Heavy-Ion Radiobiology

4

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Abstract

In biological processes after energy deposition of radiation in cells, DNA damage occurs at first to produce chromosomal breaks and then appear many cell responses. Reproductive and interphase cell death causes inactivation of cancer tissue. Traditional target theory and linear quadratic model are used to explain mechanism and treatment prediction. There are many factors to modify the efficiency of radiobiological effects such as oxygen and other chemical substances as well as physical distribution of radiation. For high-LET radiations, relative biological effectiveness is the most important factor to explain the radiobiological effects on cancer therapy as well as oxygen enhancement ratio.

Keywords

4R's • Dose response • LET • OER • RBE

4.1 Physical, Chemical, and Biological Processes

4.1.1 Processes of Radiobiological Effects

Biological effects after exposure to ionizing radiations proceed in the time sequence as follows: physical processes such as energy absorption by the atoms and molecules proceed before 10^{-15} s after the irradiation, chemical process; reaction drives direct or indirect molecular changes including free-radical production in the order of 10^{-6} s and thereby biological process; and biological effects bring on initial damages to biomolecules in the cell components (e.g., DNAs, proteins) in the order of 10^{-3} s. A part of those damages will be repaired through the biological processes to be a healthy cell, or fixed through the processes to kill the cells leading to the death of individual cells. Or the cells will live but produce a genetic change to produce a mutant or a tumor. A conceptual time sequence of the various processes initiated by radiation is shown in Fig. 4.1. The initial changes that include ionization and excitation occurring at the atomic level and DNA damage occurring at the molecular level lead to changes at a cellular level, organ level, and then total body level, eventually resulting in changes in the whole individual.

4.1.2 Direct and Indirect Actions of Radiation

Biological effects after exposure of radiation are divided into two categories, direct action and indirect action of radiation by the very early process of the energy deposition (Fig. 4.2). DNA molecules can receive energy directly from the secondary electrons produced by the incident radiation, resulting in their ionization, and are damaged by cleavage of the chemical bonds. This direct action of radiation accounts for approximately 1/3 of all biological effects after radiation. DNA molecules can also be damaged by active group of molecules (free radicals) produced by ionization of the surrounding water molecules. This is an indirect action of radiation and accounts for the remaining approximately 2/3 of the biological effects.

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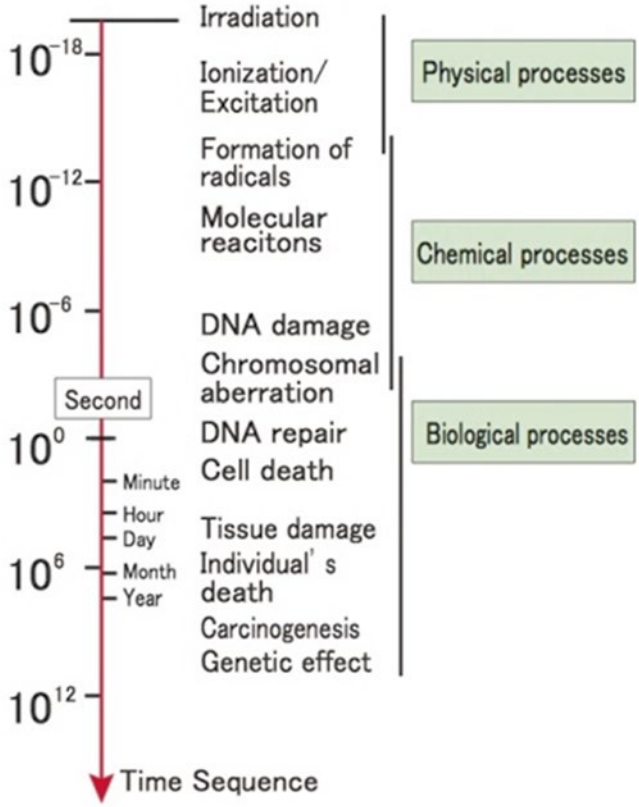

Fig. 4.1 Time sequence of radiobiological process after ionization radiation

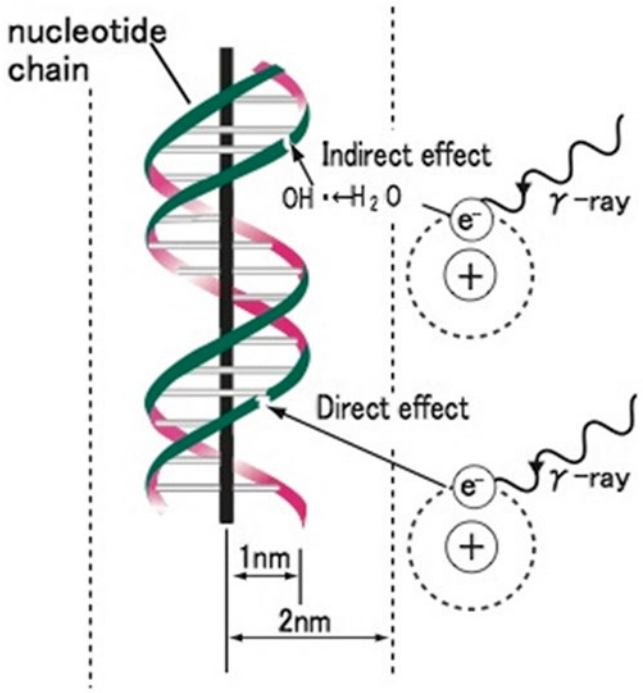

Fig. 4.2 Direct and indirect actions of radiation. Indirect action (*upper*), the secondary electron interacts with, for example, a water molecule to produce a hydroxyl radical (OH·), which produces a damage to the DNA. Direct action (*lower*), a secondary electron resulting from absorption of a photon interacts with the DNA to produce damages. Modified from [1]

The efficiency of free-radical production is expressed as a *G*-value, which is defined as the number of free-radical molecules produced per 100 eV of absorbed energy. The values are obtained as the final result of chain reactions. This process mainly consists of the chemical reactions of water molecules with the body constituents and can be affected by the surrounding various free-radical scavengers. These active species deprive a biomacromolecule (R) of a hydrogen atom (dehydrogenation) to make a radical R; the radical R binds with a hydroxyl radical (OH·) or reacts with another radical biomolecule to compose a new molecule. Consequently, the active species cause various reactions (Fig. 4.3).

4.1.3 Direct and Indirect Actions of Radiation

Low-LET radiations (photon) show a uniform, sparse spatial distribution of ionization in cells. High-LET particles bring about a dense ionization along their track through energy deposit to the medium, showing distributions called track structures (Fig. 4.4). Charged-particle beams that form the Bragg's peak in matter change the ionization density along the traveling direction, showing complexities.

