signal entities are supposed to spread out when the regions are hit.<sup>21,22</sup> Region size is also assumed to be 1  $\mu\text{m}$  in diameter in this study for the same reason as TEs. The number of hits ( $N_h$ ) per domain can be expressed by a linear-quadratic function as

$$N_h = A_b z + B_b z^2, \quad (2-16)$$

where  $A_b$  and  $B_b$  are the proportionality factors to  $z$  [Gy] and  $z^2$  [ $\text{Gy}^2$ ], respectively. Considering the probability density of  $z$ , the average number of hits per cell  $\langle N_h \rangle$  is given by

$$\begin{aligned} \langle N_h \rangle &= p_b \int_0^\infty (A_b z + B_b z^2) f(z) dz \\ &= (\alpha_b + \gamma \beta_b) D + \beta_b D^2, \end{aligned} \quad (2-17)$$

where  $p_b$  is the number of regions for NTEs in a cell, and  $\alpha_b = p_b A_b$  and  $\beta_b = p_b B_b$ . The number of incident particles which traverse regions in each cell will follow the Poisson statistics because the probability of the traverse in a micro-order area is very low, especially in the case of low-dose exposure. So, the fraction of hit cells is expressed as

$$\begin{aligned} f_h(D) &= 1 - e^{-\langle N_h \rangle} \\ &= 1 - e^{-(\alpha_b + \gamma \beta_b) D - \beta_b D^2}. \end{aligned} \quad (2-18)$$

Equation (2-18) represents the dose-dependent probability of target activation, and this LQ formula considers the probability of hit to targets, which is an alternative function to the multi-target theory or threshold-like function<sup>27</sup>.

### 2.2.3. Signal-Induced DNA Damage and Cell Survival

A series of cell responses from the signals concentration to DNA damage is summarized here. Referring to the previous models of cell-killing signals, the cell-killing signal concentration  $\rho_s(\underline{r}, t)$  in an area  $\underline{r}$   $\mu\text{m}$  away from the hit cell (in diffusion area) at time ( $t$ ) after irradiation [mol/ml] is expressed by

$$\rho_s(\underline{r}, t) = \frac{r_s \mu_s S_d(\underline{r})}{\mu_s - (\lambda + R)} \{1 - e^{-[\mu_s - (\lambda + R)]t}\} e^{-(\lambda + R)t} \quad (2-19)$$

where  $r_s$  is the reactivation coefficient to produce the cell-killing signals;  $\lambda$  [ $\text{h}^{-1}$ ] is the constant rate for the cell-killing signal that decays exponentially (lifetime  $1/\lambda$ ); and  $R$  [ $\text{h}^{-1}$ ] is the constant rate for the cell-killing signals reacting with the nucleus of the non-hit cells.

Next, based on the new assumption (iii) about DNA damage kinetics, we deduced the temporal-dependence of signal-induced PLLs in NTEs. The PLLs are assumed to be induced in proportion to the amount of cell-killing signals, and the lesions have a potential to be repaired. The average number of the signal-induced PLLs,  $x_b(\underline{r}, t)$ , per non-hit domain nearby hit domains follows the equation

$$\frac{d}{dt} x_b(\underline{r}, t) = f_b(D) k_b R \rho_s(\underline{r}, t) - (a + c_b) x_b(\underline{r}, t), \quad (2-20)$$

where  $k_b$  is the number of the PLLs per domain caused by the signals [ $(\text{mol/ml})^{-1}$ ],  $a$  is a constant rate to transform from PLL to LL [ $\text{h}^{-1}$ ] in the MK model<sup>3</sup>,  $c_b$  is a constant rate for repair in non-hit cells [ $\text{h}^{-1}$ ], and  $f_b(D)$  denotes the fraction of non-hit cells in the cell population, i.e.  $f_b(D) = 1 - f_h(D)$ . By solving Eq. (2-20), we have

$$x_b(\underline{r}, t) = \frac{R \mu_s r_s k_b S_d(\underline{r}) f_b(D)}{\mu_s - (\lambda + R)} \left\{ -\frac{1 - e^{-[\mu_s - (a + c_b)]t}}{\mu_s - (a + c_b)} + \frac{1 - e^{-[(\lambda + R) - (a + c_b)]t}}{(\lambda + R) - (a + c_b)} \right\} e^{-(a + c_b)t}. \quad (2-21)$$

Here, the average number of PLLs per domain is considered to be a spatially-dependent number. It should be noted that all the signals are released from hit cells that include hit regions. The average number of the PLLs per domain is given by

$$\begin{aligned} \langle x_b \rangle(t) &= \sum_{P f_h(D)} x_b(\underline{r}, t) \\ &= \frac{R \mu_s r_s k_b S_P f_b(D) f_h(D)}{\mu_s - (\lambda + R)} \left\{ -\frac{1 - e^{-[\mu_s - (a + c_b)]t}}{\mu_s - (a + c_b)} + \frac{1 - e^{-[(\lambda + R) - (a + c_b)]t}}{(\lambda + R) - (a + c_b)} \right\} e^{-(a + c_b)t}. \end{aligned} \quad (2-22)$$

where  $s_p$  represents the maximum amount of initial signals [mol/ml] and  $P$  is the total number of regions for the NTEs; therefore, if all regions are hit in the irradiated field,  $s_p$  is equal to  $\langle s_d(r) \rangle P$ . Thus, Eq. (2-22) and the rate equation of the average number of LLs per domain can be linked as

$$\frac{d}{dt} w_b = a \langle x_b \rangle (t), \quad (2-23)$$

and we have

$$w_b = \frac{a R r_s k_b s_p f_b(D) f_h(D)}{(\lambda + R)(a + c_b)}. \quad (2-24)$$

Let  $\langle w \rangle_{NT}$  be the average number of LLs induced by the signals per cell nucleus, and we have

$$\begin{aligned} \langle w \rangle_{NT} &= \sum_p w_b \\ &= \frac{a R r_s K_b s_p}{(\lambda + R)(a + c_b)} [1 - e^{-(\alpha_b + \gamma \beta_b) D - \beta_b D^2}] e^{-(\alpha_b + \gamma \beta_b) D - \beta_b D^2} \\ &= \delta [1 - e^{-(\alpha_b + \gamma \beta_b) D - \beta_b D^2}] e^{-(\alpha_b + \gamma \beta_b) D - \beta_b D^2}, \end{aligned} \quad (2-25)$$

where

$$\delta = \frac{a R r_s K_b s_p}{(\lambda + R)(a + c_b)} \quad (2-26)$$

and  $p$  is the number of domains per cell nucleus,  $K_b = p k_b$ . Assuming that the number of LLs per nucleus follows the Poisson distribution, the expression of cell surviving fraction by the NTEs ( $S_{NT}$ ) is given from Eq. (2-25) as

$$\begin{aligned} \langle w \rangle_{NT} &= \delta [1 - e^{-(\alpha_b + \gamma \beta_b) D - \beta_b D^2}] e^{-(\alpha_b + \gamma \beta_b) D - \beta_b D^2} \\ &= -\ln S_{NT}. \end{aligned} \quad (2-27)$$

## 2.3. Integrated Cell-Killing Model

It can be assumed that the possibility of interactions among PLLs in TEs and NTEs is very small at the domain level, so the lesions in TEs and NTEs can be treated as independent ones. To describe the surviving fraction of irradiated cells considering the TEs and NTEs, the number of LLs related with the both effects (denoted as  $\langle w \rangle_T$  and  $\langle w \rangle_{NT}$ , respectively) is written by

$$\langle w \rangle = \langle w \rangle_T + \langle w \rangle_{NT}. \quad (2-28)$$

Thus, the cell surviving fraction ( $S$ ) is given by

$$S = S_T \times S_{NT}. \quad (2-29)$$

Hereafter, we call this model the “*integrated microdosimetric-kinetic (IMK) model*” in this study.

## 2.4. Modification of the IMK Model for Medium-Transfer NTEs

The modelling of the NTEs takes account of the signal concentration and the number of DNA lesions as a function of time after irradiation. Taking advantage of this versatility, we next modified the IMK model to express the cell survival after exposure with irradiated cell conditioned medium (ICCM), namely medium transfer bystander effects (MTBEs).

As for the ICCM in a dish containing irradiated cells with dose  $D$ , the mean signal concentration in ICCM at time  $t_h$  [h] after irradiation can be expressed using Eq.(2-19) as

$$\begin{aligned} \langle \rho_s(\underline{r}, t) \rangle &= \sum_{Pf_h(D)} \left\{ \frac{r_s \mu_s \langle s_d(\underline{r}) \rangle}{\mu_s - (\lambda + R)} \left[ e^{-(\lambda + R)t_h} - e^{-\mu_s t_h} \right] \right\} \\ &= \frac{r_s \mu_s S_P f_h(D)}{\mu_s - (\lambda + R)} \left[ e^{-(\lambda + R)t_h} - e^{-\mu_s t_h} \right]. \end{aligned} \quad (2-30)$$

Then, after the transfer of the ICCM to the dish with the recipient cells, the signal concentration at time ( $t$ ) is modified as

$$\langle \rho_s(\underline{r}, t) \rangle = \frac{r_s \mu_s S_P f_h(D)}{\mu_s - (\lambda + R)} \left[ e^{-(\lambda + R)t_h} - e^{-\mu_s t_h} \right] e^{-(\lambda + R)t}. \quad (2-31)$$

Thus, the average number of the PLLs per recipient domain is deduced to

$$\frac{d}{dt} \langle x_b \rangle(t) = k_b R \rho_s(t) - (a + c_b) \langle x_b \rangle(t), \quad \langle x_b \rangle(0) = 0, \quad (2-32)$$

then

$$\langle x_b \rangle(t) = \frac{R \mu_s r_s k_b S_P f_h(D) \left[ e^{-(\lambda + R)t_h} - e^{-\mu_s t_h} \right]}{\mu_s - (\lambda + R)} \cdot \frac{e^{-(a + c_b)t} - e^{-(\lambda + R)t}}{(\lambda + R) - (a + c_b)}, \quad (2-33)$$

and we have the formula by summing up over all domains ( $p$ ) as

$$\begin{aligned}\langle w \rangle_{NT} &= \sum_p w_b \\ &= \frac{aR\mu_s r_s K_b s_P f_h(D)}{(\lambda+R)(a+c_b)} \cdot \frac{[e^{-(\lambda+R)t_h} - e^{-\mu_s t_h}]}{\mu_s - (\lambda+R)}.\end{aligned}\quad (2-34)$$

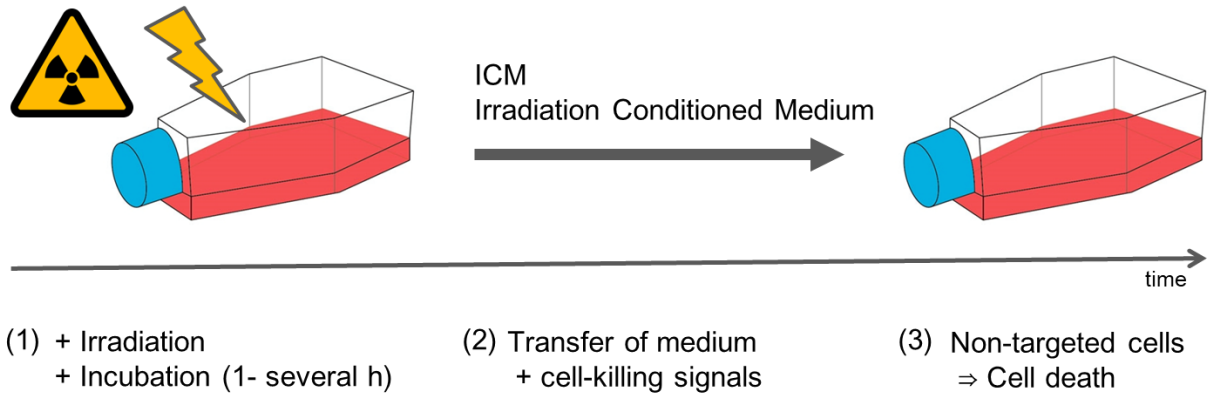
Finally, we have a relational expression of the cell surviving fraction with the recipient cell population as

$$-\ln S_{NT} = \delta_{mt} \left[ 1 - e^{-(\alpha_b + \gamma\beta_b)D - \beta_b D^2} \right]. \quad (2-35)$$

where

$$\delta_{mt} = \frac{aR\mu_s r_s K_b s_P}{(\lambda+R)(a+c_b)} \cdot \frac{[e^{-(\lambda+R)t_h} - e^{-\mu_s t_h}]}{\mu_s - (\lambda+R)}. \quad (2-36)$$

This equation represents the cell survival after exposure with ICCM, by which the probability of hit to regions in NTEs can be analyzed to interpret the underlying mechanism of signal emission quantitatively through the comparison with experimental MTBE data.



**Figure 2-7. Concept of medium-transfer bystander effects (MTBEs).** The first step is to irradiate the cultured cells and then to incubate the irradiated cell culture medium for 1- several h. The second step is to transfer the irradiation conditioned medium (ICM) to non-targeted (bystander) cells. And the final step is to observe the biological effects such as clonogenic survival or genomic instability.

## 2.5. Summary

The integrated model considering cell responses after exposure to ionizing radiations was developed based on lots of biological evidences. Mentioned earlier, the several factors to modify radio-sensitivity were incorporated into the model so far, newly calling it the “*IMK model*”. In this study, the four parts of the IMK model, i.e., (i) microdosimetry, (ii) DNA damage kinetics (SLDR) during and after irradiation, (iii) cell-cycle distribution (DNA contents and SLDR) and (vi) NTEs (intercellular signalling), were used for evaluating the cell survival after low-dose/ low-dose-rate protracted exposure to standard radiation of 250 kVp X-rays. Whilst the dose-rate effects on cell survival in relation to cell-cycle change is investigated by using the modelling of TEs in chapter 3, the cell survival after low-dose exposure is analysed by the combination of TEs and NTEs in chapter 4.

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## Chapter 3

# Dose-Rate Effects and Cell-Cycle Distribution under Protracted Exposure

During exposure at lower dose-rates, induction of toxic DNA lesions along the particle track competes with DNA damage repair, which leads to reduced cell-killing,<sup>1</sup> so called dose-rate effects (DREs).<sup>2</sup> This tendency can be explained by considering the sub-lethal damage repair (SLDR)<sup>3,4</sup> which was incorporated in the previous models.<sup>5-7</sup> However, described in the introduction section, enhancement of radio-sensitivity in a lower dose-rate range of 10-100 cGy/h has been reported, so called “inverse dose-rate effects (IDREs)”.<sup>8-10</sup> After acute exposure to low dose, mammalian cells exhibit hyper radio-sensitivity (HRS) to doses with < 30 cGy which is interpreted as a result from failure to arrest in G<sub>2</sub>.<sup>11,12</sup> Although the involvement of low-dose HRS has been presumed as the key phenomenon leading to the IDREs, another potential factor of cell-cycle change during the protracted exposure cannot be ignored. Re-evaluation of the DREs on cell survival including IDREs is a crucial issue from the standpoints of radiation therapy and radiation protection.<sup>13</sup>

Under the confluent condition of cells represented as plateau phase (similar to conditions in tissue),<sup>14</sup> the cell-cycle distribution is mainly composed of cells in G<sub>1</sub> phase. There have been some reports that the fraction of cells in G<sub>2</sub>/M phase gradually increases during protracted irradiation, i.e., at 60 cGy/h in tumour cell line of T98G (derived from human glioblastoma multiforma) and U373MG (derived from human glioblastoma astrocytoma).<sup>10</sup> Radio-sensitivity depends on cell conditions in cell cycle.<sup>15,16</sup> In this regard, radio-sensitivity during exposure can be potentially modulated by not only intercellular signalling as suspected recently but also changes in cell-cycle distribution including cell multiplication.<sup>17,18</sup> Thus, it is

necessary to investigate the cell-cycle change for various dose-rates at the level of *in vitro* experiments.

The recovery of cell survival during inter-fractionation time and continuous exposure has been described using the linear-quadratic (LQ) model with Lea-Catcheside time factor<sup>7</sup> or microdosimetric-kinetic (MK) model<sup>5,6</sup> considering SLDR during irradiation at the level of cell populations. However, the recent model analysis using the MK model suggests that rate of SLDR depends on dose-rate, in which the SLDR rate decreases as dose-rate lowers.<sup>19</sup> This interpretation may be linked to repair function depending on cell cycle. However, there is currently no report with evidence to support that SLDR changes depending on dose-rate. Thus, the interest in this study is directed to the consideration of SLDR depending on dose-rate associated with experimentally determined cell-cycle distribution during irradiation. In addition, from the standpoint of DNA-targeted effects, the radio-sensitivity might depend on the amount of DNA packaged in a cell nucleus.<sup>20,21</sup> The phase difference of the cell in terms of DNA amount should be incorporated based on the MK model.<sup>22</sup> Collectively, the further model analysis in relation to cell-cycle dynamics is necessary for investigating the biological effects after long-term exposure with ionizing radiation.

In this chapter, the Chinese Hamster Ovary (CHO)-K1 cell line that does not exhibit low-dose hyper-radio-sensitivity (HRS)<sup>23</sup> was used, and the dose-rate dependence of cell survival in relation to the change of cell-cycle distribution during irradiation was investigated. Combined with the survival data in reference, here we re-evaluated the radio-sensitivity at the endpoint of cell survival. This study finally provides: (i) important information of cell-cycle checkpoints under the long-term exposure, (ii) reasonable approach to describe the dose-rate effects on cell survival in consideration of cell-cycle changes and (iii) interpretation of the IDREs associated with cell-cycle dynamics.

## 3.1. Materials and Methods

### 3.1.1. Cell Culture and Irradiation Condition

A mammalian cell line, Chinese Hamster Ovary (CHO)-K1 was obtained from RIKEN Bio Resource Center, Japan (RBC0285). This type of cell line was selected because of it does not exhibit low-dose HRS.<sup>23</sup> The cells were maintained in Dulbecco's modified Eagle's (DMEM, Sigma Life Science) supplemented with 10% fetal bovine serum (FBS, Equitech-Bio Inc.) and 1% penicillin/streptomycin (Sigma Life Science) at 37°C in humidified 95% air and 5% CO<sub>2</sub>.

To investigate cell responses after a long-term exposure, five days prior to irradiation  $4 \times 10^5$  cells were seeded onto the cell culture dish with 60 mm diameter (Nippon Genetics) to obtain the cells under plateau phase. In parallel, to quantify the dependence of SLDR rates on cell cycle distribution, we prepared two cell-cycle distributions for plateau phase and logarithmic growth phase five days and one day after seeding, respectively.

### 3.1.2. Irradiation Condition

Standard radiation, 250 kVp X-rays (Stabilipan, Siemens, Concord, CA), was used to irradiate the cultured cells. The dose rate in air at the surface of cell culture was measured by using Farmer-type ionizing radiation chamber (model NE2581, Nuclear Enterprises Ltd) and was converted to the dose rate in water (4.31 Gy/min) according to the dose protocol TRS277.<sup>27</sup> From the comparison between Eq. (2-11) and Eq. (2-13), the practical fractionation regimens equivalent to the average dose rates were determined, which were 0.186 Gy per fraction at 1h interval for 0.186 Gy/h, 1.0 Gy per fraction at 1h interval for 1.0 Gy/h, 1.5 Gy per fraction at 30min interval for 3.0 Gy/h and 2.0 Gy/h at 20min interval for 6.0 Gy/h. To make the irradiation regimens, the cell-specific model parameters were used, which were determined as reported previously.<sup>1</sup>

- (1)  $F$  for  $T = 10$  min was calculated from  $(a+c) = 0.704 \pm 0.118$  in Eq. (2-14b),
- (2)  $(\alpha_0 + \gamma\beta_0) [= (\alpha_1 + \gamma\beta_1)]$  and  $\beta_0 (= \beta_1)$  were determined by fitting the LQ formula in

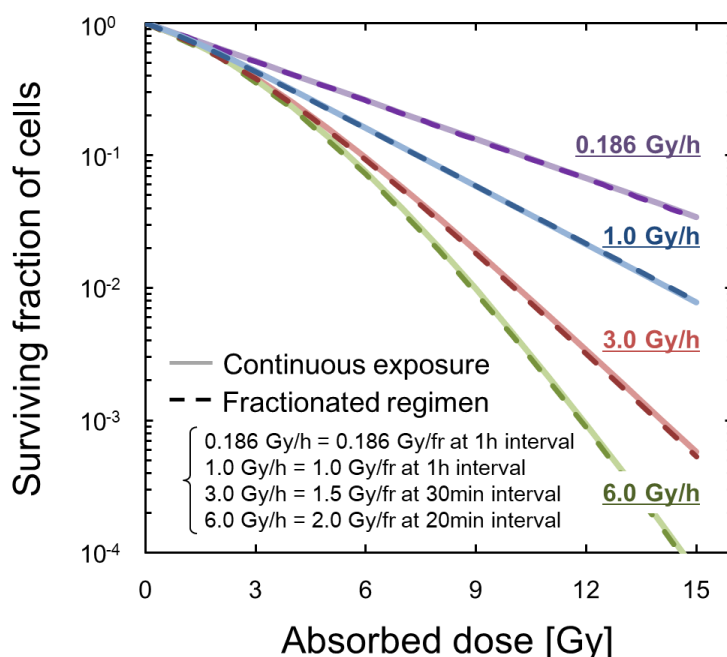
Eq. (2-13) to the cell survival data (for single-dose irradiation for 10 min).

