CONTENTS

VIDEO CONTENTS

1

The Biological Basis of Radiation Oncology

Elaine M. Zeman

WHAT IS RADIATION BIOLOGY?

In the most general sense, radiation biology is the study of the effects of electromagnetic radiation on biological systems. Three aspects of this definition deserve special mention. First, *effects* may include everything from DNA damage to genetic mutations, chromosome aberrations, cell killing, disturbances in cell cycle transit and cell proliferation, neoplastic transformation, early and late effects in normal tissues, teratogenesis, cataractogenesis, and carcinogenesis, to name but a few. *Electromagnetic radiation* refers to any type of radiant energy in motion with wave and/or particulate characteristics that has the capacity to impart some or all of its energy to the medium through which it passes. The amount of energy deposited can vary over some 25 orders of magnitude, depending on the type of electromagnetic radiation. For example, 1 kHz radio waves have energies in the range of 10^{-11} to 10^{-12} eV, whereas x-rays or γ-rays may have energies upwards of 10 MeV or more. The more energetic forms of electromagnetic radiation, the ionizing radiations, deposit energy as they traverse the medium by setting secondary particles in motion that can go on to produce further ionizations. Finally, *biological systems* may be, for example, quite simple cell-free extracts of biomolecules, or increasingly complex, from prokaryotes to single-celled eukaryotes, to mammalian cells in culture, to tissues and tumors in laboratory animals or humans, to entire ecosystems.

Radiotherapy-oriented radiobiology focuses on that portion of the electromagnetic spectrum energetic enough to cause ionization of atoms. This ultimately results in the breaking of chemical bonds, which can lead to damage to important biomolecules. The most significant effect of ionizing radiation in this context is cell killing, which directly or indirectly is at the root of nearly all of the normal tissue and tumor responses noted in patients.

Cytotoxicity is not the only significant biological effect caused by radiation exposure, although it will be the main focus of this chapter. Other important radiation effects—carcinogenesis, for example—will also be discussed, although the reader should be aware that radiation carcinogenesis is a large discipline in and of itself, involving investigators from fields as diverse as biochemistry, toxicology, epidemiology, environmental sciences, molecular biology, tumor biology, health and medical physics, as well as radiobiology. Most radiation protection standards are based on minimizing the risks associated with mutagenic and carcinogenic events. Therefore radiological health professionals are de facto educators of and advocates for the general public when it comes to ionizing radiation, who need to be fully conversant in the potential risks and benefits of medical procedures involving radiation.

The majority of this chapter will be devoted to so-called "foundational" radiobiology, that is, studies that largely predate the revolution in molecular biology and biotechnology during the 1980s and 1990s. While the reader might be tempted to view this body of knowledge as rather primitive by today's standards, relying too heavily on phenomenology, empiricism, and descriptive models and theories, the real challenge is to integrate the new biology into the already-existing framework of foundational radiobiology. Chapter 2 endeavors to do this.

RADIOTHERAPY-ORIENTED RADIOBIOLOGY: A CONCEPTUAL FRAMEWORK

Before examining any one aspect of radiobiology in depth, it is important to introduce several general concepts to provide a framework for putting the information in its proper perspective.

The Therapeutic Ratio

The most fundamental of these concepts is what is termed the therapeutic ratio—in essence, a risk-versus-benefit approach to planning a radiotherapy treatment regimen. Many of the radiobiological phenomena to be discussed in this chapter are thought to play important roles in optimizing, or at least "fine tuning," the therapeutic ratio. In theory, it should be possible to eradicate any malignant tumor simply by delivering a sufficiently high dose of radiation. Of course, in practice, the biological consequences for normal tissues that are necessarily irradiated along with the tumor limit the total dose that can be safely administered. As such, a balance must be struck between what is deemed an acceptable probability of a radiation-induced complication in a normal tissue and the probability of tumor control. Ideally, one would hope to achieve the maximum likelihood of tumor control that does not produce unacceptable normal tissue damage.