Various physical and chemical processes are involved in the biological effects as direct and indirect effects of radiations and details of these processes can be found in other materials [2]. Particle beams have more different characteristics than photon beams that originate from the differences in their ionization-density distributions. Unlike low-LET photon beams, for example, high-LET particle beams show an ionization density higher than necessary for cell killing in some area in matter and also produce concentrated damages such as multiple DSBs or complex lesions. Thus, particle beams have different and severe biological effects from those of low-LET radiations.

4.2 Biological Elementary Processes

The organs comprising a human body are composed of tissue, which is a collection of cells with various functions to achieve specific objectives. The impact of ionizing radiations on a human body is initiated in the cells. Ionizing radiations delivered to a human body have the most serious impact on the genes that contained cell nucleus or DNA molecule. DNA damage causes the transmission of erroneous information,

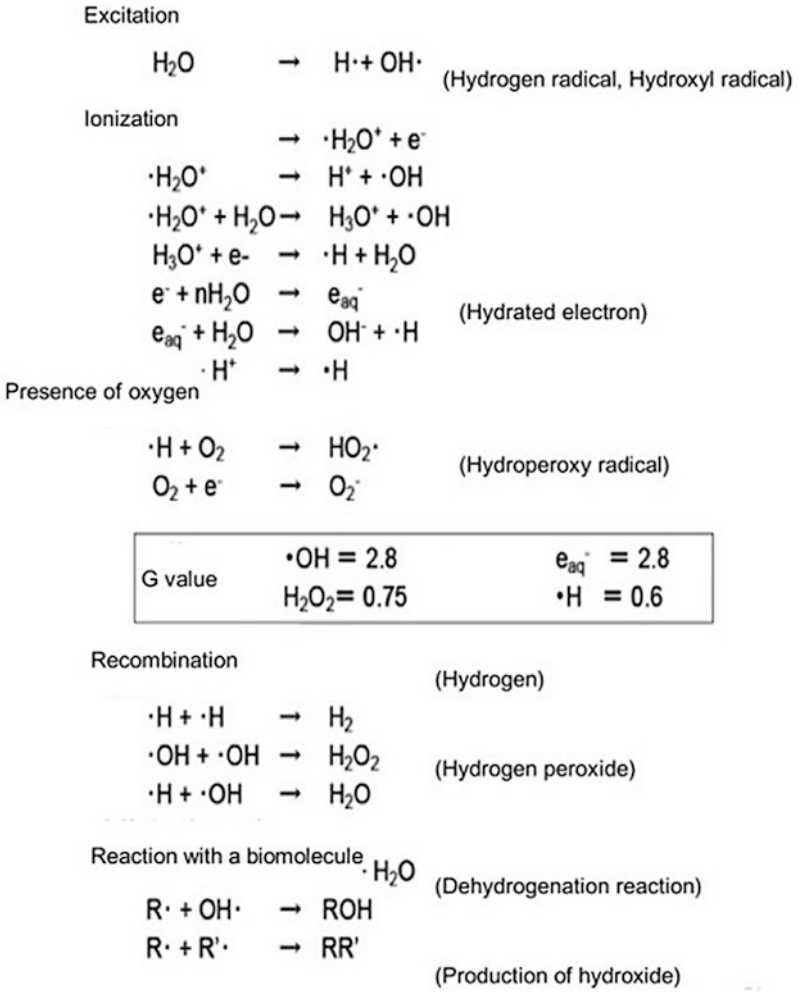

Fig. 4.3 Radiation chemical processes of water molecules after ionization radiation and the efficiency, G-value of major products

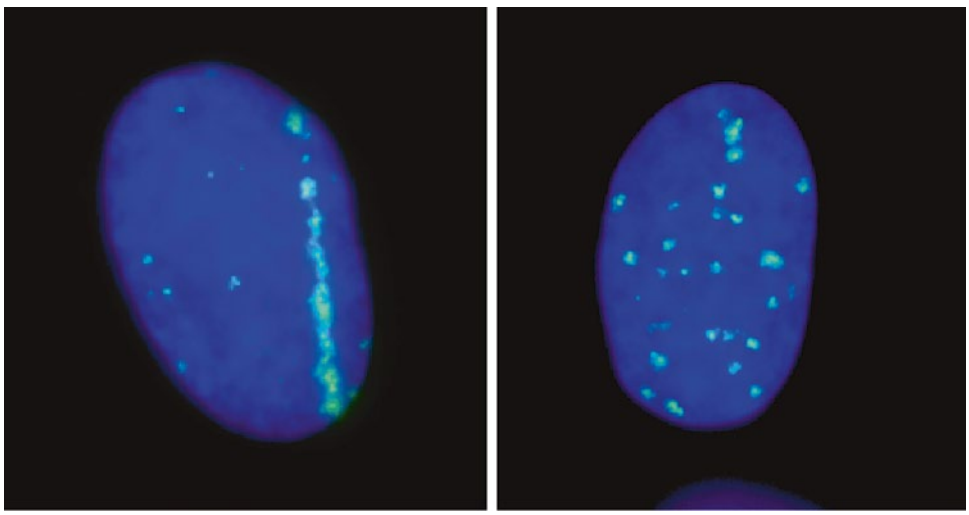

Fig. 4.4 Biological track structure of heavy-ion traversal in cell nuclei. DNA double-strand breaks were visualized after very high-LET horizontal iron-ion beam (*left*) and X-rays (*right*). Clear structure after iron beam traversal was observed, but no such structure for X-rays

makes cell growth difficult unless repaired, and produces abnormal conditions such as mutations or carcinogenesis. This damage in a cell impairs functions of the organ as damage to the tissue, eventually influencing the individual. A DNA molecule consists of a pair of backbone chains with the four (ATGC) nucleobases, which are involved in the encoding of genetic information. DNA is a long molecule encoding a huge amount of genetic information. The DNA contained in one human cell weighs approximately 6 pg, and the human genetic information is written as 4-bit data in approximately 32 hundred million base pairs.

A process of cell proliferation is carried out through a "cell cycle" sequence consisting of the four ($M/G_1/S/G_2$) phases. Certain types of cells are usually proliferating by repeatedly dividing to supply functional cells. In contrast, many somatic cells (nonreproductive cells) usually stay in another G1-like interphase (G_0 phase) without proceeding to the S phase, performing their own functions.

4.2.1 DNA Damages

The most important biological damage of cells after exposure of ionizing radiation is believed to be DNA damage. Many kinds of DNA damages will generate in a cell [3]. Damages or losses of nucleotide bases and DNA single-strand breaks (SSBs) are potentially to be repaired without errors of genetic information because of the existing right genetic information in the complementary DNA strand. Numbers of double-strand breaks (DSBs) are also rejoined to reconstruct DNA strand by the nonhomologous end joining (NHEJ) and homologous recombination (HR) systems. Damaged site is reconstructed by using the genetic information in complementary strand of sister chromatid by the HR system and shows error-free repair. Some nucleotide bases near the damage site are removed to clean up the damaged end, rejoin the strands without correct genetic information in the NHEJ process; thus it will be an error-prone rejoining. In addition, productions of complex lesion of DNA breaks are expected for heavy-ion exposure, because the density of ionization must be very high at around the traversal of the ion.