(3)  $\alpha_0$  ( $= \alpha_1$ ) was calculated by the use of the parameters ( $(\alpha_0 + \gamma\beta_0)$ ,  $\beta_0$  and  $\gamma = 0.924$ ) from Eq. (2-14a).

The values of the parameters are summarized in Table 3-1. The example of irradiation regimen used in this study is summarized in Fig.3-1 and Table 3-2.

| Model Parameters                                                   | Cell Condition              |                          |
|--------------------------------------------------------------------|-----------------------------|--------------------------|
|                                                                    | Plateau Phase               | Logarithmic Growth Phase |
| $\alpha_1 = A_1 \langle G_1 \rangle (= \alpha_0) [\text{Gy}^{-1}]$ | 0.155 $\pm$ 0.027           | 0.075 $\pm$ 0.025        |
| $\beta_1 = B_1 \langle G_1^2 \rangle (= \beta_0) [\text{Gy}^{-2}]$ | 0.048 $\pm$ 0.003           | 0.028 $\pm$ 0.008        |
| $(a+c_1) \cong c_1 (= c) [\text{h}^{-1}]$                          | 0.704 $\pm$ 0.118           | 1.782 $\pm$ 0.441        |
| Relative $\langle G_1 \rangle (= \langle G \rangle)$               | 1.000 $\pm$ 0.003           | 1.227 $\pm$ 0.009        |
| Relative $\langle G_1^2 \rangle (= \langle G^2 \rangle)$           | 1.000 $\pm$ 0.007           | 1.457 $\pm$ 0.050        |
| $\rho [\text{g}/\text{cm}^3]$                                      | 1.000 (water)               | 1.000 (water)            |
| $r_d [\mu\text{m}]$                                                | 0.500                       | 0.500                    |
| $\gamma = \gamma_D / (\rho \pi r_d^2) [\text{Gy}]$                 | 0.924 (250 kVp)             | 0.924 (250 kVp)          |
| Each Phase                                                         | Cell Cycle distribution [%] |                          |
|                                                                    | Plateau Phase               | Logarithmic Growth Phase |
| $G_0/G_1$                                                          | 72.7 $\pm$ 6.6              | 37.6 $\pm$ 3.8           |
| S                                                                  | 14.9 $\pm$ 2.3              | 52.4 $\pm$ 4.5           |
| $G_2/\text{M}$                                                     | 12.4 $\pm$ 3.9              | 11.4 $\pm$ 0.7           |

**Table 3-1. Model parameters for two cell conditions in CHO-K1 cell line.** The set of values for plateau phase was obtained from our previous report. In these parameters,  $a$  is a few percentage of  $c$ .<sup>5,19</sup> Relative mean DNA amount per nucleus was normalized by that for plateau phase. The set of parameters for logarithmic growth phase was converted from that for plateau phase (SD was deduced by error propagation).



**Figure 3-1. Comparison between fractionation regimen and continuous exposure.** The regimen of dose fractionation was determined from the comparison between Eq. (2-11) and Eq. (2-13). The regimens used in this study are summarized in Table 3-1.

| Dose rate on average [Gy/h] | Absorbed dose per fraction [Gy/fraction] | Inter-fractionation time [min] |
|-----------------------------|------------------------------------------|--------------------------------|
| 0.186                       | 0.186                                    | 60                             |
| 1.00                        | 1.00                                     | 60                             |
| 3.00                        | 1.50                                     | 30                             |
| 6.00                        | 2.00                                     | 20                             |

**Table 3-2. Irradiation regimen equivalent to continuous exposure at a constant dose-rate.** The regimen were determined according to the MK model considering the DNA damage induction and constant SLDR rate approximately expressed by  $(a+c) = 0.704 \pm 0.118 \text{ [h}^{-1}\text{]}$ .<sup>1</sup>

### 3.1.3. Flow Cytometric Analysis of Cell-Cycle Distribution

For each dose rate exposure,  $10^6$  cells were harvested at 0, 2, 4, 6, 8, 10, 12 h after the start of irradiation and fixed with 70% ethanol, and then kept at 4°C for at least 2 h. After a centrifugation, the cells were re-suspended in 1ml phosphate-buffered saline (PBS) (–). After a centrifugation again, the DNA was stained with 0.5 ml FxCycle™ propidium iodide (PI)/RNase staining solution (Life Technologies) including 0.2% v/v triton X for 15 min in the

dark at room temperature. Cell-cycle distribution was then obtained by using the Attune acoustic focusing flow cytometer (Applied Biosystems by Life Technologies TM).

The fluorescence intensity emitted from the DNA in a nucleus was normalized by that from the DNA contained in a cell in  $G_0/G_1$  phase. The cell cycle distribution (fractions of the cells in  $G_0/G_1$ , S and  $G_2/M$ ) was then obtained from the DNA profile. All sets of cell-cycle study were performed three times. By using the Tukey-Kramer test, we evaluated if there is significant difference in the change of cell-cycle distribution from the control group (before irradiation at  $t=0$  [h]).

### 3.1.4. Clonogenic survival assay

After exposure to the regimen listed in Table 3-1, irradiated cells were trypsinized immediately and the appropriate number of cells was reseeded into a cell culture dish with 60 mm diameter (Nippon Genetics). Culture medium was exchanged every two days and the cells were cultured for 10-14 days. The colonies were fixed with methanol and were stained with 2% Giemsa solution (Kanto Chemical Co. Inc.) Surviving fraction was obtained from the ratio of irradiated cells and non-irradiated cells (control cells). The values are corrected by the plating efficiency for control cells of  $38.8 \pm 9.2\%$  (mean  $\pm$  standard deviation). The number of plated dishes for each data point was three and the experiments for each dose-rate were performed three times.

### 3.1.5. Determination of SLDR rate from a split-dose cell recovery curve

The constant rate of SLDR was obtained from cell recovery curve of cell survival in a split-dose experiment. Let us consider a case of exposing a cell population to equal acute doses with  $D_1 (= \langle z_1 \rangle)$  [Gy] and  $D_2 (= \langle z_2 \rangle)$  [Gy] at the interval of  $\tau (= \Delta T)$  [h]. The surviving fraction for a split-dose exposure is given by

$$-\ln S(\tau) = \sum_{n=1}^2 [(\alpha_0 + \gamma\beta_0)D_n + \beta_0 D_n] + 2\beta_0 e^{-(a+c)\tau} D_1 D_2. \quad (3-1)$$

The SLDR rate can be deduced by using the surviving fractions taking the limits of period of exposure interval ( $\tau \rightarrow 0$ ,  $\tau \rightarrow \infty$ ). Based on Eq. (2-16),  $S(0)$  and  $S(\infty)$  can be given by

$$-\ln S(0) = \sum_{n=1}^2 [(\alpha_0 + \gamma\beta_0)D_n + \beta_0 D_n^2] + 2\beta_0 D_1 D_2, \quad (3-2)$$

$$-\ln S(\infty) = \sum_{n=1}^2 [(\alpha_0 + \gamma\beta_0)D_n + \beta_0 D_n^2]. \quad (3-3)$$

On one hand, subtracting Eq. (2-18) from Eq. (2-17) gives

$$-\ln S(0) - (-\ln S(\infty)) = 2\beta_0 D_1 D_2. \quad (3-4)$$

On the other hand, taking the derivative of Eq. (2-16) and taking the limit of  $dS/d\tau$  as  $\tau$  tends to 0, we have

$$\lim_{\tau \rightarrow 0} \frac{1}{S} \frac{dS}{d\tau} = 2\beta_0 D_1 D_2 (a+c). \quad (3-5)$$

Thus we can deduce the cell-specific parameter of SLDR rate by using the following equation,

$$(a+c) = \frac{\lim_{\tau \rightarrow 0} \frac{1}{S} \frac{dS}{d\tau}}{\ln \frac{S(\infty)}{S(0)}}. \quad (3-6)$$

Cell recovery curves for a split-dose experiment are always influenced by re-distribution and cell proliferation.<sup>6</sup> To avoid the influences of re-distribution and repopulation, the initial slope  $dS/d\tau$  and  $S(\infty)$  were determined from experimental surviving fraction by taking the gradient from 0 to 1 h, and maximum survival was taken at the 2h time interval. Whilst the SLDR rate for plateau phase of CHO-K1 was taken from our previous report,<sup>1</sup> that for logarithmic growth phase was deduced by Eq. (3-6) and a cell recovery curve reported in the literature.<sup>28</sup>

### 3.1.6. Change of DNA Amount and SLDR Rate During Irradiation

To evaluate the contribution of the change of DNA amount per cell to the cell survival, the relative DNA amount per nucleus was incorporated into the IMK model expressed in Eq. (2-10). The relative DNA amount per cell nucleus at irradiation round  $n$ ,  $\langle G_n \rangle / \langle G_1 \rangle$  and

$\langle G_n \rangle^2 \Phi_n / \langle G_1 \rangle^2 \Phi_1$ , was calculated by

$$\frac{\langle G_n \rangle}{\langle G_1 \rangle} = \frac{\int G_n f(G_n) dG_n}{\int G_1 f(G_1) dG_1}, \quad (3-7)$$

$$\frac{\langle G_n \rangle^2 \Phi_n}{\langle G_1 \rangle^2 \Phi_1} = \frac{\langle G_n^2 \rangle}{\langle G_1^2 \rangle} = \frac{\int G_n^2 f(G_n) dG_n}{\int G_1^2 f(G_1) dG_1}, \quad (3-8)$$

where  $f(G_n)$  is the probability density of the cells which have a DNA amount  $G_n$  per nucleus,  $\Phi_n$  is the dimensionless parameter. The relative DNA contents per nucleus for each period of dose-delivery time  $T$  was obtained from the DNA histogram measured by flow-cytometric analysis with PI.

Additionally, in this study it is assumed that SLDR rate represented by  $c_n$  [ $\text{h}^{-1}$ ] changes during irradiation depending on fraction of cells in S phase, which has a high repair efficiency<sup>29</sup> leading to lower radio-sensitivity.<sup>16,30</sup> We estimated the differential rate of SLDR, and then deduced the change of SLDR rate per fraction of cells in S phase during exposure from experimental cell-cycle distributions using the following equation

$$c_n = c_0 + \frac{dc}{dN_S} \Delta N_S(t), \quad (3-9)$$

where  $t$  is the time after the start of irradiation [h],  $c_0$  is the SLDR rate at  $t=0$ ,  $dc/dN_S$  is the differential rate of SLDR per fraction of cells in S phase, and  $N_S$  is the fraction of cells in S phase. In this study,  $dc/dN_S$  was determined from the subtractions of SLDR and fraction of cell in S phase for plateau and logarithmic growth phases, and the change of cell fraction in S phase  $\Delta N_S$  was obtained from cell-cycle study during fractionated exposures.

### 3.1.7. Comparison Between Model and Measured Cell Survival

To investigate the influence of change of repair rate associated with cell-cycle distribution on cell survival curve, we compared the dose-response curves estimated by Eq. (2-10) with measured clonogenic cell survival data. The model parameters used in this study are listed in Table 3-1. Here, we assumed that the constant rates of  $a$  [ $\text{h}^{-1}$ ] and  $b_d$  [ $\text{h}^{-1}$ ] are cell-specific parameters independent of the cell-cycle distribution for simplicity, and the cell survival

curve was calculated considering the change of mean DNA amount per nucleus and SLDR rate during the exposure at various dose-rates.

The fit quality of the model used in this study was evaluated from reduced chi-square value expressed by

$$\chi^2 = \frac{1}{n} \sum_{i=1}^n \frac{(S_{i \text{ exp}} - S_{i \text{ model}})^2}{\sigma_{i \text{ exp}}^2} \quad (3-10)$$

where  $S_{\text{exp}}$  is measured cell survival,  $S_{\text{model}}$  is cell survival estimated by the present model,  $\sigma_{\text{exp}}$  is the standard deviation of measured cell survival.

### 3.1.8. Mean Inactivation Doses.

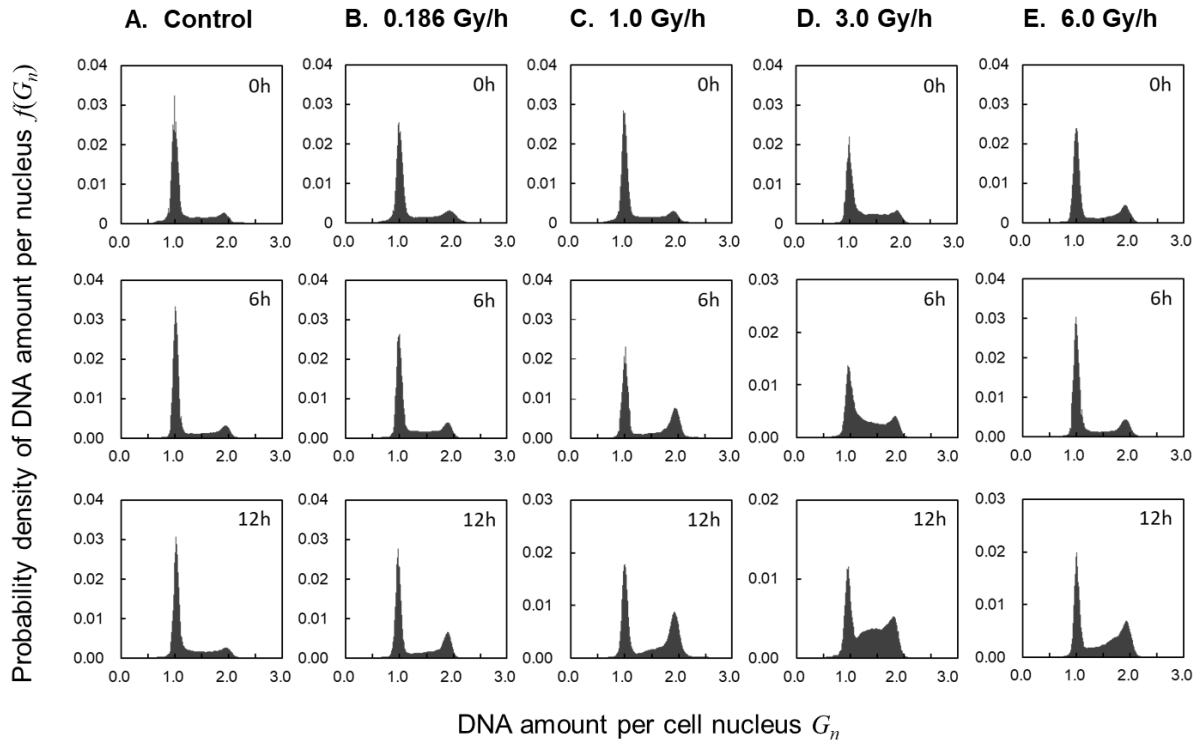
To investigate survival curves of the CHO-K1 cells, we further used the concept of the mean inactivation dose  $\bar{D}$ ,<sup>31</sup> which is recommended by ICRU Report 30.<sup>32</sup> In this quantity, dose-response curve is treated as a probability distribution of cell killing with absorbed dose. Considering the survival probability  $S(D)$  as an integral probability distribution, the mean dose necessary to inactivate cells (so-called mean inactivation dose)  $\bar{D}$  is given as,

$$\bar{D} = \int_0^{\infty} S(D) dD. \quad (3-11)$$

The  $\bar{D}$  values for various dose-rates of 18.6-60.0 Gy/h were calculated for experimental survival data, model prediction at constant SLDR of  $c \cong (a+c) = 0.704 \text{ [h}^{-1}\text{]}$  based on Eq. (2-13), and the prediction considering changes of mean DNA contents per nucleus and S-phase dependent SLDR rate based on Eq. (2-10). The  $\bar{D}$  values predicted by the model were compared with the experimental data by using  $R^2$  value given by

$$R^2 = 1 - \frac{\sum_{i=1}^n (exp_i - est_i)^2}{\sum_{i=1}^n (exp_i - \langle exp \rangle)^2}, \quad (3-12)$$

where  $exp$  and  $est$  represent the experimental value and the estimated value by the model, respectively, and  $n$  is the number of data.

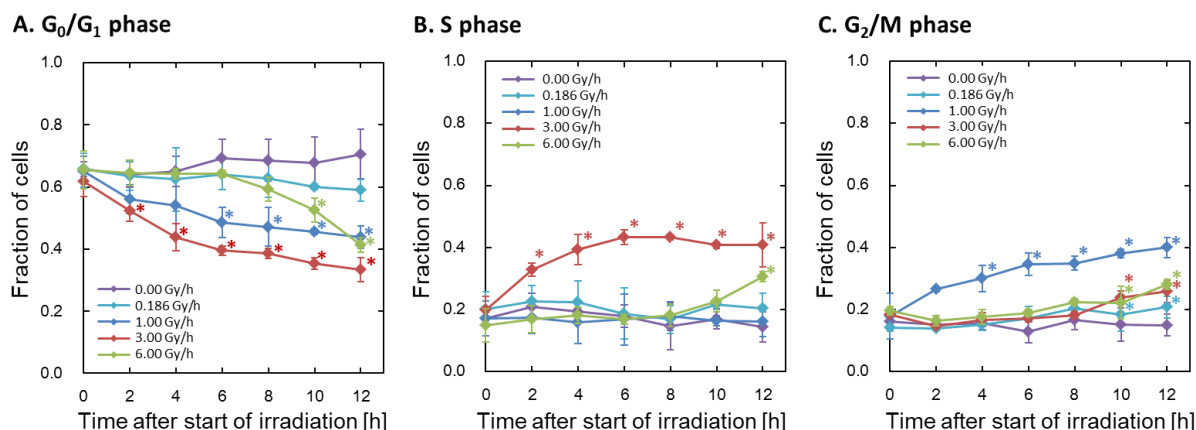


**Figure 3-2. DNA histogram during the irradiation at various dose-rates.** Cell-cycle dynamics during the exposure were measured via flow-cytometric analysis with propidium iodide (PI)/ RNase solution. The DNA profiles were used not only for incorporating the relative DNA amount per nucleus into the IMK model but also for obtaining the cell-cycle distribution, i.e., fractions of cells in  $G_0/G_1$ , S,  $G_2/M$  phases

## 3.2. Results

### 3.2.1. Change in Cell-Cycle During Exposure for Various Dose Rates

Four fractionated regimens equivalent to the continuous exposures with 0.186, 1.0, 3.0 and 6.0 Gy/h were performed to investigate DREs on cell-cycle distribution during exposure. Figures 3-2 and 3-3 shows the DNA histogram and the change in cell-cycle distribution during the exposure for various dose-rates of 0.186, 1.0, 3.0 and 6.0 Gy/h. The non-irradiated group (control) was also prepared to investigate the DNA damage checkpoints dependent of dose-rates.