The concept of therapeutic ratio is best illustrated graphically, by comparing dose-response curves for both tumor control and normal tissue complication rates plotted as a function of dose. Examples of this approach are shown in Fig. 1.1 for cases in which the therapeutic ratio is either "unfavorable," "favorable," or "optimal," bearing in mind that these are theoretical curves. Actual dose-response curves derived from experimental or clinical data are much more variable, particularly for tumors, which tend to show much shallower dose responses.[1] This

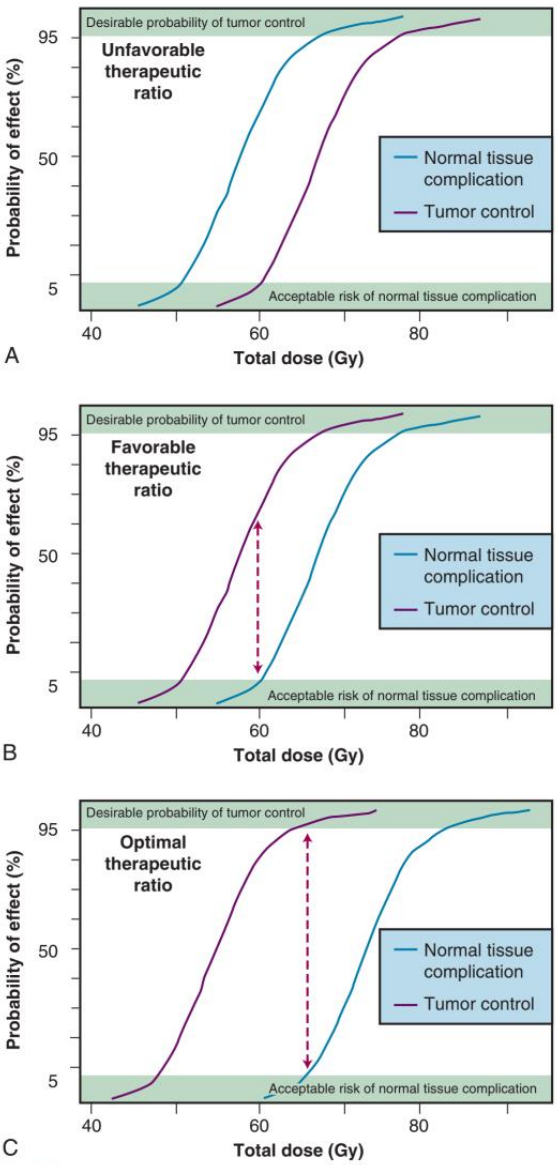

Fig. 1.1 Illustrating the concept of therapeutic ratio under conditions in which the relationship between the normal tissue tolerance and tumor control dose-response curves is unfavorable (A), favorable (B), and optimal (C).

serves to underscore how difficult it can be in practice to assign a single numerical value to the therapeutic ratio in any given situation.

Many of the radiobiological properties of cells and tissues can have a favorable or adverse effect on the therapeutic ratio. Therefore, in planning a course of radiation therapy, the goal should be to optimize the therapeutic ratio as much as possible; in other words, using our graphical approach, increase the separation between the tumor control and normal tissue complication curves. This can be accomplished either by shifting the tumor control curve to the left with respect to the dose axis (toward lower doses, i.e., radiosensitization) or shifting the normal tissue complication curve to the right (toward higher doses, i.e., radioprotection) or, perhaps, some combination of both. The key, however, is to shift these curves differentially, not necessarily an easy task given that there are not that many exploitable differences in the radiobiology of cells derived from tumors and those derived from normal tissues.

The Radiation Biology Continuum

There is a surprising continuity between the physical events that occur in the first few femtoseconds after ionizing radiation interacts with the atoms of a biomolecule and the ultimate consequences of that interaction on tissues. The consequences themselves may not become apparent until days, weeks, months, or even years after the radiation exposure. Some of the important steps in this radiobiology continuum are listed in Table 1.1. The orderly progression from one stage of the continuum to the next—from physical to physicochemical to biochemical to biological—is particularly noteworthy not only because of the vastly different time scales over which the critical events occur but also because of the increasing biological complexity associated with each of the endpoints or outcomes. Each stage of the continuum also offers a unique radiobiological window of opportunity: the potential to intervene in the process and thereby modify all of the events and outcomes that follow.