When cells are exposed to ionizing radiation, DNA damages in the form of SSBs, DSBs, base damage, or their combinations are frequent events. It is known that the complexity and severity of DNA damage depend on the quality of radiation and the microscopic dose deposited in small segments of DNA, which is often related to the linear transfer energy (LET) of the radiation. Experimental studies have suggested that under the same dose, high-LET radiation induces more small DNA fragments than low-LET radiation, which affects Ku binding with DNA end efficiently and might be a main reason for high-LET radiation-induced RBE since DNA DSB is a major cause for radiation-induced cell death. In this work, we proposed a mathematical model of DNA fragments rejoining according to NHEJ mechanism.

4.2.2 Chromosome Aberrations

One set of chromosomes in a human individual consists of 22 pairs of autosomes (any of the chromosomes in a cell other than the sex chromosomes) and two sex chromosomes, all of which are inherited from one's parents. In the DNA synthetic phase of the cell cycle, one copy of the genetic information is created and associated with the original one as a pair of sister chromatids, bound at the centromere, in order that the same genetic information may be distributed to two daughter cells in the coming mitotic phase. In the metaphase of the mitotic phase, DNA, distributed homogeneously within a cell nucleus in other phases, condenses to form 46 structures that can be microscopically observed as chromosomes. In the anaphase, 46 pairs of sister chromatids are pulled apart to transmit the genetic information to the two daughter cells.

In the cells of which DNA has been damaged by radiation exposure, abnormality can be observed as a chromosomal aberration which is effective as an indicator of radiation exposure as it is easy to quantify and correlates well with radiation-caused cell death. Various types of chromosomal aberration (Fig. 4.5) are caused by radiations, depending on the phase of the cell when its DNA is damaged. If it occurs during the period from the first pause (G1 phase) to the early DNA synthetic phase (early S phase), the so-called chromosomal aberration can be observed, which appears to be caused by simultaneous cleavage of both of the sister chromatids. If a cleaved chromosome is not reunited, "terminal deletion" and "fragment formation" are observed. After a chromosome is cleaved at the two sites, the center section turns over and is then reunited with the remaining fragments. This phenomenon is called "inversion." If the termini of this center section rejoin with one another, a "circular chromosome" is formed, with the fragments left over. After two chromosomes are cleaved, they can rejoin with each other to form a dicentric chromosome, which has two kinetochores (structures of protein associated with DNA located at the centromere region). This phenomenon is called "mutual translocation." On the other hand, if DNA damage occurs late in the DNA synthetic phase (late S phase) or the second pause (G2 phase), chromatid aberration is observed, with damage showing on either of the sister chromatids (Fig. 4.5).

4.2.3 Cellular Effects

Many of the multiplied human cells undergo cell cycle about once a day, with one parent cell dividing into two daughter cells; the number of the cells doubles again and again.

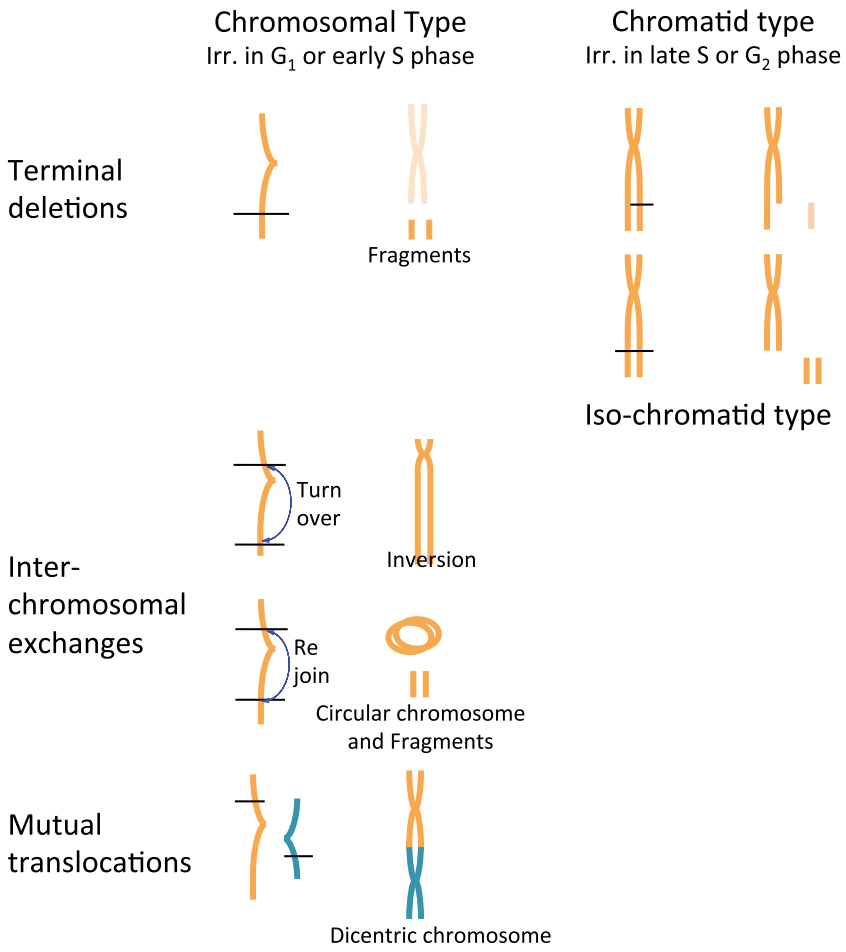

Fig. 4.5 Types of chromosomal aberrations. Many iso-chromatid-type aberration can be seen after high-LET radiation

A tens-of-micron single cell proliferates into a macroscopic colony with a diameter of several millimeters after a dozen days or so. When these cells are irradiated, some of them die owing to the resulting damage, while others survive. The surviving cells can form a colony, but the colonies show different morphology from those made by unirradiated cells, and the number of colonies they form is usually smaller. There are several cell death modes, but they are generally classified into reproductive death and interphase death.

4.2.3.1 Reproductive Death

Even after irradiation, the surviving cells can continue proliferating; cells have the ability to repair damage to their DNA or chromosomes and many cells can remove these damage. After repair, the cell cycle goes into a temporary pause, which is called G_1 block or G_2 block. These additional pauses in the cell cycle result in mitotic delay, which is often observed for the first division after irradiation. Approximately 1-Gy irradiation often causes a 1-hour mitotic delay.