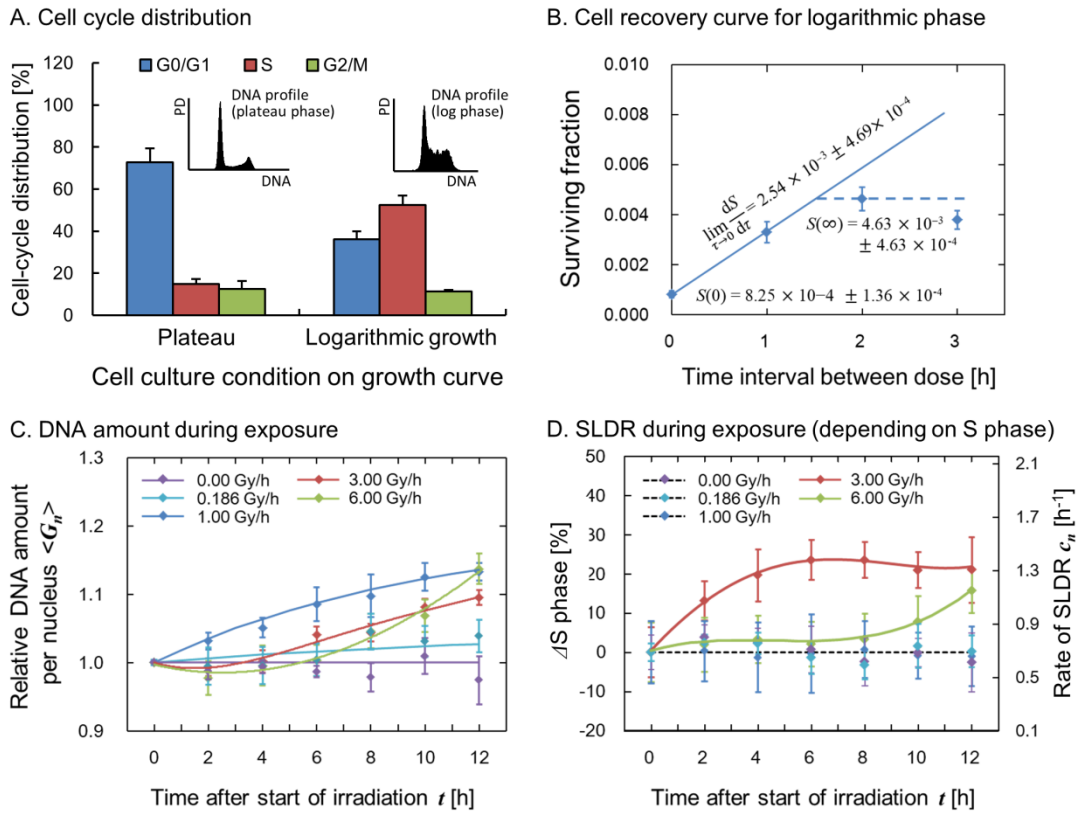
**Figure 3-3. Cell-cycle distribution during exposure at various dose-rates.** (A) is for the change of cell fraction in  $G_0/G_1$  phase, (B) is for that in S phase and (C) is for that in  $G_2/M$  phase. The data for 3.0 and 6.0 Gy/h were newly measured in the present flow-cytometric analysis. The symbol \* represents  $P < 0.05$  significant change compared with the data at just start of irradiation (0h). The error bar represents the standard deviation deduced from three independent experiments.

Whilst there is no significant differences of the cell-cycle for control group, the cell fraction in  $G_2/M$  phase increased slightly by 6.7% for 0.186 Gy/h and gradually by 22.1% for 1.0 Gy/h with increasing fractions. In contrast, during the exposure with 3.0 Gy/h, significant increases of cell fraction in S phase were observed up to 6h after the start of fractionated irradiation. During the exposure with the highest dose rate, 6.0 Gy/h, there is no difference in cell-cycle distribution until 8h after the start of irradiation. However, at 10h after the start of irradiation, significant increases of cell fraction in S phase and  $G_2/M$  phase were observed.

### 3.2.2. DNA contents and SLDR Rate During Exposure

To investigate the influence of the change in cell-cycle distribution on cell survival, we measured the change of mean DNA amount per nucleus and about the SLDR rate during exposure from flow-cytometric analysis of DNA profiles. In the upper panels of Fig. 3-4, the DNA profile for plateau phase and for logarithmic growth phase (Fig. 3-4A) and the procedure to deduce the SLDR rate for logarithmic growth phase from Eq. (3-6) with a split-dose cell recovery<sup>28</sup> (Fig. 3-4B) are presented. The details of cell-cycle distribution for both phases and SLDR rates  $c_1$  for two cell conditions (plateau and logarithmic growth

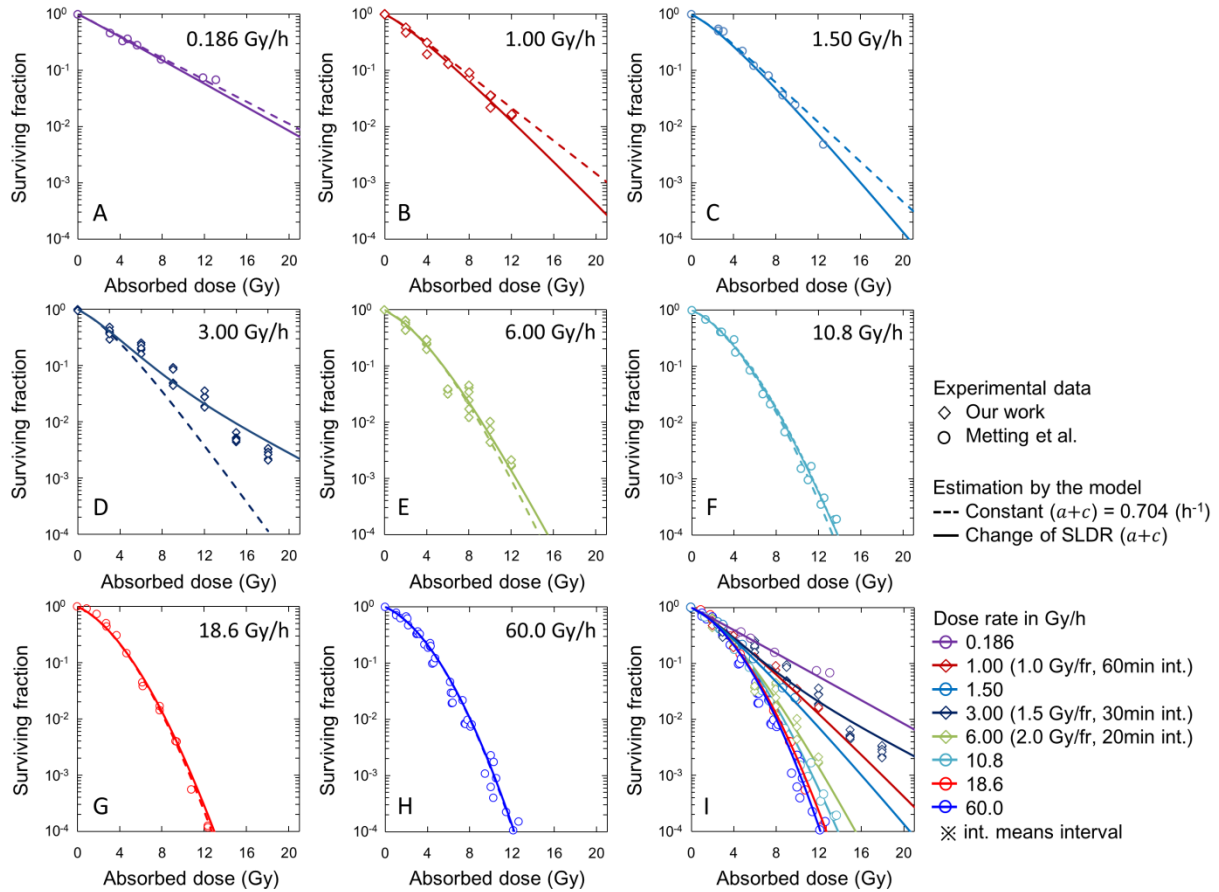
phases) were also listed in Table 3-2. Calculating the relative DNA amount in the cell population, the mean values  $\langle G_n \rangle / \langle G_1 \rangle$  for various dose-rates are shown in Fig. 3-4C, which were calculated by using the data in Fig. 3-2 and Eqs. (3-7) and (3-8). Based on the cell-cycle study (Fig. 3-4A) and the rate of SLDR via cell-killing model (Fig. 3-4B), we estimated the S-phase dependent SLDR rate during exposure according to Eq. (3-9), as shown in Fig. 3-4D. From the experimental data about fraction of cells in S phase and SLDR rate for plateau and logarithmic growth phases (Table 3-2), we deduced the value of  $dc/dN_S = 0.0287 \pm 0.0128$  [h<sup>-1</sup>/%].



**Figure 3-4. Cell condition (DNA profile and SLDR rate) input into the present model.** A shows DNA profile and cell-cycle distribution for plateau and logarithmic growth phase in CHO-K1 cell line, B shows procedure to deduce the rate of SLDR for logarithmic growth phase calculated by using Eq. (3-6) and cell recovery data,<sup>28</sup> C and D show the change of average DNA amount per nucleus and SLDR rate during protracted exposure, respectively, for various dose-rates. In Figs. 3C and D, whilst the symbols represent measured mean DNA contents and variation of S-phase fraction, the lines are the interpolated curve by spline. The constant rates for the exposure to 0.0, 0.186 and 1.0 Gy/h of SLDR were adopted because there is no significant change of S-phase fraction.

### 3.2.3. Prediction of Dose-Rate Effects on Cell Survival

Figure 3-5 shows the comparison between the cell survival curve described by the present model (IMK model) and the clonogenic survival data for various dose-rates of 0.186-60.0 Gy/h. This includes both newly obtained dose-response curves after the exposure with 1.0, 3.0 and 6.0 Gy/h (Fig. 3-5B, 3-5D and 3-5E) and re-analysed curves in comparison with reference data (Fig. 3-5A, 3-5C and 3-5F-3-5H).<sup>33</sup> In Fig. 3-5, dotted lines and solid lines represent the model prediction according to Eq. (2-13) with a constant rate of  $(a+c) = 0.704 \text{ [h}^{-1}\text{]}$  and Eq. (2-10) with the experimental-based variable DNA contents per nucleus and SLDR rate during exposure, whilst symbols denote the experimental cell survival including reference data.<sup>33</sup> In a large absorbed dose range of dose-response curve at 0.186, 1.0, 1.5 Gy/h, the surviving fraction described by the model considering the mean DNA contents per nucleus (solid line) features a higher radio-sensitivity than that by the previous MK model (dotted line) in Fig. 3-5A-3-5C. In Fig. 3-5D for 3.0 Gy/h, unexpected radio-resistance (increase of cell survival) was observed compared to the curve predicted by Eq. (2-13). This is attributable to both the change of SLDR rate and DNA amount per nucleus during exposure (Fig. 3-4C and 3-4D). The cell survival curves (in Figs. 3-5B, 3-5D and 3-5E) described by the IMK model for 1.0, 3.0 and 6.0 Gy/h with the both factors were in better agreement with experimental data (Table 3-3). Applying the time course of DNA contents and SLDR rate under 6.0 Gy/h into the model prediction of cell survival for a higher dose rate of 10.8 Gy/h, the surviving fraction estimated by the IMK model is slightly higher than that by the previous model (Fig. 3-5F). Figures 3-5G and 3-5H represent the cases in higher dose rates (18.6 and 60.0 Gy/h), where the dose-delivery time is relatively short. We applied the cell-cycle kinetics under higher dose-rate of 6.0 Gy/h to predict the dose-response curve for 18.6 and 60.0 Gy/h.



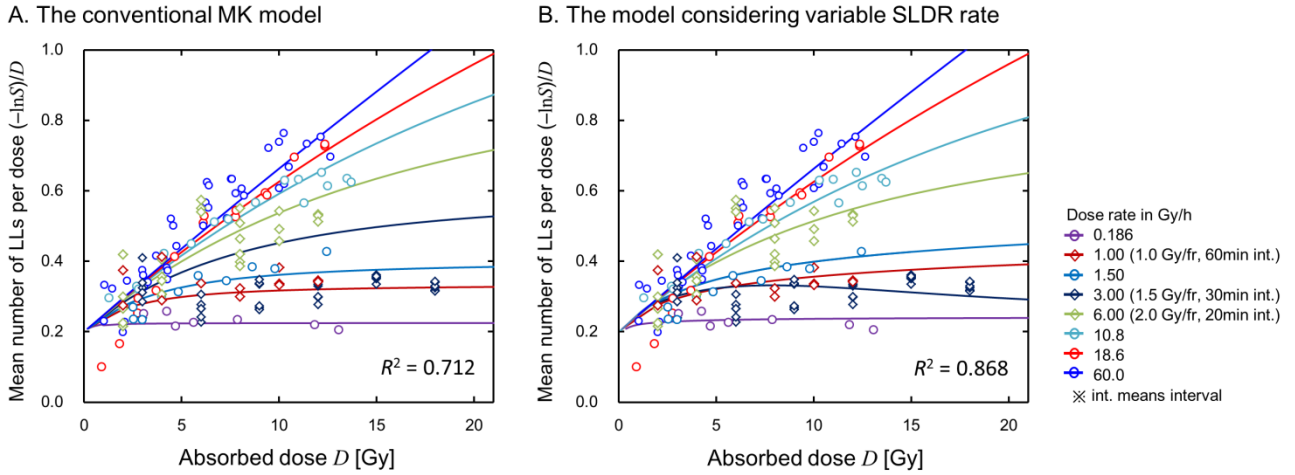
**Figure 3-5. Comparison between the clonogenic survival data and the model prediction.** Whilst the symbols denote the survival data by our work and reference data,<sup>33</sup> dotted line and solid line represent the model estimations with a constant rate of  $(a+c) = 0.704 \text{ [h}^{-1}\text{]}$  and with changing DNA contents  $\langle G \rangle$  and rate of  $c$  based on Fig. 3-4C and 3-4D, respectively.

Figure 3-5I shows the collection of all of the parts of dose-response curve estimated by the present model (following Eq.(2-10) and change of cell conditions given by Fig. 3-4C and 3-4D) in comparison with the experimental data. The cell survival increases as the dose-rate decreases by virtue of SLDR during dose-delivery time. However, focusing on the dose-rate range of 1.0-3.0 Gy/h, the inverse dose-rate effects (IDREs) can be observed, in which a radio-resistance resulted from cell accumulation in S phase for 3.0 Gy/h whilst a higher radio-sensitivity induced by cell accumulation in G<sub>2</sub>/M phase for around 1.0 Gy.

| Dose Rate (DR)     | $\chi^2$ value                     |                               |
|--------------------|------------------------------------|-------------------------------|
|                    | Constant SLDR<br>( $a+c$ ) = 0.704 | DNA amount &<br>changing SLDR |
| 1.00 Gy/h          | $9.27 \times 10^{-1}$              | $5.33 \times 10^{-1}$         |
| 3.00 Gy/h          | $1.09 \times 10^1$                 | $3.41 \times 10^0$            |
| 6.00 Gy/h          | $1.18 \times 10^0$                 | $8.28 \times 10^{-1}$         |
| Sum $\chi^2$ value | $1.30 \times 10^1$                 | $4.77 \times 10^0$            |

**Table 3-3. Statistical evaluation of fit quality of the present model.** The  $\chi^2$  value was calculated by using Eq.(3-10).

The log of surviving fraction per absorbed dose, which is equivalent to the mean number of lethal lesions per cell nucleus, enables us to evaluate a more realistic appreciation of the dose-survival relation. In this study, the dose-rate effects (DREs) on cell survival were further evaluated by this quantity. Figure 3-6 shows the comparison of the log of surviving fractions among the model with a constant ( $a+c$ ) including sub-lethal damage repair (SLDR)  $c$  [ $\text{h}^{-1}$ ], the model considering variable DNA amount (represented by  $\langle G \rangle$  and  $\langle G^2 \rangle$ ) and SLDR rate ( $c_n$ ) (shown in Figs. 3C and 3D), and the experimental data (our work and Metting *et al.* (1985)<sup>33</sup>). The  $R^2$  value for the present IMK model prediction (Fig. 3-6B) is larger than that of the conventional MK model with a constant ( $a+c$ ) value (Fig. 3-6A), which means the cell conditions such as DNA mount per nucleus and SLDR rate are important factors for modulating the dose-response curve, as discussed in the main text.

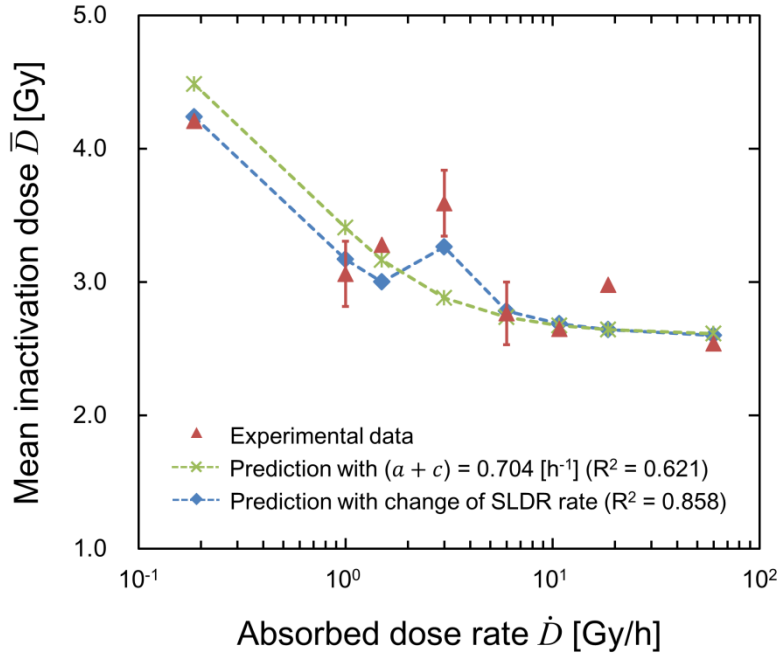


**Figure 3-6. Comparison of log of cell survival per dose by the model predictions with experimental data.** The symbols and solid lines represent experimental data and the curve by model estimation, respectively. Figure 3-6A is for the model in consideration of a constant rate of  $(a+c) = 0.704 \text{ [h}^{-1}\text{]}$  compared with the experiment, and 3-6B is for the model considering both the change of DNA amount per nucleus (Fig. 3-4C) and the SLDR rate (Fig. 3-4D) based on the flow-cytometric results measured in this study. The present model (B) reproduces the experimental data better than the conventional MK model (A) in terms of the  $R^2$  value.

### 3.2.4. Evaluation of Dose-Rate Effects on Mean Inactivation Dose

The DREs on cell survival shown in Fig. 3-5 were next evaluated by means of the mean inactivation dose  $\bar{D}$ ,<sup>30</sup> which is recommended by ICRU Report 30.<sup>32</sup> Figure 3-7 shows the relationship between the absorbed dose rate in Gy/h and mean inactivation dose  $\bar{D}$ , in which experimental  $\bar{D}$  was represented as red symbol. In this study, the DREs were predicted by using two SLDR approach, one for a constant  $(a+c)$  value of  $0.704 \text{ [h}^{-1}\text{]}$  and the other for the variable  $(a+c)$  values during irradiation shown in Fig. 3-4D. In Fig. 3-7, whilst the  $\bar{D}$  value predicted with  $(a+c) = 0.704 \text{ [h}^{-1}\text{]}$  becomes higher as the dose-rate is lower (green symbols and dotted line), the predicted  $\bar{D}$  with variable SLDR rate (blue symbols and dotted line) agrees better with the experimental  $\bar{D}$ . In comparison between the experimental and model predicted values, it was shown that the IMK model with variable  $(a+c)$  leads to a peak of resistance at dose-rates around 1.5-3.0 Gy/h in agreement with the experimental result. The experimental  $\bar{D}$  value at 10.8 Gy/h is higher than that by the model, suggesting a higher radio-resistance. However, the experimental data is calculated from just one data set result taken from reference.<sup>33</sup> Regarding the about 5 % inherent uncertainties of  $\bar{D}$  value<sup>31</sup> and the

possibility of experimental outliers, we cannot clearly judge if there is a reversal in radio-sensitivity at dose-rates around 10.8 Gy/h.

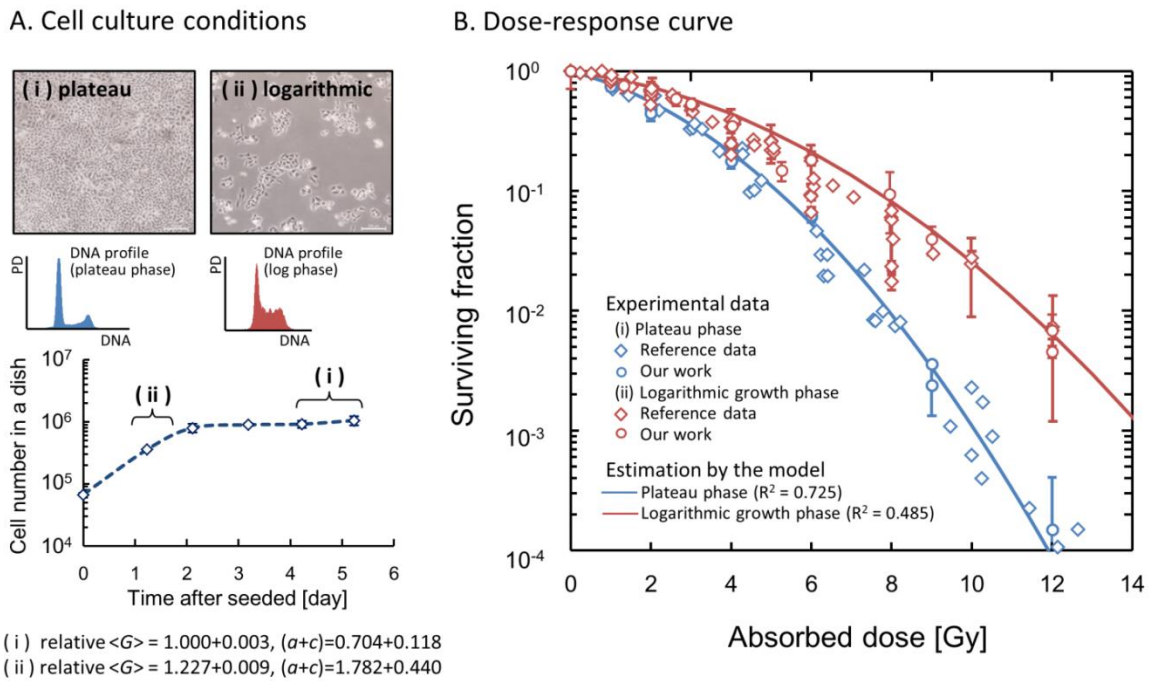


**Figure 3-7. Mean inactivation dose  $\bar{D}$  for evaluating the DREs.** Red symbols denote the experimental  $\bar{D}$ , green line (with symbols) is the  $\bar{D}$  calculated by using the cell-killing model formula Eq. (2-13) with constant  $(a+c)$  value of  $0.704 \text{ [h}^{-1}\text{]}$ , and blue line (with symbols) is the  $\bar{D}$  calculated by using the model (Eq. (2-10)) with variable repair rate,  $c$ , during the exposure. It is noted that there is concave characteristics in dose-rate range of around 1.5-3.0 Gy/h. The  $\bar{D}$  and  $R^2$  values were calculated by using Eqs. (3-11) and (3-12), respectively.