Levels of Complexity in Radiobiological Systems

Another important consideration in all radiobiological studies is the nature of the experimental system used to study a particular phenomenon, the assay(s) used, and the endpoint(s) assessed. For example, one investigator might be interested in studying DNA damage caused by ionizing radiation, in particular, the frequency of DNA double-strand breaks (DSBs) produced per unit dose. As an experimental system, the investigator might choose DNA extracted from irradiated mammalian cells and, as an endpoint, use pulsed field gel electrophoresis to measure the distance and rate at which irradiated DNA migrates through the gel compared with unirradiated DNA. The DNA containing more DSBs migrates farther than DNA containing fewer breaks, allowing a calibration curve to be generated that relates migration to the dose received. A second investigator, meanwhile, may be interested in improving the control rate of head and neck cancers with radiation therapy by employing a nonstandard fractionation schedule. In this case, the type of experiment would be a clinical trial. The experimental system would be a cohort of patients, some of whom are randomized to receive nonstandard fractionation and the rest receiving standard fractionation. The endpoints assessed could be one or more of the following: locoregional control, long-term survival, disease-free survival, normal tissue complication frequency, and so forth, evaluated at specific times after completion of the radiation therapy.

In considering both the strengths and weaknesses of these two investigators' studies, any number of pertinent questions may be asked. Which is the more complex or heterogeneous system? Which is the more easily manipulated and controlled system? Which is more relevant for the day-to-day practice of radiation oncology? What kinds of results are gleaned from each and can these results be obtained in a timely manner? In this example, it is clear that human patients with spontaneously arising tumors represent a far more heterogeneous and complex experimental system than extracted mammalian DNA. However, the DNA system is much more easily manipulated, possible confounding factors can be more easily controlled, and the measurement of the desired endpoint (migration distance/rate) plus the data analysis can be completed within a day or two. Obviously, this is not the case with the human studies, in which numerous confounding factors can and do influence results,

TABLE 1.1 Stages in the Radiobiology Continuum

Time Scale of Events ("Stage")	Initial Event	Final Event	Response Modifiers/Possible Interventions
10^{-16} to 10^{-12} second ("Physical")	Ionization of atoms	Free radicals formed in biomolecules	Type of ionizing radiation; shielding
10^{-12} to 10^{-2} second ("Physicochemical")	Free radicals formed in biomolecules	DNA damage	Presence or absence of free radical scavengers, molecular oxygen and/or oxygen-mimetic radiosensitizers
1.0 second to several hours ("Biochemical")	DNA damage	Unrepaired or misrejoined DNA damage	Presence or absence of functioning DNA damage recognition and repair systems; repair-inhibiting drugs; altering the time required to complete repair processes
Hours to years ("Biological")	Unrepaired or misrejoined DNA damage	Clonogenic cell death, apoptosis, mutagenesis, transformation, carcinogenesis, "early and late effects" normal tissues, whole body radiation syndromes, tumor control, etc.	Cell-cell interactions, biological response modifiers, adaptive mechanisms, structural and functional organization of tissues, cell kinetics, etc.

manipulation of the system can be difficult, if not impossible, and the experimental results typically take years to obtain.

The issue of relevance is an even thornier one. Arguably, both studies are relevant to radiation oncology in so far as the killing of cells is at the root of radiation's normal tissue and tumor toxicity, and that cell killing usually is, directly or indirectly, a consequence of irreparable damage to DNA. As such, any laboratory findings that contribute to the knowledge base of radiation-induced DNA damage are relevant. Clearly, however, clinical trials with human patients not only are a more familiar experimental system to radiation oncologists but also, efficacy in conducting trials with cancer patients is ultimately what leads to new standards of care in clinical practice and becomes the gold standard against which all newer therapeutic strategies are judged.