Reproductive death is the mode of death of the irradiated cells that have lost reproductive ability after several cell divisions. These cells may still retain ability to synthesize DNA and proteins. These cells, however, have various functional aberrations; for example, the nucleus divides but fail in cytokinesis (the last stage of cell division following the nuclear division, division of cytoplasm) and the cell grows but cannot divide or shows abnormal morphology. In radiobiological observations after irradiation, which aim at counting the reproductive cell deaths, a colony consisting of 50 or more cells (after 5 to 6 divisions) is regarded as a colony originating from a surviving cell. The cell survival rate is determined from the ratio of the number of colonies formed in a plate to the number of irradiated cells seeded in the plate.

4.2.3.2 Interphase Death

With this mode, irradiated cells die without dividing. Interphase death, therefore, cannot be determined by the

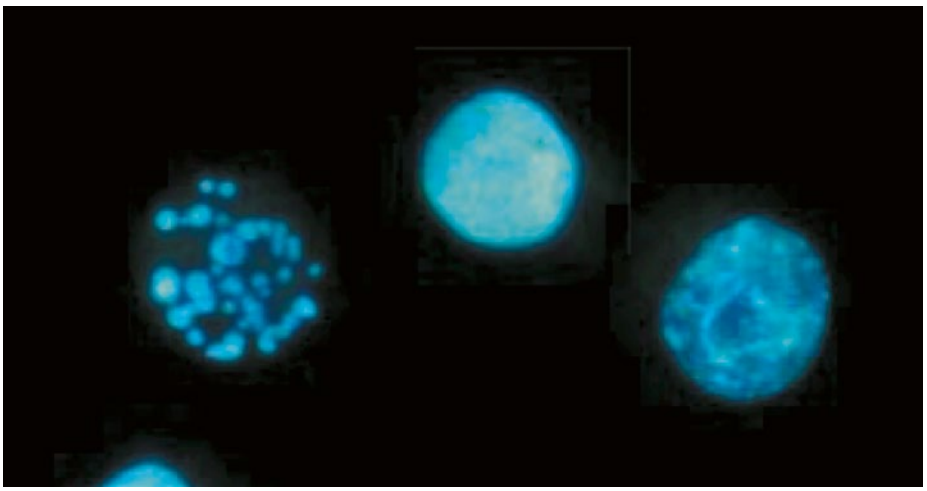

Fig. 4.6 Apoptotic cells. A finely fragmented (*left*), condensed (*middle*), and being fragmented (*right*) DNA

colony formation method. The cells are usually observed by morphological methods such as the dye exclusion method and fluorescence staining. Apoptosis and necrosis are mostly known as active and passive interphase death.

Apoptosis is an interphase death occurring even at very low doses, often observed in lymphoid stem cells. It is an active programmed cell death and a mode to exclude unnecessary cells. Cells with irreparable damage to their genetic information are identified and actively excluded to make spaces into which the surrounding normal cells can proliferate, resulting in maintenance of the entire function they are involved in. Cells undergoing apoptosis become round and are divided into units called "apoptotic vesicles." The nuclei are condensed, with DNA being cleaved into nucleosomes. The fundamental units of chromatin, the material of which eukaryotic chromosomes are made, consisting of cores of histone proteins around which are coiled about 160 bp of DNA. The divided cells are then scavenged by phagocytes such as macrophages, so that spaces for proliferation are created.

Necrosis is often observed in cells exposed to very high doses of radiations where the cells lose their functions, melt or break, and die. Necrosis is observed in nerve, muscle, and other cells, but unlike programmed cell death such as apoptosis, necrosis is not accurately controlled and means the passive death of nonfunctional cells (Fig. 4.6).

4.3 Models for Radiobiological Effects

In many cases, the biological effects of ionizing radiations are determined from the exposure dose. These effects can be expressed as a function of the dose, with the dose-effect relationship shown graphically, which enables these effects to analyze quantitatively. Plotting the rate of surviving cells against the irradiation dose can draw survival curves, and models to analyze these curves have been proposed with which the effects of ionizing radiations can be quantitatively studied.

4.3.1 Target Theory

In order to quantitatively describe the properties of radiobiological effects using survival curves, models to explain the shapes of these curves have been proposed. The target theory is the most classical model. This model assumes "parts of a cell are sensitive to ionizing radiations and regarded as targets for radiations"; "the targets are much smaller than the entire cell, but essential for survival of the cell"; and "when these targets are hit by radiations, the cell can lose its functions to become inactivated (killed)." This is the idea of "all or nothing"; cells can survive, unless they are hit. The linear quadratic model takes the place of this theory now especially in the field of radiotherapy.

4.3.2 Linear Quadratic Model

Although a DNA single-strand break leads to neither chromosomal aberration nor cell death, a DNA double-strand break could cause lethal damage. Taking this into account, in addition to the classical model described above, another model has been devised through analysis based on microscopic dose distributions. Probability of a single event that an electron track originating from one ionizing particle

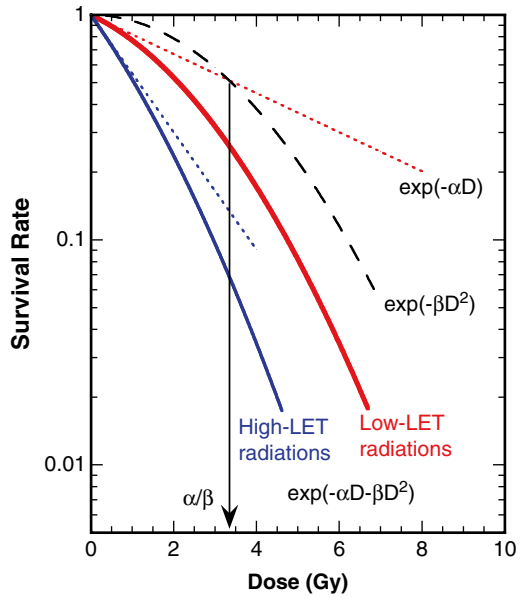

Fig. 4.7 Survival curves and parameters in the linear quadratic model. Overall cell survival curves (*solid lines*) for low- and high-LET radiations are plotted with each component in the LQ equations as the linear components (*dotted lines*) and quadratic components (*broken line*). The dose of the crossing points indicates the α-/β-value. Survival curve for high-LET radiation is drawn under the assumption of 3 times higher α-value with the same β-value to the low-LET radiation

passing near DNA cleaves both strand is proportional to the first-order term of the dose. Although the first ionizing event breaks one strand of DNA but does not lead to inactivation of the cell, the second event cleaves another strand leading to inactivation. The probability of this composite event is proportional to square of the dose. Considering these two causes, the radiation effect on a cell population can be expressed as the survival probability $S = \exp(-\alpha D - \beta D^2)$, where two constants α and β have been introduced. According to this model, the survival curves look like as shown in Fig. 4.7. Although the third and fourth events may also be taken into consideration, they are ignored for simplicity because of their small contribution. This model is called "LQ model (linear quadratic model)." In addition, there are different theories expressed with the same formula: one that a composite event consisting of the first and second radiation doses causes lethal damage and another considering damage due to one event, its repair, and the primary factors. Some theories different from the original have been proposed for interpretation and opinion is divided as to whether they accurately explain the radiobiological effects. Presently, however, the LQ model is often used for analysis of experimental data because of its greater consistency with experimental data, compared with the classical model.