### 3.2.5. Testing the Assumption of Model Parameters

Here we assumed that the constant rates of  $a \text{ [h}^{-1}\text{]}$  and  $b_d \text{ [h}^{-1}\text{]}$  are cell-specific parameters independent of the cell-cycle distribution. To check if this assumption is correct or not, the comparison of Eq. (2-15) with measured cell survival after acute exposure was added under two cell phases, plateau phase and logarithmic growth phase (Fig. 3-8A). The set of parameters  $(\alpha_0, \beta_0)$  for plateau phase was converted to those in logarithmic growth phase by using the both ratios of DNA amount and SLDR rate, which is listed in Table 3-2. In Fig. 3-8B, we also compared the experimental surviving fractions for both phases<sup>1, 33-39</sup> with the

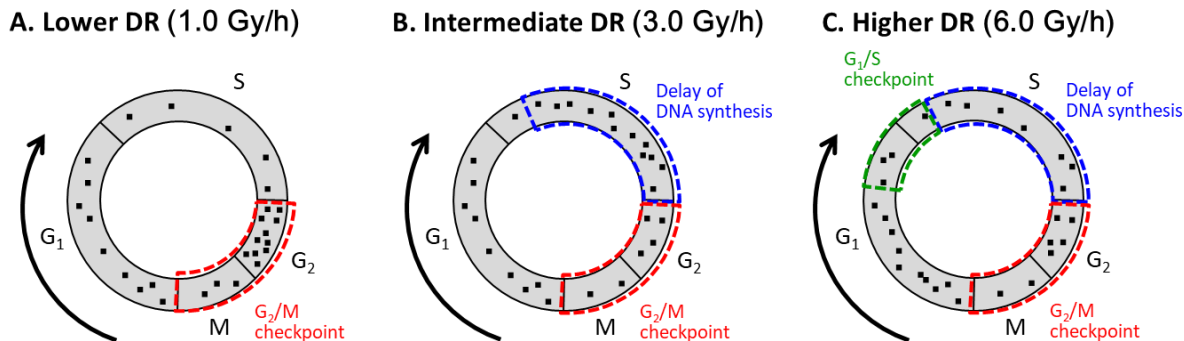
estimation by using the converted model parameters for logarithmic growth phase. In this comparison, the difference between dose-response curves in plateau phase and logarithmic growth phase is explainable by taking account of the mean DNA mount per nucleus and SLDR rate, which validates partly that the values of  $a$  [ $\text{h}^{-1}$ ] and  $b_d$  [ $\text{h}^{-1}$ ] do not depend on the cell condition.



**Figure 3-8. Dose-response curves for different cell culture conditions.** A: Upper panels represent microscopic images and DNA profiles for (i) plateau and (ii) logarithmic growth phases, respectively. Bottom figure is cell growth curve of CHO-K1 cell line to make the two cell conditions. B: Dose-response curves were predicted by considering DNA contents per nucleus and the rate of SLDR for the two different phases. The set of parameters for logarithmic growth phase listed in Table 3-2 was deduced from that for plateau phase with the cell condition (DNA content and SLDR rate). The estimated curves were compared with experimental data after irradiation with 250 kVp X-rays<sup>1, 33-39</sup> to check the assumption in this study.

### 3.3. Discussions

CHO-K1 cells show the following responses: (i) accumulation of cells in  $G_2$  during exposure with 1.0 Gy/h, (ii) delay of DNA synthesis and accumulation of the cells in S and  $G_2$  during exposure with 3.0 Gy/h and (iii) no significant change of cell-cycle distribution until 10h after the start of exposure to 6.0 Gy/h (Fig. 3-3). The increase of cells in S phase during the exposure to 3.0 Gy/h might be attributed to the DNA damage response in S phase checkpoint.<sup>40</sup> In contrast, according to previous investigations,<sup>41</sup> the threshold dose for blocking cell cycle progression at the  $G_1/S$  checkpoint is considerably higher dose. For the case of CHO cells, Lee *et al* reported a p53-independent damage-sensing checkpoint which operates to prevent late  $G_1$  or early S-phase.<sup>42</sup> In this regard, we interpreted that whilst the  $G_1/S$  checkpoint system was not activated under exposure with 3.0 Gy/h, it was activated under exposure with 6.0 Gy/h.



**Figure 3-9. Illustration of DNA damage checkpoints dependent of dose-rate.** A shows the accumulation of cells in  $G_2/M$  phase during the exposure at lower dose-rate such as 0.186 and 1.0 Gy/h. B represents the delay of DNA synthesis during the exposure at 3.0 Gy/h, leading to the cell accumulation in S phase. And C is the activation of the checkpoints of  $G_1/S$ , S and  $G_2/M$ .

From the measured cell-cycle distribution (Fig. 3-3), S-phase dependent SLDR rates was estimated (Fig. 3-4D) and subsequently cell survival curves were described (solid line in Fig. 3-5) in comparison with the MK model with a constant SLDR rate of  $(a+c) = 0.704 \text{ [h}^{-1}\text{]}$  (dotted line in Fig. 3-5). From the statistical evaluation by using the model (Table. 3-2), the

changing rate of repair,  $c$ , reasonably describes DREs on cell survival. This suggests that the magnitude of DNA damage might trigger the series of repair proteins activation, depending on dose rate.<sup>43</sup> Here, the misrepair rates,  $a$  [ $\text{h}^{-1}$ ] and  $b_d$  [ $\text{h}^{-1}$ ] were assumed to be constant cell-specific parameters not depending on cell condition. On this basis, we converted the set of parameters for plateau phase to that for logarithmic growth phase (Table.3-2), to reproduce the surviving fraction under the both phases in the methodology of the IMK model (Fig. 3-8B). According to the previous model assessment by Hawkins, the value of  $(a+c)$  is mainly composed of non-homologous end joining (NHEJ).<sup>19</sup> However, it is known that Homologous Recombination, a more accurate repair process, becomes more important in S phase, which may contribute to the observed increased resistance in S phase.

The further validation about that assumption to this was described at the discussion level here. From the previous reports of CHO-K1 cell line<sup>37,38</sup>, we took dataset of cell survival and cell-cycle information under the hypoxic (94.5%  $\text{N}_2$ , 0.5%  $\text{O}_2$ , 5%  $\text{CO}_2$ ) and anoxic (95%  $\text{N}_2$ , 5%  $\text{CO}_2$ ) conditions. The type of X-rays is the same standard radiation of 250 kVp. By using the different cell-cycle distributions under chronic hypoxia and chronic anoxia, re-confirming the assumption that model parameters of  $a$  and  $b_d$  are cell specific was made as follows:

- (i) The sets of model parameters ( $\alpha_0$ ,  $\beta_0$ ) for acute hypoxia and acute anoxia are determined by applying the model (Eq. (2-13)) to experimental data<sup>37,38</sup> via a maximum likelihood method with a Monte Carlo technique (blue line in Fig. 3-10A)
- (ii) Based on the cell-cycle information (DNA amount per nucleus and S-fraction dependent SLDR rate (Eq. (3-9))) taken from the reference (histogram in Fig. 3-10)<sup>38</sup>, the sets of parameters for acute hypoxia and acute anoxia are converted into those for chronic hypoxia and chronic anoxia, respectively
- (iii) The estimated cell survivals under chronic hypoxia and chronic anoxia are compared with the experimental data (red symbol and line in Fig. 3-10).

It should be noted that the relative DNA amount per nucleus (represented as  $\langle G \rangle$  and  $\langle G^2 \rangle$  in the present model) was deduced by Eqs. (3-13) and (3-14), assuming that the relative DNA contents per nucleus ( $G$ ) in  $G_1$ , S,  $G_2/\text{M}$  phases are 1.0, 1.5 and 2.0, respectively, as

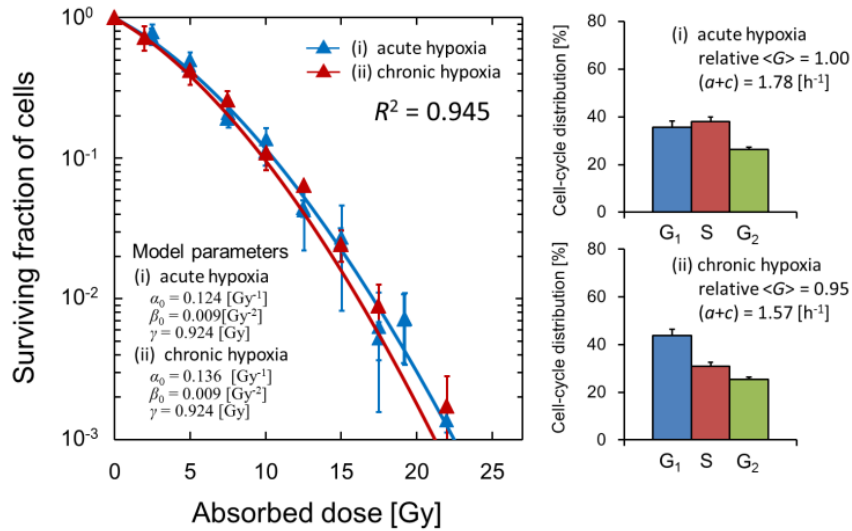
$$\langle G \rangle = 1.0 \times f_{G1} + 1.5 \times f_S + 2.0 \times f_{G2} \quad (3-13)$$

$$\langle G^2 \rangle = 1.0^2 \times f_{G1} + 1.5^2 \times f_S + 2.0^2 \times f_{G2} \quad (3-14)$$

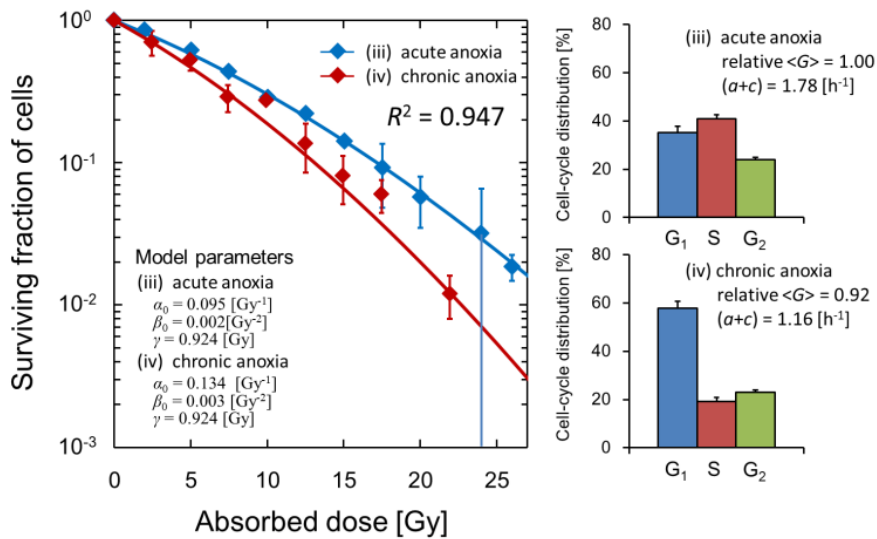
where  $f_{G1}$ ,  $f_S$  and  $f_{G2}$  are the fraction of cells in  $G_1$ , S and  $G_2$  phase, respectively. Figure 3-10 shows the comparison results between the dose-response curves described by the present model and experimental data.<sup>37,38</sup> In the model description, the conversion method of model parameters is the same as that described in the discussion part (mainly Fig. 3-8). In Fig. 3-10A, the difference of S-phase fraction  $\Delta S$  and relative  $\langle G \rangle$  under chronic hypoxia are  $-7.23\%$  ( $(a+c)=1.574 \text{ [h}^{-1}\text{]}$ ) and  $0.95$ , respectively, in which the model estimation under chronic hypoxia exhibits a radio-sensitivity not so higher than that under acute hypoxia. On the contrary, as shown in Fig. 3-10B, the  $\Delta S$  value and relative  $\langle G \rangle$  was  $-21.7\%$  and  $0.865$ , respectively. Thus, for the case of chronic anoxia, the lower cell survival was reproduced by the present model. Collectively, even under the different oxygen conditions, the dose-response curves by experiments were reproduced well by the model considering the mean amount of DNA packaged in cell nucleus and S-fraction dependent the repair rate of SLD, which supports that the two factors might play the key role in the cell-cycle dependence on cell survival.

The model proposed in this study follows the linear-quadratic (LQ) formalism, which is convenient for calculating  $\alpha/\beta$  and biological effective dose (BED)<sup>44</sup> considering the Lea-Catcheside time factor.<sup>7,45</sup> However, the DNA damage repair kinetics is complex and is generally quantified by two exponential components of rejoining of the broken ends of DNA.<sup>46</sup> If it is necessary to regard the complex repair kinetics, more detailed mechanistic modelling such as the computational modelling by McMahon<sup>47,48</sup> may be more suitable to understand the underlying radiation biology. As for clarifying the underlying mechanism of damage repair system, further investigation about the relation between SLDR and repairs function (NHEJ, HR, etc.) must be necessary.

A. Hypoxia (94.5% N<sub>2</sub>, 0.5% O<sub>2</sub>, 5% CO<sub>2</sub>)



B. Anoxic condition (95% N<sub>2</sub>, 5% CO<sub>2</sub>)



**Figure 3-10. Additional validation of model assumption through the comparison of the model estimation with experimental data for various cell-cycle conditions under the same oxygen condition.** (A) is under the acute and chronic hypoxia with 94.5% N<sub>2</sub>, 0.5% O<sub>2</sub> and 5% CO<sub>2</sub>. (B) is under acute and chronic anoxia with 95% N<sub>2</sub> and 5% CO<sub>2</sub>. By using the different cell conditions made after the treatment with hypoxia or anoxia, the assumption of cell-specific parameters ( $a$ ,  $b_d$ ) set was checked in this study. Here, the sets of parameters for acute hypoxia and acute anoxia were converted into those for chronic hypoxia and chronic anoxia, respectively, where the mean amount of DNA per nucleus and SLDR rate were considered. The symbols are experimental data taken from reference<sup>37,38</sup> and the solid lines are the estimation by the present IMK model.

The model and data exhibit IDREs in the dose-rate range of 1.0-3.0 Gy/h, attributable to increases in SLDR during irradiation. According to the previous investigation about dependence of cell phase on cell killing,<sup>16</sup> S phase (including late S phase) is the most radio-resistant. This tendency was found in the comparison between the experiment and the model in Fig. 3-5D-3-5F. In addition, the accumulation of cells in the relatively more radio-sensitive phase of G<sub>2</sub>/M<sup>16,49</sup> contributes to the modulation of cell survival curve, leading to the reversal of DREs as shown in Fig. 3-5I. Supported by the dose-response curve in Fig. 3-5I, the mean inactivation dose in Fig. 3-6 indicates the existence of IDREs as well. Considering these results, the combination of the accumulations of cells in S phase and G<sub>2</sub>/M phase is possibly responsible for IDREs.

IDREs on cell killing have been previously observed in dose-rate range of below 1.0 Gy/h,<sup>10,50</sup> and other reports have shown higher mutant frequencies at dose-rates lower than 0.1–1 cGy/min.<sup>51</sup> This previously observed dose-rate range is different from that we observed in this study. Other potential mechanisms, such as cumulative low-dose HRS after fractionated exposures has a possibility to induce this reversal in radio-sensitivity,<sup>50-53</sup> however the CHO-K1 cell line does not exhibit this behaviour.<sup>23</sup> From the present study and the previous reports, it is likely that the dose-rate range for IDREs related to cell-cycle effects is higher than that related with low-dose HRS. Further model development for the cumulative low-dose HRS based on more detailed mechanistic evidence about the time course of the HRS is necessary.

### 3.4. Summary

In summary, the radio-sensitivity after the protracted exposure for various dose rates was investigated. Focusing on cell-cycle distribution, the experimental results suggested that the CHO-K1 cells show following responses: (1) an accumulation of the cells in G<sub>2</sub> during exposure with lower dose rate (e.g., 1.0 Gy/h), (2) the delay of DNA synthesis and an accumulation of the cells in S/G<sub>2</sub> during the exposure with intermediate dose rates (e.g., 3.0

Gy/h), and (3) no significant change of cell-cycle distribution until 10h after the start of exposure to higher dose rate (e.g., 6.0 Gy/h). A greater radio-resistance after the exposure with 3.0 Gy/h was observed and this tendency was interpreted as increases in SLDR rate associated with the fraction of cells in S phase. Taking account of both higher radio-sensitivity under 1.0 Gy/h exposure and the radio-resistance after exposure to 3.0 Gy/h, the changes in cell cycle distribution during exposure modulate the cell survival curve and are possibly responsible for IDREs.

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## Chapter 4

# Radio-Sensitivity Considering Targeted and Non-Targeted Effects

The involvement of cell-cycle dynamics in inverse radio-sensitivity (cell survival) was investigated in the chapter 3. As discussed in chapter 3, the cell survival curve after exposure with X-rays was described by the modelling based on targeted effects (TEs).<sup>1</sup> However, radio-sensitivity after low-dose exposure is also affected by non-targeted effects (NTEs).<sup>2-4</sup> The NTEs are phenomena that non-irradiated cells near the radiation hit cells also show the same biological effects as radiation hit cells, so-called radiation-induced bystander effects (RIBEs), or in some cases low-dose hyper radio-sensitivity (HRS).<sup>5,6</sup> The NTEs have been interpreted as a consequence of intercellular communication with cell-killing signals.<sup>5</sup> However, the mechanisms of these effects remain unclear, particularly at low-dose exposure much less low-dose-rate exposure.<sup>7</sup> Biological effects after low-dose (below 0.5 Gy) or low-dose-rate irradiation on mammalian tissue have recently been highlighted in the field of radiation biology.<sup>8</sup> Thus, in this chapter, to estimate radio-sensitivity after low-dose or low-dose-rate exposure, the integrated microdosimetric-kinetic (IMK) model for the NTEs was verified and the underlying mechanisms of cell responses for NTEs was then investigated.

Whilst the mechanisms that induce low-dose HRS are still under investigation, clues are being obtained from the previous reports by many investigators. After irradiation, cell-killing signals such as cytokines, calcium and nitric oxide (NO)<sup>9-16</sup> are emitted from radiation hit cells, which transfer to non-irradiated cells via gap junctions or culture medium<sup>17</sup> and induce DNA damage in cell nucleus.<sup>18</sup> The size of the target to release the signals is on the micrometer order, i.e., 1.0-2.0  $\mu\text{m}$  diameter,<sup>19,20</sup> which may be related to target sensitivity<sup>21</sup> and the target may also be linked to mitochondria<sup>22</sup>. Whilst the induced DNA damage in

non-irradiated cells may be repaired,<sup>23</sup> the damage sometimes persists for a prolonged time<sup>24</sup> presumably because of different repair function of DNA damage.<sup>25-28</sup> At the current state, it has been interpreted that an increased radio-resistance (IRR) emerges if the repair capacity overcomes the low-dose HRS in a dose up to 0.3 Gy<sup>29</sup>.

Cell responses for not only DNA-TEs but also for NTEs (intercellular communication) have been modelled by many investigators.<sup>29-33,38-45</sup> Particularly focusing on NTEs, temporal characteristic of cell-killing signals has been modeled by Kunderát *et al*<sup>42</sup>, and stochastic model of signal-induced mutation and cell death was proposed by McMahon *et al*.<sup>41</sup> However, there is no single-frame model of cell responses after irradiation such as signal amount, DNA damage kinetics and cell survival. Thus, in the chapter 2, the integrated model has been developed so as to consider DNA damage kinetics considering different repair efficiency of non-hit cells.