There is a time and place both for relatively simple systems and more complex ones. The relatively simple, homogeneous, and easily manipulated systems are best suited for the study of the mechanisms of radiation action, such as measuring DNA or chromosomal damage, changes in gene expression, activation of cell cycle checkpoints, or the survival of irradiated cells in vitro. The more complicated and heterogeneous systems, with their unique endpoints, are more clinically relevant, such as assays of tumor control or normal tissue complication rates. Both types of assay systems have inherent strengths and weaknesses, yet both are critically important if we hope to improve the practice of radiation therapy based on sound biological principles.

Heterogeneity

Why is radiation therapy successful at controlling one patient's tumor but not another's when the two tumors in all other clinical respects seem identical? Why are we generally more successful at controlling certain types of cancers than others? The short answer to such questions is that, although the tumors may appear identical "macroscopically," their component cells may be quite different genotypically and phenotypically. Also, there could be important differences between the two patients' normal tissues.

Because normal tissues by definition are composed of more than one type of cell, they are necessarily heterogeneous. However, tumors, owing both to the genomic instability of individual cells and to microenvironmental differences, are much more so. Different subpopulations of cells isolated from human and experimental cancers can differ with respect to differentiation, invasive and metastatic potential, immunogenicity, and sensitivity to radiation and chemotherapy, to name but a few. (For reviews, see Heppner and Miller[2] and Suit et al.[3]) This heterogeneity is manifest both within a particular patient and, to a much greater extent, between patients with otherwise similar tumors. Both intrinsic and extrinsic factors contribute to this heterogeneity. Intrinsic factors can include inherent radiosensitivity, genomic instability, gene expression patterns, DNA repair fidelity, mode(s) of cell death, cell cycle regulation, and how the tissue is structurally and functionally arranged. Extrinsic factors, on the other hand, are related to microenvironmental differences between tissues, such as the functionality of the vasculature, availability of oxygen and nutrients, pH, presence or absence of reactive oxygen species, cytokines and immune cells, energy charge, and cell-cell and cell-extracellular matrix interactions.

What are the practical implications of normal tissue and tumor heterogeneity? First, if one assumes that normal tissues are the more uniform and predictable in behavior of the two, then tumor heterogeneity is responsible, either directly or indirectly, for most radiotherapy failures. If so, this suggests that a valid clinical strategy might be to identify the radioresistant subpopulation(s) of tumor cells and then tailor therapy specifically to cope with them—although, admittedly, this approach is much easier said than done. Some clinical studies—both prospective and retrospective—now include one or more determinations of, for example, extent of tumor hypoxia[4,5] or potential doubling time of tumor clonogens[6] or specific tumor molecular/genetic factors. The hope is that these and other biomarkers can identify subsets of patients bearing tumors with different biological characteristics and that, accordingly, patients with particular characteristics can be assigned prospectively to different treatment groups.

Another consequence of tissue heterogeneity is that any radiobiological endpoint measured in an intact tissue necessarily reflects the sum total of the individual radiosensitivities of all of the subsets of cells, plus all other intrinsic and extrinsic factors that contribute to the overall response of the tissue. Since data on normal tissue tolerances and tumor control probabilities are also averaged across large numbers of patients, heterogeneity is even more pronounced.

Powers of Ten

Tumor control is achieved only when all clonogenic cells are killed or otherwise rendered unable to sustain tumor growth indefinitely. In order to estimate the likelihood of cure, it is necessary to know, or at least have an appreciation for, approximately how many clonogenic cells the tumor contains, how radiosensitive these cells are (i.e., some measure of killing efficiency per unit radiation dose), and what the relationship is between the number of clonogenic cells remaining after treatment and the probability of recurrence. The latter is perhaps the easiest to ascertain given our knowledge of both the random and discrete nature of radiation damage and the general shape of dose-response curves for mammalian cells and tissues. For a given number of surviving cells per tumor, the probability of local control can be derived from Poisson statistics using the equation $P = e^{-n}$, where P is the tumor

control probability and n is the average number of surviving clonogenic tumor cells. For example, when an average of one clonogenic cell per tumor remains at the end of radiation therapy, the tumor control rate will be about 37%. This means that about 6 out of 10 tumors of the same size and relative radiosensitivity will recur. Should the treatment reduce clonogenic cell numbers to an average of 0.1 per tumor, the tumor control probability would increase to 90%; 0.05 per tumor, 95%; and 0.01 per tumor, 99%, respectively.