4.4 Modifying Factors of Cell Sensitivity

Radiosensitivities of cells are modified by physical (radiation quality; LET, dose rate, etc.), chemical (oxygen, scavengers, protectors, etc.), and biological (metabolism, cell cycle, repair, etc.) conditions.

4.4.1 Biological Factors

The cell damage caused by ionizing radiations is classified into irreparable damage, or lethal damage (LD), and reparable damage. The reparable damage is divided into sublethal damage (SLD) and potentially lethal damage (PLD). SLD can be repaired within several hours under normal circumstances, but additional SLD before repair is completed leads to LD owing to interaction. PLD is lethal under normal circumstances, depending on the postirradiation condition of cell circumstances. There are two main ways to repair the DNA damage by which damaged DNA segments are resynthesized after being removed. One is SLD repair/recovery (SLDR), which is also known as Elkind recovery; and the other is PLD repair/recovery (PLDR).

4.4.1.1 Sublethal Damage

SLD is considered slight nonlethal damage. When fractionated irradiation is performed, damage caused by the first irradiation becomes lethal owing to the second irradiation. Because of the first irradiation to cells, some cells are lethally damaged so as to die, and others undergo SLDs and survive. Damaged cells with SLD surviving the first irradiation can recover to become exactly like they were as original normal cells with no damage, when they have sufficient time to repair the damage to themselves before the second irradiation. Cells undergoing fractionated irradiation twice with a certain dose show a higher survival rate than those undergoing sequential irradiation with the same dose. A survival curve for the second irradiation after sufficient time has passed is similar to that for the first irradiation from dose zero. When the SLDR occurs, the slope of the lineal part of the survival curve is reproduced universally, and the "shoulder" of the survival curve appears again to various extents depending on the time allowed to pass. Cells undergoing more instances of fractionated irradiation show a higher survival rate, under the condition that the given dose is constant (Fig. 4.8). When high-LET radiation is applied, the cell survival curve will be a straighter line and shows a small shoulder, because repair efficiency is small and shows a larger α-value and a smaller β-value of LQ model. SLDR is also small for high-LET radiations.

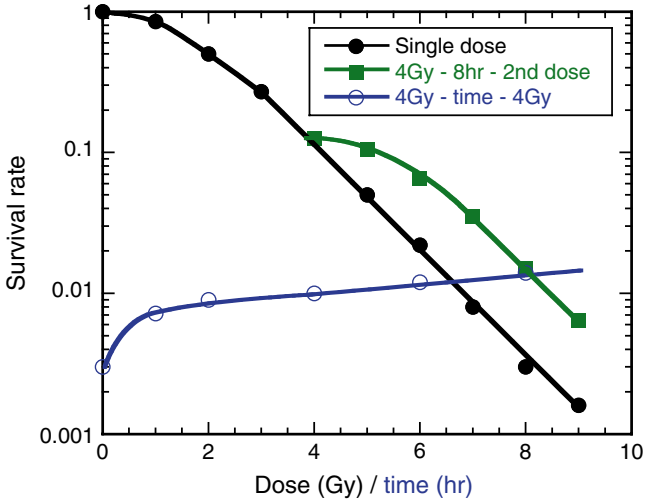

Fig. 4.8 Sublethal damage repair and survival rate. Fresh cells were irradiated with different single doses (*closed circle*), cells irradiated with 2nd challenging doses following 4-Gy priming doses and 6-h incubation (*closed square*), or survival after different interval time between 4-Gy priming dose and 4-Gy challenging dose

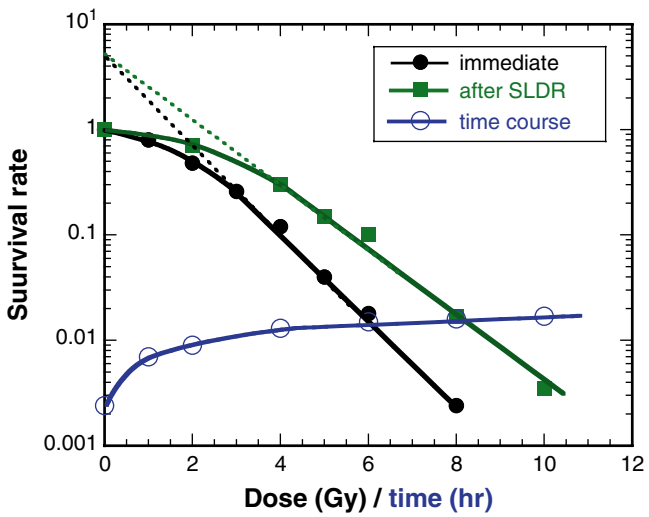

Fig. 4.9 Potentially lethal damage repair and survival rate. Cells were irradiated with different single doses plated immediately after irradiation (*closed circle*) or after 24-h incubation (*closed square*). Cell survival after 8-Gy irradiation and different incubation time (*open circle*)

4.4.1.2 Potentially Lethal Damage

PLD is essentially lethal. Recovery from PLD can be observed in cells placed in special circumstances, though the mechanism for this is little understood. Probably a difference occurs in the survival rate, if sufficient time can be provided for damage repair, with cell growth arrested, or if the conformation of DNA or a cell nucleus can be changed. This recovery can be observed in irradiated cells placed in special circumstances, especially inadequate conditions for cell growth. For example, recovery can be markedly observed in cells that are placed in poor nutritional conditions such as saline rather than in a growth medium containing sufficient nutrition. Recovery can also be observed in a population of steady-state cells that are too crowded to proliferate.

The potentially lethal damage repair/recovery (PLDR) after irradiation can occur with poor nutrition, low pH, and hypoxic conditions that arrest the cell cycle (proliferation) and in an extracellular environment that inhibits cell proliferation. According to the repair time, a fast type (<1 h), a slow type (2–6 h), and a very slow type (>8 h) have been observed. Fixation of PLD where PLD is converted into lethal damages and then the damage type is fixed has also been observed after treatment with an isotonic sodium chloride solution, caffeine, or some anticancer drugs. In these cases the slope of the survival curve changes in regard to the linear part (Fig. 4.9) or decreases the α-value. PLDR becomes smaller as well as SLDR and less prominent for high-LET radiations.

4.4.1.3 Cell Cycle

Tissues are classified into two groups according to their activity for multiplication. One group comprises cells that repeat proliferation vigorously in the proliferative tissue, including the bone marrow, skin, intestinal crypt, and genital gland. The other group comprises nonproliferative tissues including the cranial nerve, bone, muscle, and lung. Importantly, tissues that are proliferating vigorously have high radiosensitivity in general, and cells that are not proliferating have radiation resistance.