In this chapter 4, by applying the IMK model to reference data of intercellular signals, DNA damage kinetics and surviving fraction after irradiation, the IMK model for NTEs (intercellular signaling) was verified and the mechanisms of NTEs were investigated. As a remarkable outcome of this model analysis, it was found that the low-dose hyper-radiosensitivity (HRS) is enhanced more as the DNA repair efficiency in non-hit cells is lower.

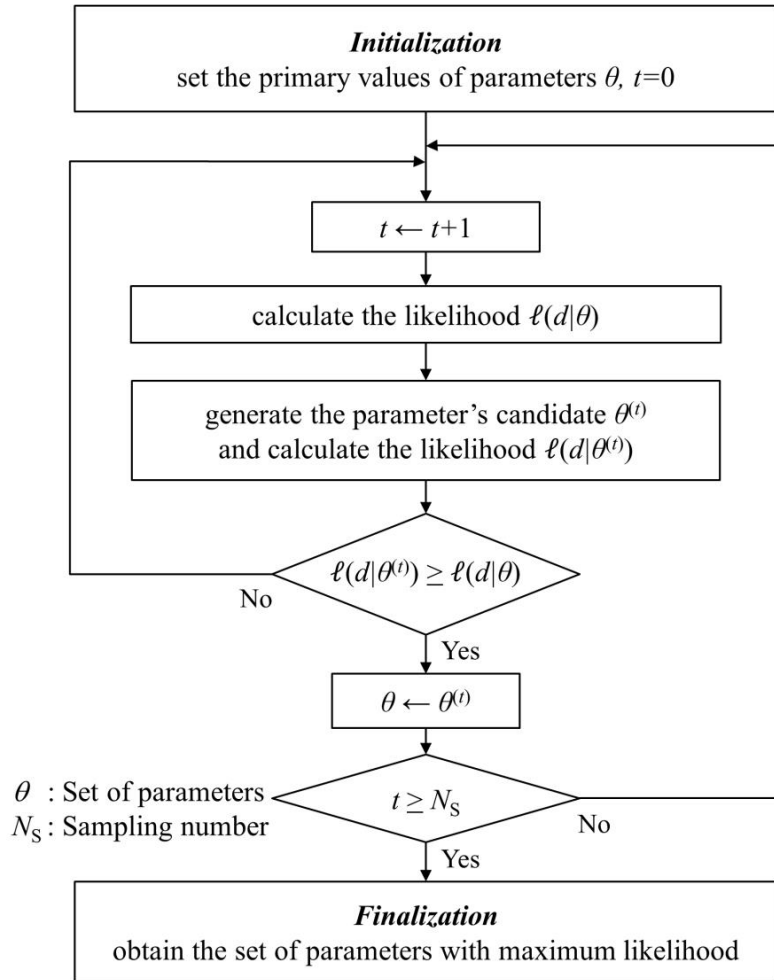
## 4.1. Materials and Methods

### 4.1.1. Application of the IMK Model to Experimental Data

To determine the cell-specific parameters in the IMK model, the formulae were fitted to the data by using the maximum-likelihood procedure with a Monte Carlo technique. The likelihood function is given by

$$\begin{aligned}\ell(d, \theta) &= \prod_{i=1}^N [\ell(d_i, \theta)] \\ &= \prod_{i=1}^N \left\{ \frac{1}{\sqrt{2\pi\sigma_i^2}} e^{-\frac{(Mod_i - Exp_i)^2}{2\sigma_i^2}} \right\},\end{aligned}\tag{4-1}$$

where  $\ell(d, \theta)$  is the likelihood function,  $N$  is the number of experimental data,  $\sigma$  is the standard deviation of experimental value,  $Exp$  stands for the experimental value and  $Mod$  for the value calculated by the model. Using Eq. (4-1) and the algorithm illustrated in Fig. 4-1, we determined the set of model parameters for describing cell responses of signal, DSB kinetics and cell survival.



**Figure 4-1. Algorithm of the maximum likelihood method used in this study.** We assumed that experimental value follows normal distribution, and the likelihood was calculated by using the likelihood function by Eq. (SI-11). In this study, we set that the sampling number  $N_s$  is equal to  $1 \times 10^7$ .

#### 4.1.2. Fitting to Intercellular Signalling and Induced DNA Damage Data

Responses to intercellular signaling are characterized by  $r_s s_d(r)$ ,  $\mu_s$  and  $(\lambda+R)$  in Eq. (2-19). We fit Eq. (2-19) to the relative signal concentration data reported by Lyng *et al.* (2002) for calcium as the first messenger of the signals<sup>46</sup> and by Han *et al.* (2007) for NO as the final messenger<sup>47</sup>. We then obtained the parameters,  $\mu_s$  and  $(\lambda+R)$ , for calcium and NO.

DNA damage induction and its repair kinetics are characterized by  $a$ ,  $b_d$ ,  $c$ ,  $\gamma$  and  $k_d <g>$  for TEs and  $c_b$ ,  $Rr_s k_b s_p$ ,  $\mu_s$ ,  $(\lambda+R)$ ,  $\alpha_b$ ,  $\beta_b$  and  $\gamma$  for NTEs. The damage kinetics at the domain level in TEs and NTEs can be expressed by Eqs. (2-2), (2-4), (2-20) and (2-23). By using these equations for the cell nucleus composed of  $p$  domains, we compared the average number of DSB per nucleus estimated by the model with experimental DSB data in primary normal human fibroblasts from the lung, MRC-5<sup>23</sup>. The response parameters of DNA damage link to cell survival parameters of  $\alpha_0$  and  $\beta_0$ , thus these can be determined backward from the parameters featuring cell survival according to the following procedure:

- (i) The  $\gamma$  value, as a representative of microdosimetric quantity for both effects, for 200 kVp X-rays was taken from the previous report<sup>49</sup>.
- (ii) The values of  $(a+c)$  and  $k_d <g>$  were obtained from two reports on mammalian cell lines<sup>33,36</sup>. Then, by using Eqs. (2-6a), (2-6b), (2-9a) and (2-9b),  $a$ ,  $b_d$ , and  $c$  values were deduced backward from the survival-specific parameters ( $\alpha_0 = 0.358 \text{ [Gy}^{-1}\text{]}$ ,  $\beta_0 = 0.0618 \text{ [Gy}^{-1}\text{]}$ ) in normal human fibroblast cell line<sup>50</sup>. Noted that we used the values,  $p = 9.55 \times 10^2$  calculated from the report on cell size<sup>51</sup> and  $\Phi = 1.04$  for the plateau phase from the cell-cycle data<sup>48</sup>.
- (iii) After fixation of the parameters in TEs ( $a$ ,  $b_d$ ,  $c$ ,  $\gamma$ ,  $k_d <g>$ ), we determined the cell specific parameters  $c_b$ ,  $Rr_s k_b s_p$ ,  $\alpha_b$ ,  $\beta_b$  by using  $\mu_s$ ,  $(\lambda+R)$  in the specific case of calcium in NTEs by fitting the formulae (Eqs. (2-2), (2-4), (2-20) and (2-23)) to the DSB data<sup>23</sup>.

| Concentration of intercellular signalling |                       |                       |
|-------------------------------------------|-----------------------|-----------------------|
| $\mu_s$                                   | 80.4                  | [h <sup>-1</sup> ]    |
| $\lambda+R$                               | 79.3                  | [h <sup>-1</sup> ]    |
| DNA damage kinetics (in case of calcium)  |                       |                       |
| $a$                                       | $9.37 \times 10^{-3}$ | [h <sup>-1</sup> ] *  |
| $b_d$                                     | $1.15 \times 10^{-1}$ | [h <sup>-1</sup> ] *  |
| $a+c$                                     | $7.04 \times 10^{-1}$ | [h <sup>-1</sup> ] ** |
| $p$                                       | $9.55 \times 10^2$    | [per nucleus]***      |
| $k_d <g>$                                 | $2.83 \times 10^{-2}$ | [Gy <sup>-1</sup> ]   |
| $\Phi$                                    | 1.04                  | (dimensionless)       |
| $a+c_b$                                   | $1.09 \times 10^{-2}$ | [h <sup>-1</sup> ]    |
| $R r_s k_b s_p$                           | $4.61 \times 10^{-1}$ | [h <sup>-1</sup> ]    |
| Number of hit probability for NTEs        |                       |                       |
| $\alpha_b$                                | 5.38                  | [Gy <sup>-1</sup> ]   |
| $\beta_b$                                 | 5.41                  | [Gy <sup>-2</sup> ]   |
| $\gamma$                                  | 0.923                 | [Gy] (200 kVp X-rays) |

**Table 4-1. Parameters associated with signal concentration (calcium) and the number of DNA lesions in human fibroblast cells.** \* $a$  and  $b_d$  were deduced by using Eqs. (2-6) and (2-9) with the parameters ( $\alpha_0$ ,  $\beta_0$ ,  $k_d p <g>$ ). \*\*( $a+c$ ) value in mammalian cells was taken from the reference.<sup>36</sup> \*\*\* $p$  was calculated from the sizes of domain ( $\phi$  1.0  $\mu$ m) and volume of nucleus (500  $\mu$ m<sup>3</sup>) reported by the previous report.<sup>51</sup>

#### 4.1.3. Fitting to Dose-Response Curve and MTBE Data

The dose-response curve of cell survival (hereafter, the cell survival curve) is characterized by 7 parameters,  $\alpha_0$ ,  $\beta_0$  and ( $a+c$ ) in TEs,  $\alpha_b$ ,  $\beta_b$  and  $\delta$  in NTEs, and  $\gamma$  in both effects. We applied Eqs. (2-13), (2-27) and (2-29) to the cell survival data for Chinese Hamster lung fibroblast cell line V79-379A<sup>52-57</sup> and human breast carcinoma cell line T-47D.<sup>58-61</sup> The  $\gamma$  value was taken from the previous reports<sup>36,49</sup>, and the residual cell-specific parameters were determined all at once. By using these parameters, we illustrated cell survival curves in comparison with the experimental survival data for the V79-379A<sup>52-57</sup> and T-47D cell lines<sup>58-61</sup>.

The other SF data of HPV-G cells and E48 cells after exposure with ICCM<sup>18,62-65</sup> and that

of CHO-K1 cells after irradiation of broad beam X-rays<sup>66</sup> were also fitted by the IMK model, and all parameters in the model were determined simultaneously. Whilst the data in MTBEs was used to evaluate the linear-quadratic relation of target activation to release signals, a set of SF data in CHO-K1 cells was used to investigate the relation between the low-dose HRS and the inactivation of the repair in non-hit cells.

#### 4.1.4. Fit Quality

To check the fit quality of the IMK model to the experimental data of signals, DNA damage kinetics and cell survival, we calculated  $R^2$  value given by

$$R^2 = 1 - \frac{\sum_{i=1}^n (Exp_i - Mod_i)^2 / (n-m-1)}{\sum_{i=1}^n (Exp_i - \langle Exp \rangle)^2 / (n-1)}, \quad (4-1)$$

where  $Exp$  represents the experimental value and  $Mod$  is for the calculated value by the IMK model,  $n$  is the number of data,  $m$  is the number of parameters in the model, and  $n-m-1$  represents the degrees of freedom. In addition to the  $R^2$  value, for evaluating model selection in NTEs we calculated the chi-square value and Akaike's information criterion (AIC)<sup>67</sup> for the data in MTBEs. The chi-square and AIC values are defined by

$$\chi^2 = \sum_{i=1}^n \frac{(S_{exp_i} - S_{model_i})^2}{\Delta S_{exp_i}^2}, \quad (4-2)$$

$$AIC = n \ln \left[ \frac{\sum_{i=1}^n (S_{exp_i} - S_{model_i})^2}{n} \right] + 2m, \quad (4-3)$$

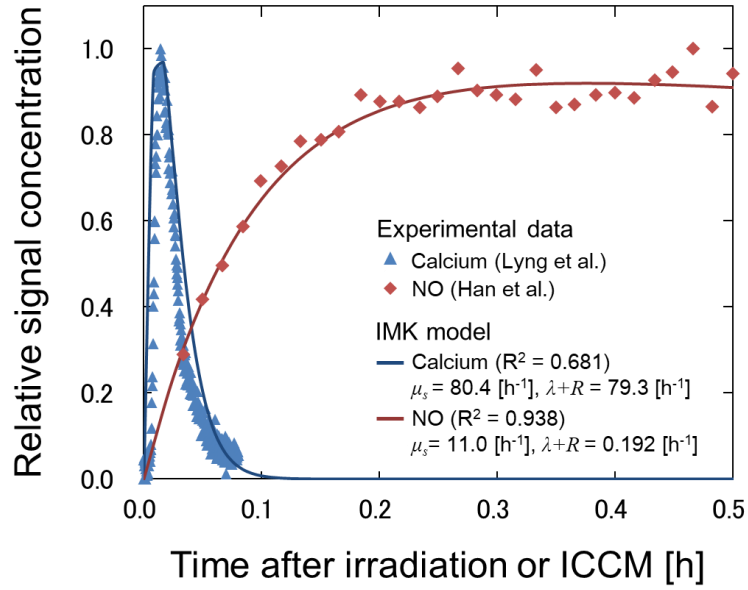
where  $S_{exp}$  is the experimental surviving fraction,  $S_{model}$  is the surviving fraction calculated by the model and  $\Delta S_{exp}$  is the experimental uncertainty.

## 4.2. Results

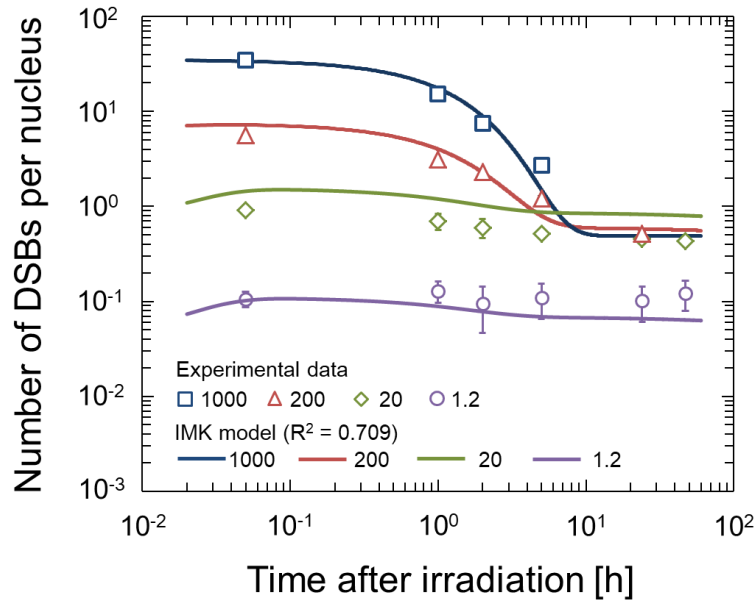
### 4.2.1. Temporary-Dependence on Response to Signals and DSBs

Figure 4-2A shows the changes in cell-killing signal concentration of calcium and NO after irradiation or ICCM, in which the model well reproduces experiments<sup>46,47</sup> with reasonable  $R^2$  values. The parameters for cell-killing signal,  $\mu_s$  and  $(\lambda+R)$ , for calcium are 80.4 [ $\text{h}^{-1}$ ] and 79.3 [ $\text{h}^{-1}$ ] and those for NO are 11.0 [ $\text{h}^{-1}$ ] and 0.192 [ $\text{h}^{-1}$ ], respectively. According to Hu *et al.* (2006)<sup>18</sup>, the number of DSBs in irradiated and in non-irradiated cells reaches its peak at 30 min after radiation. Since the damage induction in the NTEs may have occurred at an earlier time after irradiation, we next tried to reproduce the kinetics of the number of DSBs per nucleus induced by NTEs, assuming that the first messenger of calcium induces the damage. Figure 4-2B shows fitting results of the IMK model to the DSB kinetics data in MRC-5 (human normal fibroblast cell line<sup>23</sup>), in which the DSB kinetics curve in the IMK model was described using the parameters summarized in Table 4-1. The lines and symbols in Fig. 4-2B represent the curves by the IMK model (Eqs. (2-2), (2-4), (2-20) and (2-23)) and experimental data<sup>23</sup>, respectively. The number in Fig. 4-2B represents the prescribed dose in mGy. The curves by the IMK model in consideration of inactivation of the repair in non-hit cells agree well with the experimental data.

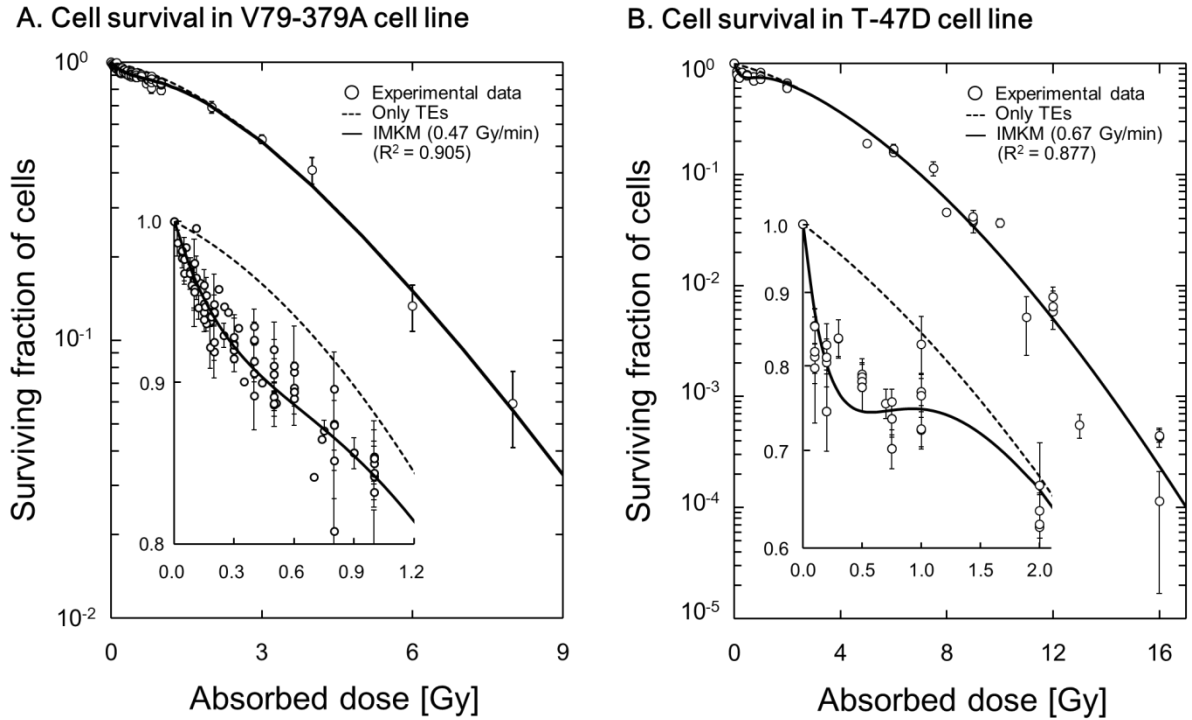
### A. Concentration of cell-killing signals



### B. Number of DNA lesions (PLLs + LLs)



**Figure 4-2. Model performance for cell-killing signals and DNA damage kinetics.** Whilst A is the fitting curve by the IMK model to experimental data of cell-killing signal kinetics, B show the comparison of the IMK model curve with experimental data for temporary dependence of DNA double-strand breaks (DNA-DSBs) per nucleus. In Fig. 4-2A, the specific signals, calcium as the first messenger<sup>46</sup> and NO as the final messenger<sup>47</sup>, were adopted. Response parameters to DNA-DSBs are listed in Table 4-1. In Fig. 4-2B, the number represents the prescribed dose to cells in mGy. Under the assumption that the repair in non-hit cells is inactivated, the IMK model reproduced the time dependency of DNA-DSBs in good agreement with experimental data<sup>23</sup>.