The tumor control probability for a given fraction of surviving cells is not particularly helpful when the total number of cells at risk is unknown; this is where an understanding of logarithmic relationships and exponential cell killing is useful. For example, estimates are that a 1-cm^3 (1-g) tumor mass contains approximately 10^9 cells,[7] admittedly a theoretical (and incorrect) value that assumes that all cells are perfectly packed and uniformly sized and that the tumor contains no stroma. A further assumption, that all such cells are clonogenic (rarely, if ever, the case), suggests that at least 9 logs of cell killing would be necessary before any appreciable tumor control (about 37%) would be achieved, and 10 logs of cell killing would be required for a high degree of tumor control (i.e., 90%).

After the first log or two of cell killing, however, some tumors respond by shrinking, a so-called partial response. After two to three logs of cell killing, the tumor may shrink to a size below the current limits of clinical detection, that is, a complete response. While partial and complete responses are valid clinical endpoints, a complete response does not necessarily equal a tumor cure. At least six more logs of cell killing would still be required before any significant probability of cure would be expected. This explains why radiation therapy is not halted if the tumor disappears during the course of treatment; this concept is illustrated graphically in Fig. 1.2.

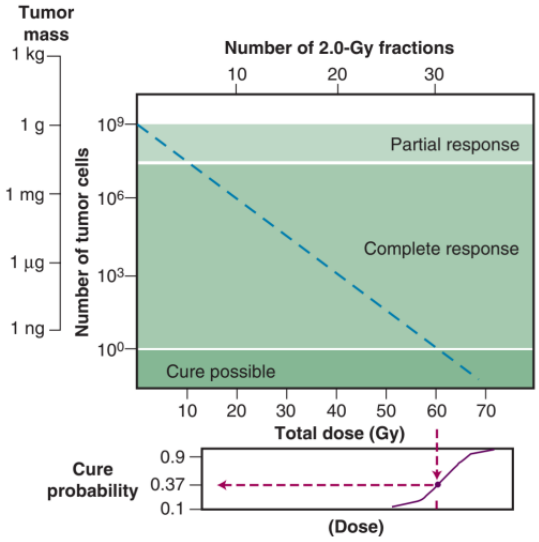

Fig. 1.2 The relationship between radiation dose and tumor cell survival during fractionated radiotherapy of a hypothetical 1-g tumor containing 10^9 clonogenic cells. Although a modest decrease in cell-surviving fraction can cause the tumor to shrink (partial response) or disappear below the limits of clinical detection (complete response), few if any cures would be expected until at least 9 logs of clonogenic cells have been killed. In this example, a total dose of at least 60 Gy delivered as daily 2-Gy fractions would be required to produce a tumor control probability of 0.37, assuming that each dose reduced the surviving fraction to 0.5. (Modified from Steel G, Adams G, Peckham M, eds. *The Biological Basis of Radiotherapy*. New York: Elsevier; 1983.)

Finally, it should be noted that while the goal of curative radiation therapy is to reduce tumor cell survival by at least nine logs, even for the smallest tumor likely to be encountered, it is much less clear how many logs of cell killing a particular normal tissue can tolerate before it loses its structural and/or functional integrity. This would depend on how the tissue is organized structurally, functionally, and proliferatively, which constituent cells are the most and least radiosensitive, and which cells are the most important to the integrity of the tissue. It is unlikely, however, that many normal tissues could tolerate a depletion of two logs (99%) of their cells, let alone nine or more logs.

RADIATION BIOLOGY AND THERAPY: THE FIRST 50 YEARS

In fewer than 4 years after the discovery of x-rays by Roentgen,[8] radioactivity by Becquerel,[9] and radium by the Curies,[10] the new modality of cancer treatment known as radiation therapy claimed its first cure of skin cancer.[11] Today, more than 120 years later, radiotherapy is most commonly given as a series of small daily dose fractions of approximately 1.8 to 2.0 Gy each, 5 days per week, over a period of 5 to 7 weeks to total doses of 50 to 75 Gy. While it is true that the historical development of this conventional radiotherapy schedule was empirically based, there were a number of early radiobiological experiments that suggested this approach.