Cells are also known to change their radiosensitivity during the cell division process with the cell cycle consisting of G_1 (G_0), S, G_2, and M phases. Cells in the early S and M phases undergo DNA synthesis and cell division, respectively, and radiosensitivity of the cells in these active periods is higher. Cells in the G_1 and G_2 phases are less active and have less sensitivity to X-rays. However, high-LET radiation increases the sensitivity and decreases the variation through the cell cycle (Fig. 4.10). It gives an advantage on high-LET heavy-ion radiotherapy, because there are little resistant cells in target.

4.4.2 Chemical Factors

Among processes through which radiations produce biological effects, the indirect effect causes damage through chemical reactions, including reactions of DNA with active free radicals of water molecules, which accounts for about 2/3 of

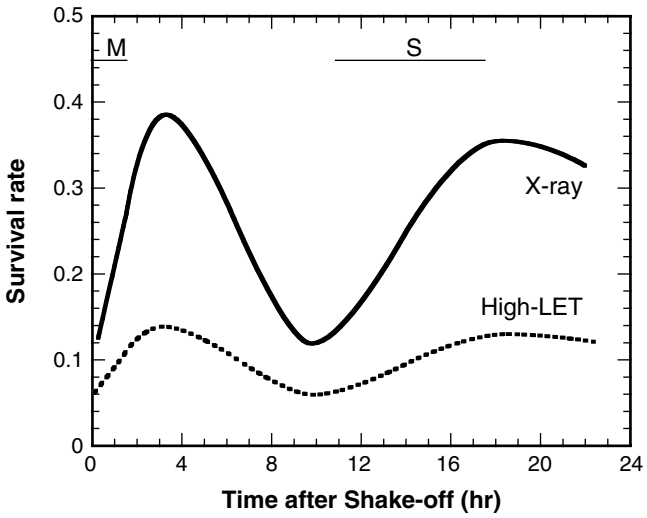

Fig. 4.10 Cell cycle and radiosensitivity. Cells exposed to three X-rays or high-LET radiations after shake-off (synchronization at M phase). M and S with bars correspond to the cell cycle at M phase and S phase after shake-off. Modified and reproduced from [4]

a cell. The radiation effect can be chemically augmented or attenuated by modifying substances participating in the reaction paths. In order to describe the change of radiosensitivity by these chemical substances, the dose reduction factor (DRF, mainly protectors) and the dose-modifying factor (DMF, mainly sensitizers) are used as well as the oxygen enhancement ratio (OER). The OER, DRF, and DMF are expressed as the ratio of isoeffective doses. Usually, a combination of the denominator and numerator of these fractional expressions is determined so that the quotient can exceed one.

$$DRF = D_{(with\ protectors)} / D_{(radiation\ alone)}$$

$$DMF = D_{(radiation\ alone)} / D_{(with\ sensitizers)}$$

$$OER = D_{(under\ hypoxic)} / D_{(under\ oxic)}$$

It is thought that those chemical modifiers are less effective on high-LET radiations. Since serious DNA damages are produced prominently by the direct action of radiation and the complexity of the damages after irradiation of high-LET radiations.

4.4.2.1 Oxygen

Oxygen produces the maximal radiosensitization effect in many parts of the mechanism. The presence of oxygen produces more free radicals to increase its chemical yield in a water solution. However, because the chemical yield is approximately doubled, the fact that the oxygen effect triples the biological effect cannot be explained. Thus, it is necessary to consider the contribution of other factors as well as the chemical yield. In a living body, glutathione with a thiol (SH) group, which participates in oxidation-reduction reactions, contributes to both the protection and oxygen effect for radiations. Some of the damage caused in the early stage of irradiation can be repaired chemically; the reduction reaction of glutathione with some of the free radicals produced by radiations leads to chemical repairs and elimination of the damages. However, the presence of oxygen causes competition between the oxygen and glutathione, resulting in establishing the damages before chemical repairs.

4.4.2.2 Other Chemical Substances

Some chemical substances are known as radiosensitizers or radioprotectors. Radiosensitizers are sensitizing agents of radiation that can be incorporated into cells and mainly augment the radiation effect in radiotherapy are typified by halogenated pyrimidines and hypoxic cell sensitizers. The halogenated pyrimidines are analogues to nucleobases comprising DNA and incorporated into DNA to increase the efficiency of cleavage of the DNA by radiations. Bromodeoxyuridine (BUdR) is incorporated into DNA as a substitute for thymidine, and it was used for BAR therapy for approximately 10 years from 1965; however, BUdR went out of favor because there were no significant differences in the 5-year survival rate between it and radiation therapy, although the short-term survival rate was favorable. Radioprotectors attenuating the radiation effect during irradiation have been known for a long time. Such attenuation is observed during exposure to radiations when drugs with antagonistic activity against subcellular radiation-produced active agents or drugs with repairing activity that brings produced active agents back into its original state are administered. Both types of the drugs are included in the radioprotective agents. Some drugs containing -SH groups and S–S bonds are well known as radical scavengers, including cysteine and cysteamine. In addition to these compounds with SH, serotonin, WR-2721, and AET are representative compounds. Alcohols, glycerins, and other coexisting substances that have high reactivity with free radicals such as·OH and·H can also scavenge and remove the free radicals, competing with oxygen to chemically repair the targeted molecules, attenuating the radiation effect.

4.4.3 Physical Factors

The magnitude of the radiation effect depends on the physical properties of the radiations as well as the radiation quality represented typically as LET, temporal (dose rate) and spatial (irradiation field) distributions of irradiation.

The dose-rate dependence of the radiation effect is known [5]. As the dose rate decreases from 1 Gy/min, radiosensitivity, which is high with high dose rates (acute irradiation,

short interval), reduces, with the survival curve becoming straighter with a decrease in the parameter β of the survival curve. For chronic irradiation of 0.2 Gy/h or less, radiation resistance increases, with the survival curve being straight ($\beta = 0$) and similar to that for fractionated irradiation. This is because the rate at which DNA damage is repaired is sufficiently higher than the rate at which the radiations cause the damage. The effect in much higher dose-rate region is not clear, because many have interest in radiation protection field and the difficulty of radiation sources. Dose rate from a high-LET particle must be extremely high when we consider single particle traversal in a cell. We confirmed the dose-rate effect on cell killing with a carbon ion beam at 70 keV/μm between 0.008 and 10 Gy/min (0.5 and 600 Gy/h) and found no significant change in those survival curves.