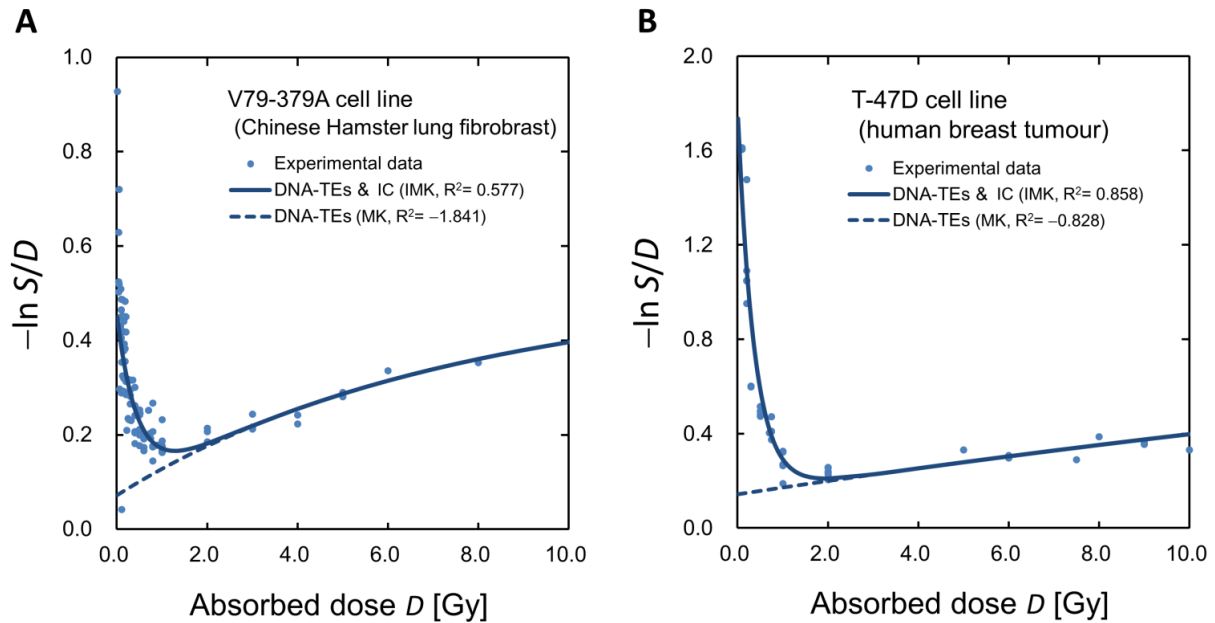
**Figure 4-3. Model performance for dose-response curve (cell surviving fraction).** C is dose-response curve in Chinese Hamster lung fibroblast cell line V79-379A and D is the curve in human breast cancer cell line T-47D. The symbols represent the experimental SF data reported by Marples *et al.*<sup>52-57</sup> and Edin *et al.*<sup>58-61</sup>. The black solid line and dotted line represent the curve described by the IMK model with or without NTEs, respectively. The parameters in the model are summarized in Table 4-2.

#### 4.2.2. Cell Survival Curve Described by the Present Model

Table 4-2 summarizes the parameters associated with cell survival in the IMK model. Figures 4-3A and 4-3B show the fitting results of the IMK model for V79-379A cell line and for T-47D cell line, respectively. In these figures, the solid line is the curve given by the IMK model (TEs and NTEs) and the dotted line is by the model considering only TEs. The symbols represent the experimental SF data<sup>52-61</sup>. Taking account of NTEs into the IMK model, the low-dose HRS for both cell lines was reproduced well by the use of Eqs. (2-13), (2-27) and (2-29). As shown in Fig. 4-3, the IMK model can precisely reproduce the low-dose HRS in terms of the  $R^2$  value.

In addition, here the log of cell survival per absorbed dose ( $-\ln S/D$ ) was also calculated

by the IMK model. The log of cell survival per dose means the average number of lethal lesions per cell induced by unit absorbed dose in this model. The survival rate in this way enables us to evaluate a more realistic appreciation of the dose-response curve than that with the usual semi-log plot of surviving fraction versus dose. By using the experimental data and the curve shown in Figs. 4-3A and 4-3B, the model performance about the low-dose hyper radio-sensitivity (HRS) was checked again. Figure 4-4 shows the log of cell survival per dose versus dose in V79-379A and T-47D cell lines. In this figure, the curve by the IMK model considering the DNA-targeted effects (TEs) and intercellular communication (IC) agrees much better with the experimental data than that by the MK model considering only the DNA-TEs in terms of  $R^2$  value.



**Figure 4-4.** Log of cell survival per dose as a function of dose in comparison with the experimental data summarized in Figs. 4-3A and 4-3B, and the prediction curve by the present model: (A) for Chinese Hamster lung fibroblast V79-379A cell line and (B) for human breast carcinoma cell line T-47D cell line. The line and symbol represent the curve by the model and experimental data, respectively. The  $R^2$  value was calculated by Eq. (4-1).

#### 4.2.3. Medium Transfer Bystander Effects Described by the Present Model

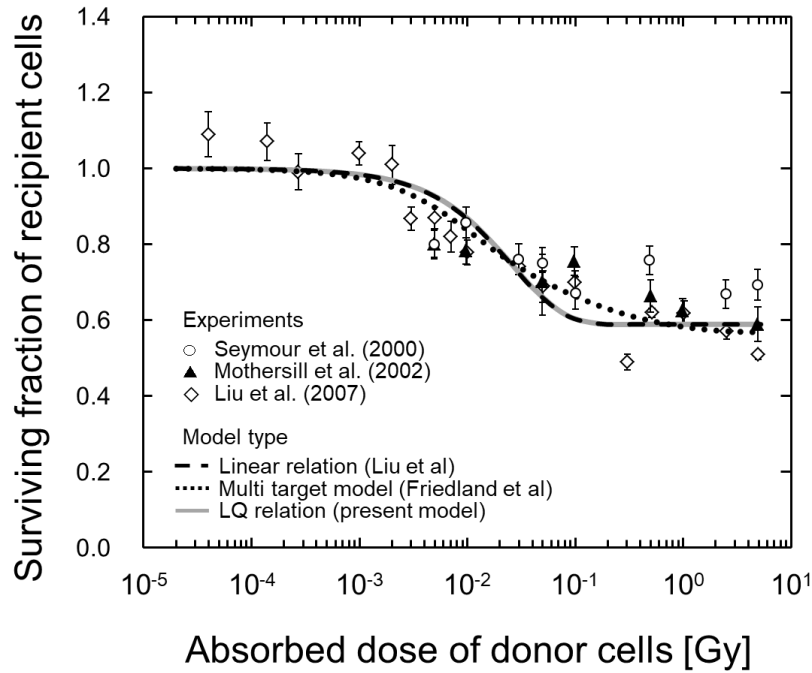
To evaluate the hypothesized mechanism of hits to targets in NTEs, the IMK model for MTBEs was further applied to the MTBE data<sup>18, 62-65</sup>, where the parameters (in Eq. 2-35) in Table 4-2 were used as well. Figures 4-5A and 4-5B show the fitting curves of the IMK model to surviving fractions (SFs) for MTBEs in comparison with the experimental data for HPV-G and E48 cell lines. In Figs. 4-4A and 4-4B, the horizontal axis is the absorbed dose in the irradiated cell population. The agreement between the resultant curves and the experimental data is also fair. The fitting results shown in Figs. 4-3, 4-4 and Fig. 4-5 suggest that the IMK model can describe not only the low-dose SF after the irradiation with broad beam but also the reduction of SF by intercellular signaling in MTBEs.

| Effect type  | Parameter                   | Cell line             |                       |                       |                         |
|--------------|-----------------------------|-----------------------|-----------------------|-----------------------|-------------------------|
|              |                             | V79-379A              | T-47D                 | HPV-G                 | E48                     |
| Targeted     | $\alpha_0 [\text{Gy}^{-1}]$ | $1.60 \times 10^{-2}$ | $1.29 \times 10^{-1}$ | -                     | -                       |
|              | $\beta_0 [\text{Gy}^{-2}]$  | $6.00 \times 10^{-1}$ | $2.90 \times 10^{-2}$ | -                     | -                       |
|              | $(a+c) [\text{h}^{-1}]$     | 6.29                  | 1.60                  | -                     | -                       |
| Common       | $\gamma [\text{Gy}]^*$      | $9.24 \times 10^{-1}$ | $4.80 \times 10^{-1}$ | $4.80 \times 10^{-1}$ | $4.80 \times 10^{-1}$   |
| Non-Targeted | $\alpha_b [\text{Gy}^{-1}]$ | 1.46                  | 1.80                  | $3.09 \times 10^1$    | $< 1.00 \times 10^{-3}$ |
|              | $\beta_b [\text{Gy}^{-2}]$  | $3.96 \times 10^{-2}$ | $3.00 \times 10^{-2}$ | $2.38 \times 10^{-1}$ | $1.72 \times 10^{-1}$   |
|              | $\delta (\delta_m)$         | $2.57 \times 10^{-1}$ | $9.02 \times 10^{-1}$ | $5.29 \times 10^{-1}$ | $5.79 \times 10^{-1}$   |

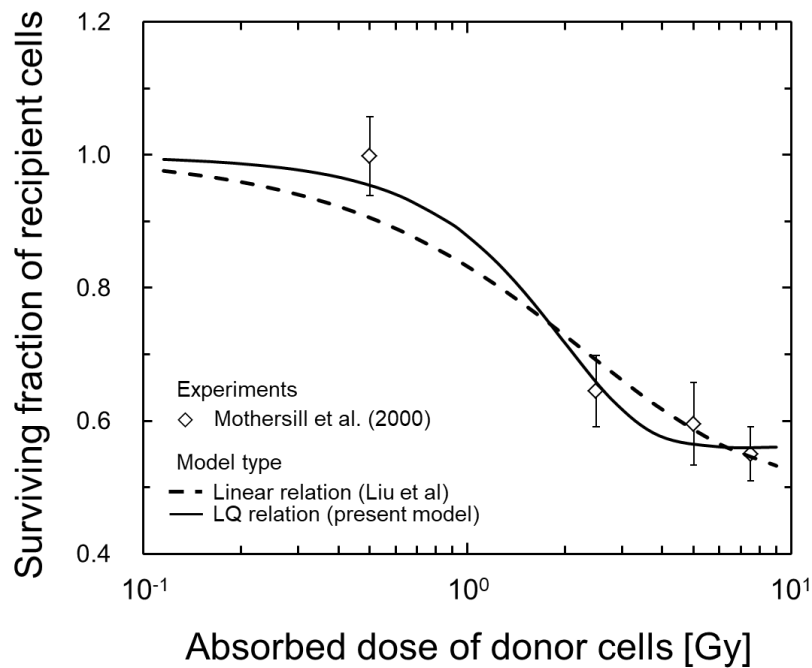
**Table 4-2.** Parameters in the IMK model determined by maximum likelihood method.

\*The  $\gamma$ -values for 250 kVp X-rays and  $^{60}\text{Co}$   $\gamma$ -rays were taken from refs<sup>35,44</sup>.

### A. MTBEs in HPV-G cell line



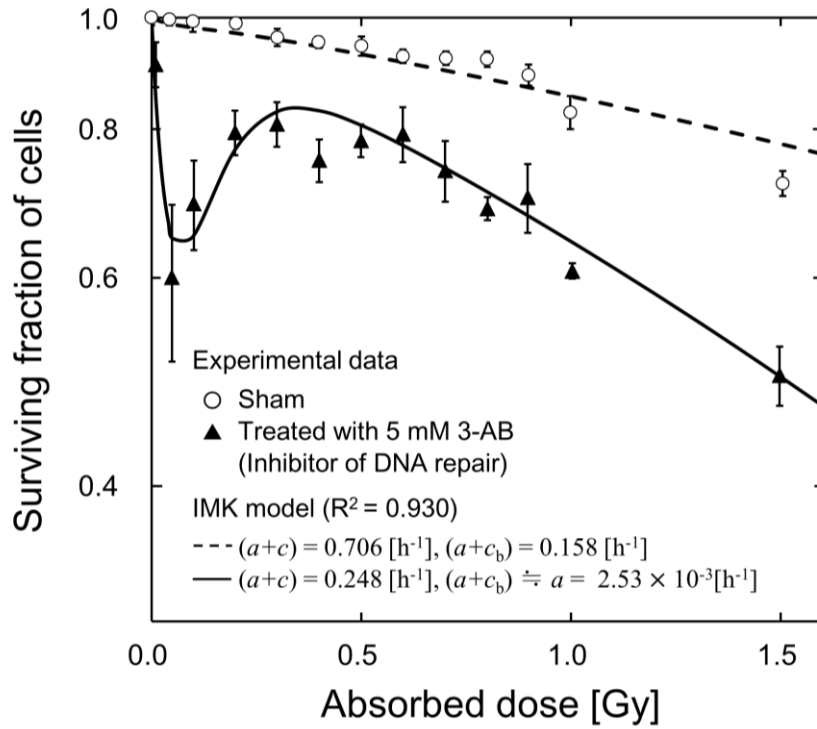
### B. MTBEs in E48 cell line



**Figure 4-5. Comparison between the modified IMK model (Eq. 2-35) and experimental SF data in MTBEs<sup>18,62-65</sup>.** The figures show the relation between the surviving fraction of recipient cells affected by the ICCM and absorbed dose in the irradiated (donor) cell population: A for HPV-G cell line and B for E48 cell line, respectively. It is noted that the quadratic term in Eq. (2-17) significantly contributes to the reproduction of cell survival in Fig. 4-5B.

### 4.2.3. Relation Between Low-Dose HRS and Repair in Non-Hit Cells

In the present model, the DNA repair function in non-hit cells is newly introduced as an inactivation factor. The assumption that the DNA repair in non-hit cells is inactivated was checked by the fitting results of DNA lesions as shown in Fig. 4-2B. Next, focussing on the inactivated repair, the IMK model was fitted simultaneously to the experimental data in both sham CHO-K1 cells (which does not exhibit low-dose HRS) and the cells treated with an inhibitor of DNA repair<sup>66</sup>. Figure 4-6 shows the fitting results of the IMK model to experimental data. The sets of response parameters in the cells are  $(\alpha_0, \beta_0) = (1.15 \times 10^{-1}, 2.20 \times 10^{-2})$  for TEs,  $(\alpha_b, \beta_b, \delta) = (9.28, 1.21, 2.79 \times 10^{-2})$  for NTEs, while the common microdosimetric quantity  $\gamma = 0.924$  is chosen for 240 kVp X-rays (as it is close to an energy of 250 kVp X-rays<sup>36</sup>). The DNA repair functions for hit and non-hit cells are characterized by  $a/(a+c)$  ( $\propto \alpha_0$ ) and  $b_d/2(a+c)$  ( $\propto \beta_0$ ) in Eqs. (2-6) and (2-9) for TEs, and  $a/(a+c_b)$  ( $\propto \delta$ ) in Eqs. (2-26) for NTEs, respectively. Here, we assumed that  $a$  and  $b_d$  are cell-specific parameters in the IMK model. This assumption was verified in dose-rate study associated with cell-cycle dependence (see chapter 3). To reproduce the experimental SF by using the IMK model, the parameters ( $\alpha_0 \propto a/(a+c)$ ,  $\beta_0 \propto b_d/2(a+c)$  and  $\delta \propto a/(a+c_b)$ ) of non-treated CHO cells to the repair-inhibited cells were determined to be  $3.52 \times 10^{-1}$  for TEs ( $\alpha_0$  and  $\beta_0$ ) and  $1.60 \times 10^{-2}$  for NTEs ( $\delta$ ). In the course of the model analysis about DNA repair efficiency of non-hit cells  $c_b$ , it is suggested that the repair in non-hit cells is almost inactivated. For this reason, we estimated the repair rate of inactivation ( $c_b$ ) from parameters,  $\alpha_0 = 1.15 \times 10^{-1} [\text{Gy}^{-1}]$ ,  $k_d p < g > = 32.1 [\text{Gy}^{-1}]$ <sup>49</sup> and  $(a+c) = 0.706 [\text{h}^{-1}]$ <sup>36</sup>, to be  $c_b = 0.155 [\text{h}^{-1}]$  with  $a = 2.52 \times 10^{-3} [\text{h}^{-1}]$ . The result in Fig. 4-6 and the estimated value of  $c_b$  suggest that the repair function in non-hit cells can be regarded as a key to reproduce the low-dose HRS in repair-inhibited CHO cells.



**Figure 4-6. Comparison between the surviving fraction in our model and the experimental data for non-treated CHO-K1 cells and repair-inhibited cells<sup>66</sup>.** The sets of response parameters in the cells are  $(\alpha_0, \beta_0) = (1.15 \times 10^{-1}, 2.20 \times 10^{-2})$  for TEs,  $(\alpha_b, \beta_b, \delta) = (9.28, 1.21, 2.79 \times 10^{-2})$  for NTEs, and the common microdosimetric quantity  $\gamma = 0.924$ . To reproduce the experimental results by using the IMK model, the parameters  $(\alpha_0 \propto a/(a+c), \beta_0 \propto b_d/2(a+c) \text{ and } \delta \propto a/(a+c_b))$  of non-treated CHO cells to the repair-inhibited cells are chosen to be  $3.52 \times 10^{-1}$  for the TEs ( $\alpha_0, \beta_0$ ) and  $1.60 \times 10^{-2}$  for the NTEs ( $\delta$ ).

## 4.3. Discussion

### 4.3.1. Mechanisms of Hit to Target for Signal Emission

The number of hits to targets to release signals in NTEs and the signal amount have been thought to be related with the mechanism as a function of absorbed dose in Gy or specific energy<sup>18,21</sup>. In the present model, a formula for the mechanism as a LQ relation was defined. To evaluate this definition, the IMK model was further compared with the previous model (linear relation) reported by Liu et al. (2002)<sup>18</sup> in SF data after exposure with ICCM. The sets of parameters by Liu *et al.* ( $z_F, \omega$ ) for MTBEs in HPV-G and E48 cell lines are  $(3.18 \times 10^{-2}, 5.29 \times 10^{-1})$  and  $(3.10, 6.67 \times 10^{-1})$ , respectively. Whilst the quadratic term is close to zero in

the HPV-G cell line (Table 4-2 and Fig. 4-5A), the linear term is close to zero ( $< 0.001$ ) in the E48 cell line (Table 4-2 and Fig. 4-5B). Table 4-3 shows that the chi-square value deduced by the LQ relation (with parameter number,  $m=4$ ) has a tendency to be smaller than the value by linear relation ( $m=2$ ).

In addition, the previously proposed model by Friedland *et al.* (2011)<sup>21</sup> was fitted to MTBE data, in which the response parameter is  $a = 5.65 \times 10^{-1}$  and the characteristic dose for signal emission  $D_c$  values lies in between 10 and 1000 mGy (40% of cells with  $D_c = 10$  mGy, 20% with 30 mGy, 20% with 100 mGy, 10% with 300 mGy and 10% with 1000 mGy). Although the model (including a lot of parameters) by Friedland *et al.* makes the chi-square value the smallest, the AIC value as the index of model selection becomes larger.

It is generally accepted that the LQ relation in hit number depends on the number of radiation particles in the LQ model<sup>69</sup>. Collectively, the number of hits to targets in NTEs may hold similarly to that in the previous TEs.

| Type of cell line | Model type                     | Parameter number | Statistical index |          |       |
|-------------------|--------------------------------|------------------|-------------------|----------|-------|
|                   |                                | $m$              | $R^2$             | $\chi^2$ | AIC   |
| HPV-G             | Liu <i>et al.</i> (2007)       | 2                | 0.708             | 222      | -158  |
|                   | Friedland <i>et al.</i> (2011) | 11               | 0.704             | 153      | -151  |
|                   | Modified IMK model             | 4                | 0.687             | 222      | -154  |
| E48               | Liu <i>et al.</i> (2007)       | 2                | 0.948             | 3.22     | -26.7 |
|                   | Modified IMK model             | 4                | 0.982             | 0.934    | -30.7 |

**Table 4-3.** Comparison of fitting properties among the models for hit mechanisms in NTEs

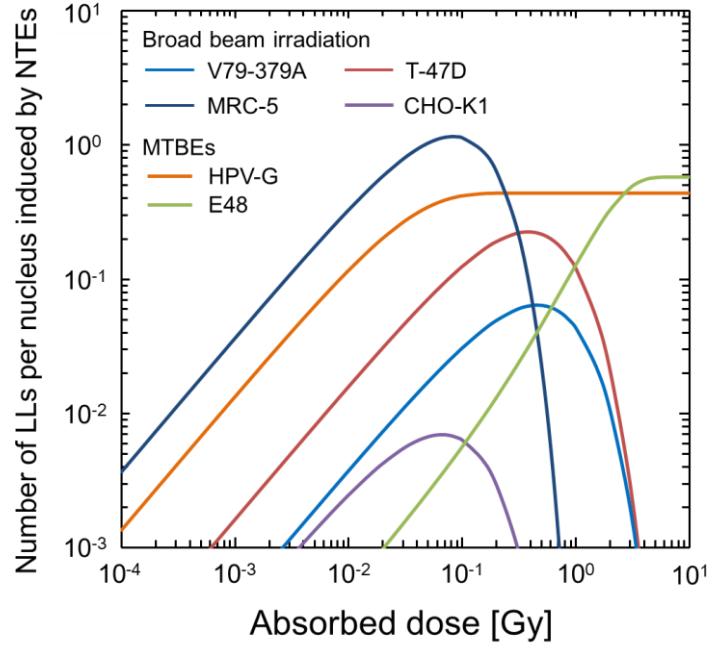
#### 4.3.2. Parameters in the IMK Model for the Non-Targeted Effects

The parameters,  $\alpha_b$  and  $\beta_b$ , were newly defined in this study. The parameters  $(\alpha_b + \gamma\beta_b)$  and  $\beta_b$  in Eq. (2-17) represent the proportionality factors to  $D$  and  $D^2$  in  $\text{Gy}^{-1}$  and  $\text{Gy}^{-2}$ , and the reciprocals of  $(\alpha_b + \gamma\beta_b)$  and  $\beta_b^{1/2}$  denote the doses to induce a signal-release hit with single particle track and the hit with a pairwise combination of two tracks, respectively. From Table

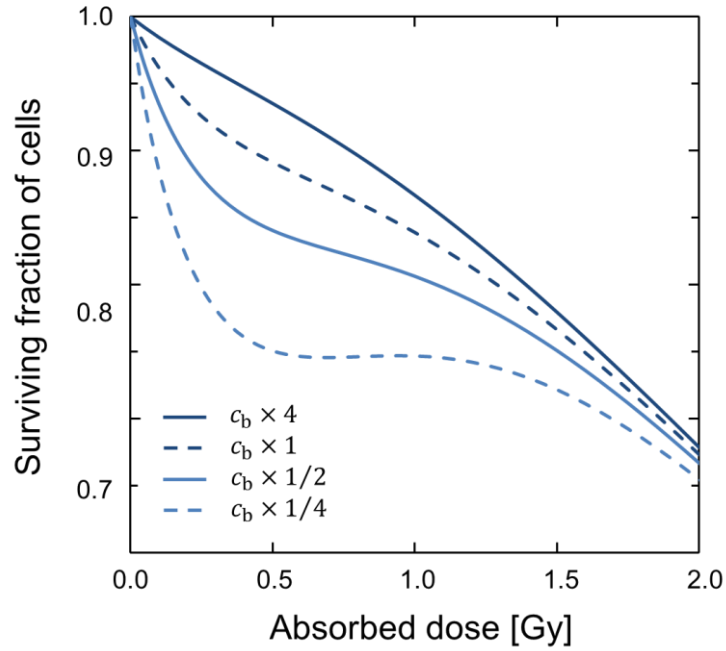
4-2, the values of  $1/(\alpha_b + \gamma\beta_b)$  are given to be 0.68 Gy for V79-379A cells and 0.56 Gy for T-47D cells, while the values of  $1/\beta_b^{1/2}$  are 5.03 Gy for V79-379A cells and 5.77 Gy for T-47D cells. As shown in Table 4-1 (for human fibroblast cell line) and Table 4-2 (for V79-379A and T-47D cell lines), the values of  $\alpha_b$  and  $\beta_b$  vary depending on the cell line. This suggests that the parameters in NTEs are cell-specific.

By using the IMK parameters listed in Tables 4-1 and 4-2, the degree of the dose-dependent NTEs based on the linear-quadratic theory was estimated. Figure 4-7A exemplifies the estimated number of LLs per nucleus for the NTEs. In Fig. 4-7A, the maximum numbers of LLs per nucleus for V79-379A and T-47D cells are 0.064 and 0.23, respectively. The maximum number of LLs is characterized by the  $\delta$  value in the present model. This value may also depend on the cell type.

A. Estimated number of LLs in NTEs



B. Low-dose HRS and repair in non-hit cells



**Figure 4-7.** The IMK model analyses with the number of LLs in NTEs and DNA repair function in non-irradiated cells: (A) for the mean number of LLs per nucleus induced by NTEs in various cell lines, (B) for the surviving fraction with a variety of DNA repair factors. In Fig. 4-7(A), we used the IMK parameters deduced in this study to estimate LL number per nucleus. In Fig. 5(B), the cell survival curves for V79-379A cells were estimated with repair function rates,  $c_b \times 4$ ,  $c_b \times 1$ ,  $c_b \times 1/2$  and  $c_b \times 1/4$  under the condition of  $a = 8.12 \times 10^{-3} \text{ [h}^{-1}\text{]}$ .

### 4.3.3. Involvement of the Repair in Low-Dose HRS

Some previous investigations indicate that the defect of DNA repair in non-irradiated cells is related to the bystander effect<sup>26,27</sup>. According to the report by Rothkamm *et al* (2006)<sup>25</sup>, the threshold value to activate DNA repair is a small dose such as 1.2 mGy, where one electron track may traverse a cell nucleus at most. Thus, it is reasonable to suppose that the DNA repair function in non-irradiated cells is inactivated. From the viewpoint of the repair capability in a cell population, it has been interpreted as that the increased radioresistance (IRR) is associated with overcoming low-dose HRS<sup>29</sup>. In contrast, we evaluated the influence of disorder of DNA repair function in non-hit cells on the NTE-related DSBs induction as shown in Fig. 4-2B and also cell death in Fig. 4-6.

Figure 4-7B illustrates the estimated curves for V79-379A cells in the IMK model with DNA repair  $c_b$  by factors of 4, 1, 1/2 and 1/4, in which the constant rate of  $a$  was determined to be  $8.12 \times 10^{-3} \text{ [h}^{-1}\text{]}$  from  $k_d p < g > = 30 \text{ [Gy}^{-1}\text{]}$ <sup>70</sup> and  $\alpha_0 = 3.89 \times 10^{-2} \text{ [Gy}^{-1}\text{]}$ , and  $c_b$  was estimated to be  $0.155 \text{ [h}^{-1}\text{]}$  from the results in Fig. 4-6. As is shown in Fig. 4-7B, the low-dose HRS is enhanced by lowering the repair factor. This suggests that the inactivation of DNA repair in non-hit cells tends to enhance cell-killing (or to decrease radioresistance) after a low-dose irradiation. As to the increase of repair by virtue of the repair function, evidences have been reported<sup>71,72</sup>.

In this study, through the analyses using the developed IMK model, we have demonstrated NTEs by the combination of a variety of processes: signal transfer from hit cells, kinetics of DSBs to enhance cell killing and disorder of the DNA repair function, to show characteristics of the radio-sensitivity of cells in conformity with experimental evidences.

## 4.4. Summary

In this chapter 4, an integrated microdosimetric-kinetic (IMK) model taking account of TEs and NTEs was applied to demonstrate the experimental data of the cell-killing signals, number of DSBs per nucleus and cell survival. From the comparison of the results by the model with experimental data, it was shown: (i) a LQ relation to express the hit probability for emitting signals is suitable to describe the cell killing in NTEs, (ii) the low-dose hyper radio-sensitivity (HRS) is attributed to the combination of the induction of DSBs by the signals and low DNA repair efficiency in non-hit cells, and (iii) the low-dose HRS is enhanced more as the DNA repair efficiency in non-hit cells is lower.

The IMK model provides quantitative formulae that enable us to analyze both TEs and NTEs based on cell-killing signals, DNA damage and DNA repair. In this analysis, it was suggested that the inactivation of DNA repair in non-hit cells is dominant in HRS for cell survival.

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## Chapter 5

# Summary

### 5.1. Conclusion

In this study, time course of cell responses and radio-sensitivity were investigated by using the hybrid method with *in vitro* experiments and model approaches. Two main subjects were discussed: one is the cell responses and radio-sensitivity under protracted exposure with X-rays at various dose-rates (chapter 3), and the other is development of mathematical modelling considering targeted and non-targeted effects in wide dose range (chapter 2) and verification of the performance of the present model (chapter 4).

Cell-cycle study showed cell-cycle dynamics of Chinese hamster ovary (CHO-K1) cells during the exposure at various dose rates and clonogenic cell survival. As a result of cell-cycle study in chapter 3, the DNA damage checkpoints through cell cycle depend on the magnitude of dose rate: (i) an cell accumulation in  $G_2$  phase during exposure at lower dose-rates (e.g., 0.186 and 1.0 Gy/h), (ii) the delay of DNA synthesis and an accumulation of the cells in  $S/G_2$  during the exposure at intermediate dose-rate (e.g., 3.0 Gy/h), and (iii) the blocks of cell cycle progressing in  $G_1/M$  and  $G_2/M$  checkpoints and the delay of DNA synthesis during the exposure at higher dose (e.g., 6.0 Gy/h). The CHO-K1 cells exhibit inverse dose-rate effects with subtle high radio-sensitivity under exposure at 1.0 Gy/h and unexpected greater radio-resistance under exposure at 3.0 Gy/h. From our model analysis, it was suggested that the change of the cell condition (i.e., amount of DNA as radiation target & DNA repair efficiency) is a key factor to modulate radio-sensitivity, which is possibly responsible for IDREs.

The present model for non-targeted effects provides quantitative formulae that enable us to describe signal kinetics, DNA repair kinetics and cell survival. The model was verified in comparison with a series of cell responses and provided a couple of new estimations for DNA

damage kinetics and dose-response curve associated with DNA repair efficiency in non-hit cells. As a result of model analysis in the chapter 4, it was found that the low-dose hyper radio-sensitivity (HRS) is enhanced more as the DNA repair efficiency in non-hit cells is lower. This model has temporal dependence of NTEs, so that it might contribute to estimating biological effects under low-dose-rate exposure.

Both investigations were conducted in the aim of estimating contribution degree of cell-cycle dynamics and low-dose HRS to reversal of radio-sensitivity after long-term exposure. Through this hybrid analysis, a couple of new interpretations for cell responses under low-dose or protracted exposure were indicated in this study. This study would contribute to more precise understandings of cell responses after the long-term exposure and low-dose exposure to ionizing radiations.

## 5.2. Acknowledge

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# PUBLICATION LIST

## Publication to Journal of the Society

1. The Report of the 2nd Educational Symposium on Radiation and Health by Young Scientists (ESRAH2015). T. Tsujiguchi, M. Yamaguchi, N. Nanashima, M. Chiba, S. Terashima, Y. Fujishima, Y. Matsuya, J. Kwon, T. Nakamura. *Jpn. J. Radiol. Technol.* 71(11), 1132-1137 (2015).
2. Report on the 2nd Educational Symposium on RADIATION AND HEALTH by Young Scientists (ESRAH2015). T. Tsujiguchi, M. Yamaguchi, N. Nanashima, M. Chiba, S. Terashima, Y. Fujishima, Y. Matsuya, J. Kwon, T. Nakamura. *Radiat. Emerg. Med.* 5(1),15-24 (2015).
3. Modeling cell survival and change in amount of DNA during protracted irradiation. Y. Matsuya, K. Tsutsumi, K. Sasaki, Y. Yoshii, T. Kimura, H. Date. *J. Radiat. Res.* 58 (3), 302-312 (2017). (Impact factor = 1.788)
4. Educational Activity for the Radiation Emergency System in the Northern Part of Japan: Meeting Report on “The 3rd Educational Symposium on Radiation and Health (ESRAH) by Young Scientists in 2016”. Y. Matsuya, T. Tsujiguchi, M. Yamaguchi, T. Kimura, R. Mori, R. Yamada, R. Saga, Y. Fujishima, H. Date. *Radiat. Res.* 187, 641–646 (2017). (Impact factor = 2.539)
5. Inorganic polyphosphate enhances radio-sensitivity in a human non-small-cell lung cancer cell line, H1299. K. Tsutsumi, Y. Matsuya, T. Sugahara, M. Tamura, S. Sawada, S. Fukura, H. Nakano, H. Date. *Tumor Biol.* 1-6. (2017). (Impact factor = 3.650)
6. Markov chain Monte Carlo analysis for the selection of a cell-killing model under high-dose-rate irradiation. Y. Matsuya, T. Kimura, H. Date. *Med. Phys.* 44 (10): 5522-5532 (2017) (Impact factor = 2.617)
7. Estimation of the radiation-induced DNA double-strand breaks number considering cell cycle and absorbed dose per cell nucleus. R. Mori, Y. Matsuya, Y. Yoshii, H. Date. *J. Radiat. Res. In Press.* (Impact factor = 1.788)

## Japanese Presentation

1. Microdosimetric analysis of the energy deposition at low dose rate X-ray irradiation. Y. Matsuya, Y. Yoshii, K. Sasaki, H. Date. The 100th Japanese Society of Medical Physics, 2015.9.18-20., Hokkaido University, Sapporo, Hokkaido.
2. Estimation of the number of DNA double-strand breaks induced by megavoltage X-ray beams. Y. Yoshii, Y. Matsuya, K. Sasaki, H. Date. The 100th Japanese Society of Medical Physics, 2015.9.18-20., Hokkaido University, Sapporo, Hokkaido.
3. Radiation effects: from physical processes to DNA damage. H. Date, Y. Yoshii, Y. Matsuya. The 100th Japanese Society of Medical Physics, 2015.9.18-20., Hokkaido University, Sapporo, Hokkaido.
4. Dose rate effects of conventional fractionated radiotherapy on the cell-killing. Kimura T, Matsuya Y, Date H. The 43rd Japanese Society of Radiological Technology, 2015.10.8-10., Kanazawa Bunka Hall, Kanazawa, Ishikawa.
5. Influence of inorganic polyphosphate on radio-sensitivity in a human non-small cell lung cancer cell line. Tsutsumi K, Sugahara T, Tamura M, Sawada S, Fukura S, Nakano H, Matsuya Y. The 38th annual meeting of the molecular biology society of Japan. 2015.12.1-4., Kobe port island, Kobe, Hyogo.
6. Influence of method to determine model parameters on cell survival curve. Yamada R, Kimura T, Matsuya Y, Date H. The 70th annual meeting of Japanese Society of Radiological Technology Hokkaido branch, 2015.11.14-15., Hokkaido University, Sapporo, Hokkaido.
7. Estimation of cell-killing based on the probability density of DNA contents in cell population. Y. Matsuya, K. Tsutsumi, K. Sasaki, Y. Yoshii, H. Date. The 111st annual meeting of Japanese Society of Medical Physics, 2016.4.14-17. Pacifico Yokohama, Yokohama.

8. Determination of the cell survival curve considering the lethal lesion number per nucleus after irradiation. Yamada R, Matsuya Y, Kimura T, Date H. The 111st annual meeting of Japanese Society of Medical Physics, 2016.4.14-17. Pacifico Yokohama, Yokohama.
9. Evaluation of the dose-rate effects on cell viability for various dose rates considering G2 accumulation of cell cycle. Y. Matsuya, K. Sasaki, Y. Yoshii, T. Kimura, R. Mori, H. Date. The 112nd annual meeting of Japanese Society of Medical Physics, 2016.9.8-10., Okinawa convention center, Okinawa.
10. A calculation model of radiation-induced complex DNA double-strand break based on clustering properties of the electron processes. Y. Yoshii, Y. Matsuya, R. Mori, K. Sasaki, H. Date. The 112nd annual meeting of Japanese Society of Medical Physics, 2016.9.8-10., Okinawa convention center, Okinawa.
11. A revisional study of the cell survival curve after irradiation: influences of the survival model and determination method of parameters. Yamada T, Kimura T, Matsuya Y, Date H. The 112nd annual meeting of Japanese Society of Medical Physics, 2016.9.8-10., Okinawa convention center, Okinawa.
12. Estimation of DNA double-strand breaks based on DNA amount per cell nucleus. Mori T, Matsuya Y, Yoshii Y, Date H. The 112nd annual meeting of Japanese Society of Medical Physics, 2016.9.8-10., Okinawa convention center, Okinawa.
13. Development of mathematical model for cell survival considering non-targeted effect. Matsuya Y, Sasaki K, Yoshii Y, Date H. The 59th annual meeting of Japanese Radiation Research Society, 2016.10.26-28., JMS asuteru plaza, Hiroshima.
14. Estimating the induction probability of DNA double-strand breaks in cell nuclei after X-rays irradiation. Mori R, Matsuya Y, Yoshii Y, Date H. The 59th annual meeting of Japanese Radiation Research Society, 2016.10.26-28., JMS asuteru plaza, Hiroshima.
15. In silico study of cell responses bases on statistical model for cell population with hit and non-hit cells. Sasaki K, Matsuya Y, Yoshii Y, Date H. The 59th annual meeting of Japanese Radiation Research Society, 2016.10.26-28., JMS asuteru plaza, Hiroshima.
16. Analysis of cell-cycle and radio-sensitivity under protracted exposure to ionizing radiation. Matsuya Y, Tsutsumi K, Sasaki K, Yoshii Y, Mori R, Oikawa J, Date H. The 72nd annual meeting of Japanese Society of Radiological Technology Hokkaido branch 2016.11.19-20, Tokachi plaza, Obihiro, Hokkaido.
17. Cell survival model for low-dose X-rays exposure in consideration of targeted and non-targeted effects. Matsuya Y, Sasaki K, Yoshii Y, Kimura T, Date H. The 72nd annual meeting of Japanese Society of Radiological Technology Hokkaido branch 2016.11.19-20, Tokachi plaza, Obihiro, Hokkaido.
18. Estimation of complex DNA double-strand breaks induced by diagnosis X-rays. Yoshii Y, Matsuya Y, Sasaki K, Mori R, Date H. The 72nd annual meeting of Japanese Society of Radiological Technology Hokkaido branch 2016.11.19-20, Tokachi plaza, Obihiro, Hokkaido.
19. Influence of cross-section of electrons for analysis of electron track in liquid water. Kimura T, Matsuya Y, Yoshii Y, Date H. The 72nd annual meeting of Japanese Society of Radiological Technology Hokkaido branch 2016.11.19-20, Tokachi plaza, Obihiro, Hokkaido.
20. Model for biological effects considering DNA amount per nucleus and energy deposition by secondary electrons. Mori R, Matsuya Y, Yoshii Y, Date H. The 72nd annual meeting of Japanese Society of Radiological Technology Hokkaido branch 2016.11.19-20, Tokachi plaza, Obihiro, Hokkaido.
21. Assessment for uncertainty of oxygen enhancement ratio in re-oxygenated tumour cells. Yamada R, Matsuya Y, Date H. The 72nd annual meeting of Japanese Society of Radiological Technology Hokkaido branch 2016.11.19-20, Tokachi plaza, Obihiro, Hokkaido.
22. Assessment for uncertainty of model parameters in LQ model in radiotherapy. Kokubun R, Kimura T, Matsuya Y, Date H. The 72nd annual meeting of Japanese Society of Radiological Technology Hokkaido branch 2016.11.19-20, Tokachi plaza, Obihiro, Hokkaido.
23. Analysis of cell-cycle dynamics under long-term exposure at low dose rate. Oikawa J, Matsuya Y, Mori R, Date H. The 72nd annual meeting of Japanese Society of Radiological Technology Hokkaido branch 2016.11.19-20, Tokachi plaza, Obihiro, Hokkaido.
24. Model estimation for DNA double-strand breaks induction in consideration of cell cycle and dose dependence. Mori R, Matsuya R, Yoshii Y, Date H. The 113rd annual meeting of Japanese Society of Medical Physics, 2017.4.13-16. Pacifico Yokohama, Yokohama.
25. Evaluation of oxygen enhancement ratio calculated by the cell survival curves based on

- microdosimetric-kinetic model. R. Yamada, Y. Matsuya, H. Date. The 113rd annual meeting of Japanese Society of Medical Physics, 2017.4.13-16. Pacifico Yokohama, Yokohama.
26. Investigation of cell survival under protracted exposure for various dose rates in consideration of cell cycle distribution. Matsuya Y, McMahon SJ, Tsutsumi K, Sasaki K, Yoshii Y, Mori R, Oikawa J, Date H, Prise KM. The 60th annual meeting of Japanese Radiation Research Society, 2017.10.25-28., Keioh Ginko Bunka Plaza, Chiba.
  27. Computational model for predicting the change of cell cycle distribution during protracted irradiation. Oikawa J, Matsuya Y, Mori R, Sakashita T, Date H. The 60th annual meeting of Japanese Radiation Research Society, 2017.10.25-28., Keioh Ginko Bunka Plaza, Chiba.
  28. Mechanistic analysis for inflammation associated radio-resistance by using biophysical model. Takahashi R, Saga R, Matsuya Y, Hosokawa Y. The 7th annual meeting of Tohoku Radiological Medical Technology, 2017.10.28-29, Link station hall Aomori, Aomori.
  29. Microdosimetric analysis with Monte Carlo simulation for photon and electrons. Yachi Y, Yoshii Y, Matsuya Y, Date H. The 72nd annual meeting of Japanese Society of Radiological Technology Hokkaido branch 2017.11.18-19, Sapporo medical university, Sapporo, Hokkaido.
  30. Analysis by *in vitro* experiments and model for spatial distribution of X-rays induced DNA double-strand breaks. Ohshima Y, Mori R, Matsuya Y, Yoshii Y, Date H. The 72nd annual meeting of Japanese Society of Radiological Technology Hokkaido branch 2017.11.18-19, Sapporo medical university, Sapporo, Hokkaido.