The radiosensitivity depends on the dose distribution in a body and becomes higher as the volume dose (g×Gy) increases. As this gets higher, the burden on the whole body becomes much greater, with recovery of the irradiated site being retarded, even though the influence of radiations on the cells in the irradiated site has not changed. If organs at risk (hematopoietic tissue, intestine) are included in the irradiated region, functions of these organs can be impaired so as to kill the individual. The treatment is associated with the idea of volume dose distribution (DVH, dose volume histogram) for treatment planning. As a DVH curve of normal tissue appears at the lower left, the risk is lower.

4.5 Characteristics of High-LET Radiations

4.5.1 Relative Biological Effectiveness: RBE

The biological effect depends on the quality of the delivered radiations. According to the LET, quality of radiation, X-rays, or γ-rays with several keV/μm or less can be classified into low-LET radiations, and particle beams with tens of keV/μm or more are classified into high-LET radiations. The biological effects of high-LET radiations are usually larger than that of low-LET radiations, where the difference between these biological effects is expressed as the relative biological effectiveness (RBE). RBE is described as dose ratio of test and reference radiations having the same biological effect:

$$RBE = D_{reference} / D_{test}$$

The RBE changes little in the low-LET region up to several keV/μm that correspond the LET region for γ-rays or X-rays, starts clear increasing after 10 keV/μm or more, and shows a peak in the LET at 100–200 keV/μm. However, the peak of the RBE depends on the particles accelerated and radiobiological end points. The RBE decreases in much higher-LET region and shows similar values to the low-LET radiations, and it goes to show less effect than one in the region above thousands of keV/μm. This is supposedly due to the fact that as LET increases, the number of particles to give the same absorbed dose decreases and the probability of hit cells also decreases (Fig. 4.11). Huge previous RBE data are summarized [8–10].

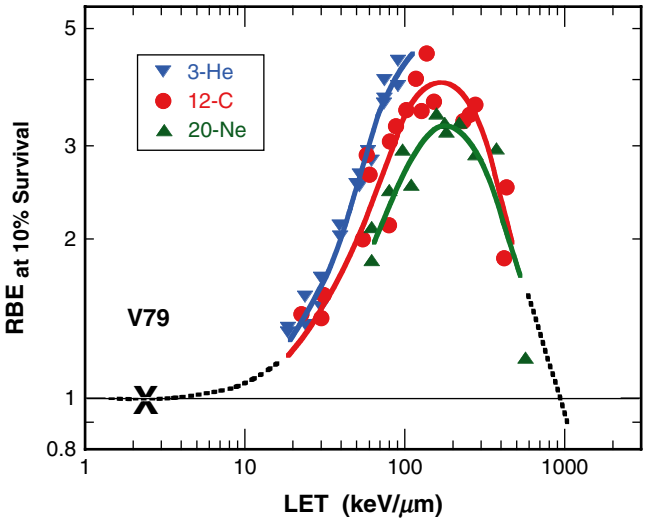

Fig. 4.11 RBE to different LETs for different ion species for cell killing of V79 Chinese hamster cells. Reproduced from [6, 7]

This all depends on cell types and nature of the biological effect observed (cell death, chromosome aberration, DNA strand breaks, mutation, carcinogenesis, and so on) and depends on the types of ionizing particles even though they have the same LET. The reason for this fact has not yet been properly explained. However, because the potential distribution of damage occurring is different owing to the difference in track structure specific to particle beams and geometrical structures of biomolecules in a cell, the types and complexity of this damage may be different. In addition, because this damage may influence the efficiency of biological repair system, geometrical distribution of damages, etc., the final influence may be different. Moreover, the dose-effect relationship of low-LET radiations is usually expressed with a straight line connecting to a quadratic curve with a nonlinear "shoulder" and that of high-LET radiations is expressed with a simple straight line. Considering the definition of RBE, or the ratio of two radiation doses to give the same biological effect, RBE depends on the level of the biological effect when the two radiation doses are compared.

It is recommended to use special X-rays showing a LET of 3 keV/μm and a dose rate of 0.1 Gy/min as a defined reference radiation from the viewpoint of radiation protection. Actually, ^{60}Co γ-rays or generated X-rays by a tube operated with several hundreds kVp are often employed as the reference radiations, since they have widely been used.

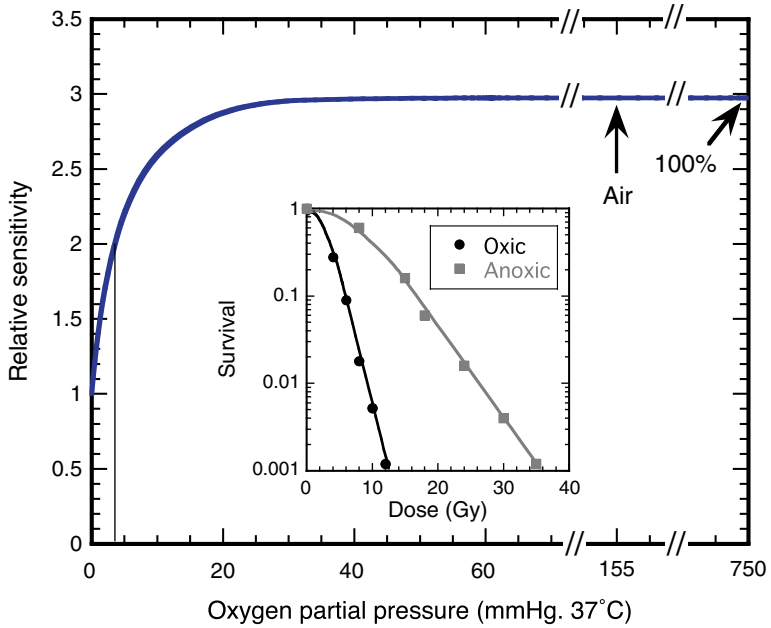

Fig. 4.12 OER for photons to oxygen concentration. Superimposed small figure shows cell survival curve at normoxic and anoxic conditions

In comparison with these X-rays, RBE of γ-rays and high-energy X-rays generated by linacs is clearly small. As radiobiological papers often show no clear definition of the reference radiation, attention should be paid to these facts.

4.5.2 Oxygen Enhancement Ratio: OER

In normal tissue, blood vascular systems are often substantial, with the oxygen partial pressure (pO_2) ranging from 10 to 80 mmHg. In the microenvironments of tumor tissue with blood vessels incompletely constructed, the oxygen concentration is low, and there are often regions with a pressure less than 5 mmHg. The oxygen effect can reduce radiation dose 3 times in normal cells to obtain the same effect (killing) or 2 at a pO_2 of 3 mmHg, for example (Fig. 4.12). Thus, cells at this site survive as radiation therapy-resistant cells, causing a repopulation of cancer cells in the treated site. In order to solve the problem of recurrence in the irradiated region, hypoxic cell sensitizers, which show a similar activity to oxygen and establish damage, were developed. Nitroimidazole agents have been the main ones developed, though at first misonidazole and etanidazole were developed but not put to practical use because tests revealed some adverse side effects. Today, however, to reduce these side effects, study into drug design is being vigorously performed.