## Refereed Papers for International Conference

1. Influence of two types of DNA repair pathways on the surviving fraction of cells exposed to radiations. Y. Matsuya, K. Sasaki, Y. Yoshii, H. Date. 15th International Congress of Radiation Research, 2015.5.25-29, Kyoto International Conference Center. (Poster)
2. Cell-killing model considering DNA damage repair and cell phase. Y. Matsuya, K. Sasaki, Y. Yoshii, H. Date. The 2nd meeting of educational symposium of radiation and health (ESRAH), 2015.05.23-24., Hirosaki, Aomori, Japan. (Poster).
3. Effects of secondary electrons on DNA double-strand breaks after photon irradiation. Y. Yoshii, Y. Matsuya, K. Sasaki, H. Date. The 2nd meeting of educational symposium of radiation and health (ESRAH), 2015.05.23-24., Hirosaki, Aomori, Japan. (Poster)
4. Dose rate effect on cell survival in the fractionated radiotherapy. T. Kimura, Y. Matsuya, H. Date. The 2nd meeting of educational symposium of radiation and health (ESRAH), 2015.05.23-24., Hirosaki, Aomori, Japan. (Poster)
5. Influence of dose delivery time on the fractionated radiotherapy. T. Kimura, Y. Matsuya, H. Date. The 2nd symposium of Faculty of Health Sciences (FHS), 2015.07.3., Sapporo, Hokkaido, Japan. (Poster)
6. Mathematical analysis of the cell viability under low dose-rate exposure: the change of DNA amount during the protracted irradiation. Y. Matsuya, K. Tsutsumi, K. Sasaki, Y. Yoshii, T. Kimura, H. Date. The 42nd Annual Meeting of the European Radiation Research Society, 2016.9.4-8., Royal Tropical Institute, Amsterdam, Netherlands. (Poster)
7. Determination of cell surviving fraction for radiotherapy in fractionated irradiation considering dose rates and repopulation of tumor cells. T. Kimura, Y. Matsuya, H. Date. The 42nd Annual Meeting of the European Radiation Research Society, 2016.9.4-8., Royal Tropical Institute, Amsterdam, Netherlands. (Poster)
8. Estimation of DNA damage kinetics in both targeted and nontargeted cells using a stochastic model with the microdosimetric analysis. K. Sasaki, Y. Matsuya, H. Date. The 62nd Radiation Research Society (RRS) Annual Meeting in Waikoloa, Hawaii, 2016.10.16-19. (Poster)
9. A Cell-killing Model of Targeted and Non-Targeted Effects Based on Microdosimetry and Biological Processes. Y. Matsuya, K. Sasaki, Y. Yoshii, G. Okuyama, T. Kimura, H. Date. The 3rd meeting of educational symposium of radiation and health (ESRAH), 2016.10.01-02., Sapporo, Hokkaido, Japan. (Oral and Poster)
10. Probability of radiation-induced DNA double-strand breaks in statistical considerations for radiation energy deposition and cell cycle. R. Mori, Y. Matsuya, Y. Yoshii, H. Date. The 3rd meeting of

- educational symposium of radiation and health (ESRAH), 2016.10.01-02., Sapporo, Hokkaido, Japan. (Oral and Poster)
11. Uncertainty assessment of OER for reoxygenated tumor. R. Yamada, Y. Matsuya, H. Date. The 3rd meeting of educational symposium of radiation and health (ESRAH), 2016.10.01-02., Sapporo, Hokkaido, Japan. (Oral and Poster)
  12. Impacts of electron collision cross sections on electron track simulations in liquid water. T. Kimura, Y. Matsuya, Y. Yoshii, H. Date. The 3rd meeting of educational symposium of radiation and health (ESRAH), 2016.10.01-02., Sapporo, Hokkaido, Japan. (Oral and Poster)
  13. Biophysical modeling for targeted and non-targeted effects on cells after irradiation. Y. Matsuya, K. Sasaki, Y. Yoshii, H. Date. The 22nd International Conference on Medical Physics (ICMP) 2016, 2016.12.09-12., Bangkok, Thailand. (e-Poster)
  14. Optimization of the irradiation scheme in radiotherapy focusing on the dose and dose rate to the tumor and organs at risk. R. Yamada, Y. Matsuya, H. Date. The 22nd International Conference on Medical Physics (ICMP) 2016, 2016.12.09-12., Bangkok, Thailand. (e-Poster)
  15. Probability estimation of radiation-induced DNA double-strand breaks in the cell nucleus. R. Mori, Y. Matsuya, Y. Yoshii, H. Date. The 22nd International Conference on Medical Physics (ICMP) 2016, 2016.12.09-12., Bangkok, Thailand. (e-Poster)
  16. Evaluation of G2-block of cell cycle progression during a low-dose-rate exposure in mammalian cell line. J. Oikawa, Y. Matsuya, R. Mori, H. Date. The 3rd symposium of Faculty of Health Sciences (FHS), 2017.07.7., Sapporo, Hokkaido, Japan. (Poster)
  17. Computational estimation of the number of DNA double-strand breaks per nucleus in mammalian cells after X-ray exposure in consideration of cell cycle. R. Mori, Y. Matsuya, Y. Yoshii, H. Date. The 3rd symposium of Faculty of Health Sciences (FHS), 2017.07.7., Sapporo, Hokkaido, Japan. (Poster)
  18. Estimation of OER in consideration of LET and oxygen partial pressure by the Microdosimetric-Kinetic model. R. Yamada, Y. Matsuya, H. Date. The 3rd symposium of Faculty of Health Sciences (FHS), 2017.07.7., Sapporo, Hokkaido, Japan. (Poster)
  19. Application of the IMK model to cell survival curves following the exposure to intensity modulated radiation fields. Y. Matsuya, SJ. McMahon, KM. Prise, H. Date. The 43rd Annual Meeting of the European Radiation Research Society, 2017.9.4-8., Universitätsklinikum Essen, Essen, Germany.
  20. A model analysis to estimate the number of DNA double-strand breaks in cells exposed to X-rays. R. Mori, Y. Matsuya, Y. Yoshii, H. Date. The 43rd Annual Meeting of the European Radiation Research Society, 2017.9.4-8., Universitätsklinikum Essen, Essen, Germany.
  21. Modelling of oxygen enhancement ratio in consideration of LET and oxygen partial pressure by using a microdosimetric-kinetic model. R. Yamada, Y. Matsuya, H. Date. The 43rd Annual Meeting of the European Radiation Research Society, 2017.9.4-8., Universitätsklinikum Essen, Essen, Germany.
  22. A simulation study for both of the targeted and untargeted effect on the uniformly irradiated cells. K. Sasaki, Y. Matsuya, Y. Yoshii, T. Sanada, Y. Yaegashi, H. Date. The 43rd Annual Meeting of the European Radiation Research Society, 2017.9.4-8., Universitätsklinikum Essen, Essen, Germany.
  23. Effects of intercellular signalling on sub-lethal damage repair between fractionation doses following intensity modulated radiation field. Y. Matsuya, Stephen J. McMahon, Mihaela Ghita, Kevin M. Prise. The 4th meeting of educational symposium of radiation and health (ESRAH), 2017.9.23-24., Hirosaki, Aomori, Japan. (Poster)
  24. Mathematical estimation of the number of DNA double-strand breaks induced by radiation exposure. R. Mori, Y. Matsuya, Y. Yoshii, H. Date. The 4th meeting of educational symposium of radiation and health (ESRAH), 2017.9.23-24., Hirosaki, Aomori, Japan. (Poster)
  25. Estimation of OER by function considering variation of LET, dose rate and oxygen partial pressure. R. Yamada, Y. Matsuya, H. Date. The 4th meeting of educational symposium of radiation and health (ESRAH), 2017.9.23-24., Hirosaki, Aomori, Japan. (Poster)

## **Seminar**

1. Meeting on “Radiation Exposure of the Ocular Lens in Medicine: Health Effects, Dose and Protection”, Conference Room E, 25th floor, Yokohama Landmark Tower, 2017.4.15.

## **Award and Prize from an academic society**

1. Poster Prize Winner (1st), “A Cell-Killing Model of Targeted and Non-Targeted Effects Based on Microdosimetry and Biological Processes”, the 3rd Educational Symposium on Radiation and Health by Young Scientists, 2016.10.02., Hokkaido University, Sapporo, Japan. (Poster)
2. Excellent Presentation Award, “Development of mathematical model for cell surviving fraction including non-targeted effect”, The 59th Japanese Radiation Research Society, 2016.10.27.
3. ICMP2016 Anchali Krisanachinda Award (1st), “Biophysical modeling for targeted and non-targeted effects on cells after irradiation”, The 22nd International Conference on Medical Physics (ICMP), 2016.12.12.
4. Excellent Presentation Award, “Investigation of cell survival under protracted exposure for various dose rates in consideration of cell cycle distribution”, The 60th Japanese Radiation Research Society, 2017.10.28.

## **Competitive funds**

1. Travel Award for students in 58th Annual meeting of Japanese Radiation Research Society, Japanese Radiation Research Society, March in 2015, ¥87,000.
2. Research Fellow of Japan Society for the Promotion of Science (JSPS Research Fellow), Japan Society for the Promotion of Science, H28.4.1-H31.3.31.
3. JSPS KAKENHI Grant Number 16J07497, H28.4.1-H29.3.31, ¥700,000.
4. JSPS KAKENHI Grant Number 16J07497, H29.4.1-H30.3.31, ¥600,000.
5. Research Award in Graduate School of Health Sciences, Hokkaido University, 2017.5. ¥300,000
6. Travel Award ERRS-GBS-2017, European Radiation Research Society (ERRS), 2017.9.20. €500.

## **Society activity and contribution to society**

1. Committee member, 2nd Educational Symposium of Radiation and Health by Young Scientist (ESRAH2015) in Hirosaki, 2015.5.23-24.
2. Chairperson, Educational Lecture 5, 2nd Educational Symposium of Radiation and Health by Young Scientist (ESRAH2015) in Hirosaki, 2015.5.24.
3. Committee member, 3rd Educational Symposium of Radiation and Health by Young Scientist (ESRAH2016) in Sapporo, 2016.10.1-2.
4. Chairperson, Educational Lecture I, 3rd Educational Symposium of Radiation and Health by Young Scientist (ESRAH2016) in Hirosaki, 2016.10.1.
5. Committee member, 4th Educational Symposium of Radiation and Health by Young Scientist (ESRAH2017) in Hirosaki, 2017.9.23-24.
6. Chairperson, Educational Lecture I, 4th Educational Symposium of Radiation and Health by Young Scientist (ESRAH2016) in Hirosaki, 2017.9.23.

## APPENDIX: Programming Code in R Language

```
#-----
# Microdosimetric-Kinetic (MK) model for multi-fractionation dose equivalent to continuous irradiation at a dose-rate
# Irradiation type: multi-split-dose irradiation
# Multi-split-dose irradiation = continuous irradiation (Gy/h)
#-----
# Graduate school of Health Sciences Hokkaido University
# Created by Yusuke Matsuya
#-----
# 2015/08/17 Version 1
# Model considers the change in mean amount of DNA per nucleus during irradiation,
# which is called a "MK-DNA model"
# 2016/10/31 Version 2
# Model considers the change in SLDR rate during irradiation
#-----

#---CHECK THE DIRECTORY---

getwd()

#---READ MODEL PARAMETERS---

xx<-matrix(scan("parameter.d"),ncol=1,byrow=T)
a<-xx[1] # a: A=alpha1/<G1>
b<-xx[2] # b: B'=beta1/G1^2>
g<-xx[3] # g: gamma=z1D (gamma=0.9236)
apc<-xx[4] # apc: (a+c)-value (SLDR rate [h-1])
head(xx,4) # check the MK parameters

yy<-matrix(scan("regimen.d"),ncol=1,byrow=T)
d<-yy[1,] # d: Dose per fraction
tau<-yy[2,] # tau: interval [h]
f<-yy<-yy[3,] # irradiation number
head(yy,10)

G<-matrix(scan("dna.d"),ncol=3,byrow=T)
G1<-G[,2] # relative <G>=SGf(G)dG (normalized by G1 phase)
G2<-G[,3] # relative <G^2>=SG^2f(G)dG
head(G,10) # check the DNA contents

CC<-matrix(scan("repair.d"),ncol=2,byrow=T)
C<-CC[,2] # DNA repair function (normalized by c1)
head(C,10) # check the DNA repair

#---END OF READ MODEL PARAMETERS---

#---CALCULATION OF CELL SURVIVAL BASED ON THE IMK MODEL---

out<-c(0,1) # Control (D,SF)=(0,1)

# Instantaneous irradiation
alpha0<-a*G[1,2]
beta<-b*G[1,3]
alpha<-alpha0+beta*g
LL<-alpha*d+beta*d*d

# Dose versus survival
D<-d
SF<-exp(-LL)
```

```

out1<-c(D,SF)
out<-rbind(out,out1)

# split-dose irradiation
for(N in 2:f){
  LL1<-0          # lethal lesion 1
  LL2<-0          # lethal lesion 2
  LL<-0           # total number of lethal lesion
  alpha0<-0       # alpha0: MK parameter [/Gy]
  alpha<-0        # alpha: MK parameter (alpha=alpha0+beta*g) [/Gy]
  beta<-0         # beta: MK parameter [/Gy^2]

  for(n in 1:N){
    alpha0<-a*G[n,2]/CC[n,2]
    beta<-b*G[n,3]/CC[n,2]
    alpha<-alpha0+beta*g
    LL1<-LL1+alpha*d+beta*d*d
  }
  # print(LL1)
  # calculation of interaction of sub-lesions
  for(n in 1:(N-1)){
    for(m in (n+1):N){
      l<-m-n
      rr<-2*CC[n,2]/(CC[n,2]+CC[m,2])
      LL2<-LL2+2*b*CC[1,2]/CC[n,2]*rr*G[n,2]*G[m,2]*(exp(-l*apc*CC[n,2]*tau))*d*d
    }
  }
  # print(LL2)
  LL<-LL+LL1+LL2
  # print(LL)
  D<-N*d
  SF<-exp(-LL)
  out2<-c(D,SF)
  out<-rbind(out,out2)
}

```

#---END OF CALCULATION OF SURVIVAL-----

```

# output the dose vs surviving fraction
print(out)
write.csv(out,"SF.csv")

```

#---PLOT THE SURVIVING FRACTION -----

```

x<-out[,1]
y<-out[,2]
xl=c(min(x),max(x))
yl=c(min(y),max(y))
plot(x,y,log="y",main="IMK model",xlim=xl,ylim=yl,col="red"
      ,xlab="Absorbed dose [Gy]",ylab="Surviving fraction")

```

#---SPLINE INTERPOLATION-----

```

sp<-smooth.spline(x,y)
x<-seq(min(x),max(x),length=6) # length: number of data for interpolation
pred <- predict(sp, x)
par (new =T)
lines(pred,col="blue")

```

```

#-----
# Output file: SF.csv, interpolation.csv
# graphics: calculated sf-values, spline interpolation
#-----

```

```

#-----
# Determining the model parameters for TEs and NTEs by using maximum likelihood methods (MLM)
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# Model type: IMK model for TEs and NTEs in log of cell survival against dose
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#---CHECK THE DIRECTORY-----

getwd()

#---READ THE EXPERIMENTAL DATA-----

xx<-matrix(scan("data.d"),ncol=2,byrow=T)
D<-xx[,1]
SF<-xx[,2]
LOGSF<-log(SF)
xl=c(min(D),max(D))
yl=c(min(LOGSF),max(LOGSF))
head(xx,100)

#---DESCRIBE THE EXPERIMENTAL DOSE-SF RELATION-----

dev.set(1)
plot(D,LOGSF,xlim=xl,ylim=yl,col="black",
      xlab="Absorbed dose [Gy]",ylab="Surviving fraction")

#---SET THE RANGE OF CANDIDATE OF MODEL PARAMETERS-----

yy<-matrix(scan("setting.d"),ncol=1,byrow=T)
la<-yy[1,]      # la: lower IMK parameter (alpha) for TEs
lb<-yy[2,]      # lb: lower IMK parameter (beta) for TEs
lapc<-yy[3,]    # lapc: lower IMK parameter (a+c) for TEs
ua<-yy[4,]      # ua: Upper IMK parameter (alpha) for TEs
ub<-yy[5,]      # ub: Upper IMK parameter (beta) for TEs
uapc<-yy[6,]    # uapc: Upper IMK parameter (a+c) for TEs
lab<-yy[7,]     # lab: lower IMK parameter alphab for NTEs
lbb<-yy[8,]     # lab: lower IMK parameter betab for NTEs
ldel<-yy[9,]    # lab: lower IMK parameter delta for NTEs
uab<-yy[10,]    # lab: Upper IMK parameter alphab for NTEs
ubb<-yy[11,]    # lab: Upper IMK parameter betab for NTEs
udel<-yy[12,]   # lab: Upper IMK parameter de;ta for NTEs
N<-yy[13,]      # N : sampling number
head(yy,13)     # check the prior information of parameters

#---READ THE IRRADIATION CONDITIONS-----

zz<-matrix(scan("IRcondition.d"),ncol=1,byrow=T)
DR<-zz[1,]      # DR: Absorbed dose rate in Gy/h
gamma<-zz[2,]   # gamma: radiation quality [Gy]

#---DETERMINE THE BEST-FIT VALUE OF MODEL PARAMETERS-----

#---INITIALIZATION-----
a<-0            # initialization of alpha
b<-0            # initialization of beta
apc<-0.01       # initialization of (a+c)
ab<-0           # initialization of alphab
bb<-0           # initialization of betab
del<-0          # initialization of delta
logL<-0         # initialization of likelihood
logL2<-0

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logL3<-0
out<-c(a,b,apc,ab,bb,del)
n<-nrow(xx)      # number of experimental data

for(i in 1:n){
  #---user setting model-----
  # this code is an example for cell-killing
  f<-2/apc/apc/D[i]/D[i]*DR*DR*(apc*D[i]/DR+exp(-apc*D[i]/DR)-1)
  mod1<-(a+gamma*b)*D[i]+f*b*D[i]*D[i]      # IMK model for TE
  fb<-exp(-(ab+gamma*bb)*D[i]-bb*D[i]*D[i])
  fh<-1-fb
  mod2<-del*fb*fh      # IMK model for NTE
  mod<-mod1+mod2
  #-----

  ExpSF<-LOGSF[i]      # Experimental data
  logL2<-(-(mod+ExpSF)^2)
  logL<-logL+logL2      # sigma^2
  print(logL)
}
#---END OF INITIALIZATION-----

#---MAIN CODE FOR DEDUCTION OF THE BEST-FIT PARAMETERS-----

for(j in 1:N){
  # generate the candidate of model parameter
  print(j)      # based on uniform distribution
  ra<-runif(1,min=la,max=ua)      # parameter alpha
  #print(ra)
  rb<-runif(1,min=lb,max=ub)      # parameter beta
  #print(rb)
  rapc<-runif(1,min=lapc,max=uapc)      # parameter (a+c)
  #print(rapc)
  rab<-runif(1,min=lab,max=uab)      # parameter alpha
  #print(rab)
  rbb<-runif(1,min=lbb,max=ubb)      # parameter beta
  #print(rbb)
  rdel<-runif(1,min=ldel,max=udel)      # parameter (a+c)
  #print(rdel)

  for(i in 1:n){
    #---user setting model-----
    f<-2/rapc/rapc/D[i]/D[i]*DR*DR*(rapc*D[i]/DR+exp(-rapc*D[i]/DR)-1)
    mod1<-(ra+gamma*rb)*D[i]+f*rb*D[i]*D[i]      # IMK model for TE
    fb<-exp(-(rab+gamma*rbb)*D[i]-rbb*D[i]*D[i])
    fh<-1-fb
    mod2<-rdel*fb*fh      # IMK model for NTE
    mod<-mod1+mod2
    #-----

    ExpSF<-LOGSF[i]      # Experimental data
    logL2<-(-(mod+ExpSF)^2)
    logL3<-logL3+logL2      # sigma^2 for danditate
    #print(logL)
  }

  if (logL3>logL){
    logL<-logL3
    out<-c(ra,rb,rapc,rab,rbb,rdel)
    #print(out)
    logL3<-0
  }else{
    logL3<-0
  }
}

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}
# print(out)

#---END OF MAIN CODE -----

#---OUTPUT THE BEST-FIT MODEL PARAMETERS-----

write.csv(out,"Parameter.csv")
# Output the csv file

#---DESCRIBE THE DOSE-RESPONSE CURVE ACCORDING TO THE IMK MODEL-----

# definition of model parameter for describing dose-response curve
a<-out[1]      # alpha for TEs
b<-out[2]      # beta for TEs
apc<-out[3]    # (a+c) value (SLDR rate)
ab<-out[4]     # alphab for NTEs
bb<-out[5]     # betab for NTEs
del<-out[6]    # delta for NTEs

IMKM<-function(x){
  return(-(a+gamma*b)*x-b*x*x2/apc/apc/x/x*DR*DR*(apc*x/DR+exp(-apc*x/DR)-1)
        -del*exp(-(ab+gamma*bb)*x-bb*x*x)*(1-exp(-(ab+gamma*bb)*x-bb*x*x)))
}
par(new=T)
plot(IMKM,xlim=xl,ylim=yl,col="black",
      xlab="Absorbed dose [Gy]",ylab="Surviving fraction")

#-----
# output file: Parameter.csv
# graphics: relation between dose and cell survival (dose-response curve)
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