In different oxygen conditions, such as normal internal body, compared with oxygen-free conditions, radiations are strong enough to have the same biological effect at an about one-third dose. Relative radiosensitivity under oxygen-rich condition is almost constant for the concentration above 30 mmHg, decreased with decreasing the concentration, and reached to be 1/3 at 0 mmHg. In order to describe the magnitude of the oxygen effect, the OER is used, defined as the ratio of a dose under testing oxygen conditions, D_{hypoxic}, to a dose having the same biological effect under oxygen-rich conditions, D_{normoxic}:

$$OER = D_{hypoxic} / D_{normoxic}$$

This maximum oxygen effect is observed as OER = 3 for low-LET radiations such as γ-rays or X-rays under oxygen-free anoxic condition and causes both a decrease in the sensitivity of intratumoral hypoxic cells and a recurrence of cancer in treated sites. Healthy cells in the normal tissues or cancer tissues having enough blood vessels are rich in oxygen and undergo the ultimate oxygen effect when exposed to radiations.

When high-LET ion beams are delivered, the hypoxic sites in tumors are unlikely to be affected by oxygen, showing similar radiosensitivity; the radiation effect on the hypoxic regions is larger. The OER decreases over tens of keV/μm for carbon beam, reaches to 2 at 100 keV/μm, and further decreases in higher-LET region (Fig. 4.13 also see [11]). We could see the difference in particles at the same LET region, and lighter ion can reduce the OER at lower LET. However, proton beams with an LET of several keV/μm may not be expected to produce this effect, because the LET is too low to reduce the OER. A combination of a high RBE and low OER is expected to have the effect of suppressing the proliferation of cancer cells during cancer treatment.

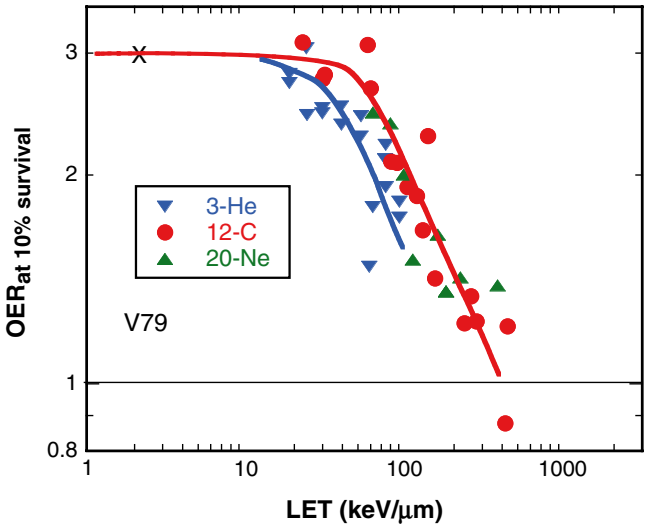

Fig. 4.13 OER to different LETs for different species for cell killing of V79 Chinese hamster cells reproduced from [6, 7]

This oxygen effect may also show a difference among ion spices accelerated. The oxygen effect is believed to cause fixation of radiation damage. The reason for decreasing the oxygen effect to high-LET radiations has different interpretations: oxygen may be produced during the recombination of ionized water molecules because of the extremely high ionization density and the radiation damage may be severe enough and thus established as local compound damage even without oxygen. However, the oxygen effect is not well understood in detail.

4.5.3 Fractionated Irradiation and 4R

Fractionated irradiation is typically performed during radiation treatment since the therapeutic ratio may be improved through various biological effects of the fractionated irradiation. This is known as the "4Rs" which consist of (1) repair, (2) repopulation, (3) redistribution, and (4) reoxygenation. The 4Rs are important issue especially for photon therapy, but they are usually a little for high-LET radiations. Those factors depend on the LET and the LET of therapeutic carbon beams are still smaller to neglect the factors. However, it is more important for carbon ion therapy that normal healthy cells that stay both at shallower part and deeper part from the target area in a patient will receive low dose of low-LET radiation and those cells will escape from radiation damage. Because there are small dose rate and lower LETs at the upper stream of the target and very small dose rate and the lowest LET from fragments behind the target. Only a less repair could be possible in target volume by irradiation with high-dose and high-LET radiation and possibly better repair in normal tissues by the ion beam with lower LET and lower dose at carbon ion radiotherapy (see [12]).

Repair is an important issue in radiotherapy. It is expected that the survival rate for normal cells is a little larger than that for cancer cells in lower irradiation doses, even though these survival rates are the same with higher irradiation doses. Thus, it is expected that this difference will increase logarithmically by SLDR to become large at total treatment dose when fractionated irradiation is performed using a dose range in which the survival rate for normal cells is larger than that for cancer cells. This is a reason why fractionated irradiation is performed during radiation treatment. The difference of survival level between normal and cancer cells at low-dose region is unfortunately not analyzed well for heavy-ion beams yet. A small fractionation treatment is applied in carbon ion therapy and obtains successful results.

Repopulation means new cell proliferation during treatment, which suppresses the entire cell growth inhibitory effect of irradiation. Repopulation is markedly observed in rapidly proliferating cancer cells, the effect of repopulation being small in cells proliferating slowly in the late-response tissue. During the final period of treatment, repopulation is promoted by growth stimulation, which becomes stronger because the number of dying cells has increased. The total dose required for treatment is constant because of non-prominent repopulation, if the total treatment period is not more than 30 days. The total dose that is required for the equivalent biological effect increases because of strong proliferation, if the total treatment period exceeds a certain value. In this point, it is difficult to find an analysis for heavy-ion therapy.

Redistribution means change in cell cycle distribution in tumor population. During irradiation to cells, cells in the highly sensitive periods such as the G2/M phase are likely to be killed, and cells in the resistant periods such as the late S phase of the cell cycle can specifically survive. After a cell suffers damage, a checkpoint at G_2 or other phases temporarily arrests the cell cycle, probably in order that the damage can be repaired. Less sensitive normal cells in the G_0 resting phase enter the cell cycle to become highly sensitive. In a cell population, the general sensitivity depends on the cell cycle distribution.

Reoxygenation is attributed to the decrease of oxygen consumption through the death of the surrounding cells, removal of killed cells, changes in the bloodstream, and other factors. Cells in the hypoxic regions of tumors are radioresistant and very likely to survive the first irradiation. However, if radiations kill the surrounding cells in oxic region or other factors change the microenvironment, the hypoxic regions are oxygenated to become sensitive to the subsequently coming radiation doses. Reoxygenation in different extent and at various times has been observed in almost all tumors. Some studies with high-LET carbon beams showed that the reoxygenation occurs earlier and is also stronger than reoxygenation caused by photon beams.

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