In the earliest days of radiotherapy, both x-rays and radium were used for cancer treatment. Due to the greater availability and convenience of using x-ray tubes and the higher intensities of radiation output achievable, it was fairly easy to deliver one or a few large doses in short overall treatment times. Thus, from about 1900 into the 1920s, this "massive dose technique"[12] was a common way of administering radiation therapy. Normal tissue complications were often quite severe and, to make matters worse, the rate of local tumor recurrence was still unacceptably high.

Radium therapy was used more extensively in France. Because of the low activities available, radium applications necessarily involved longer overall treatment times in order to reach comparable total doses. Although extended treatments were less convenient, clinical results were often superior. Perceiving that the change in overall time was the critical factor, physicians began to experiment with the use of multiple, smaller x-ray doses delivered over extended periods. By that time, there was already a radiobiological precedent for expecting improvement in tumor control when radiation treatments were protracted.

As early as 1906, Bergonié and Tribondeau observed histologically that the immature, dividing cells of the rat testis showed evidence of damage at lower radiation doses than the mature, nondividing cells of the stroma.[13] Based on these observations, they put forth some basic "laws" stating that x-rays were more effective on cells that were (1) actively dividing, (2) likely to continue to divide indefinitely, and (3) undifferentiated.[13] Since tumors were already known to contain cells that were not only less differentiated but also exhibited greater mitotic activity, they reasoned that several radiation exposures might preferentially kill these tumor cells but not their slowly proliferating, differentiated counterparts in the surrounding normal tissues.

The end of common usage of the massive dose technique in favor of fractionated treatment came during the 1920s as a consequence of the pioneering experiments of Claude Regaud.[14] Using the testes of the rabbit as a model tumor system (since the rapid and unlimited proliferation of spermatogenic cells simulated to some extent the pattern of cell proliferation in malignant tumors), Regaud showed that only through the use of multiple, smaller radiation doses could animals be completely sterilized without producing severe injury to the scrotum.[15] Regaud suggested that the superior results afforded the multifraction irradiation scheme were related to alternating periods of relative radioresistance and sensitivity in the rapidly proliferating germ cells.[16] These principles

were soon tested in the clinic by Henri Coutard, who first used fractionated radiotherapy for the treatment of head and neck cancers, with spectacularly improved results, comparatively speaking.[17,18] Largely as a result of these and related experiments, fractionated treatment subsequently became the standard form of radiation therapy.

Time-dose equivalents for skin erythema published by Reisner,[19] Quimby and MacComb,[20] and others[21,22] formed the basis for the calculation of equivalents for other tissue and tumor responses. By plotting the total doses required for each of these "equivalents" for a given level of effect in a particular tissue, as a function of a treatment parameter—such as overall treatment time, number of fractions, dose per fraction, and so forth—an isoeffect curve could be derived. All time-dose combinations that fell along such a curve theoretically would produce tissue responses of equal magnitude. Isoeffect curves, relating the total dose to the overall treatment time, derived in later years from some of these data,[23] are shown in Fig. 1.3.

The first published isoeffect curves were produced by Strandqvist in 1944[24] and are also shown in Fig. 1.3. When transformed on log-log coordinates, isoeffect curves for a variety of skin reactions and the cure of skin cancer were drawn as parallel lines, with common slopes of 0.33. These results implied that there would be no therapeutic advantage to using prolonged treatment times (i.e., multiple small fractions versus one, or a few, large doses) for the preferential eradication of tumors while simultaneously sparing normal tissues.[25] It was somewhat ironic that the Strandqvist curves were so popular in the years that followed, when it was already known that the therapeutic ratio did increase (at least to a point) with prolonged, as opposed to very short, overall treatment times. However, the overarching advantage was that these isoeffect curves were quite reliable at predicting skin reactions, which were the dose-limiting factors at that time.

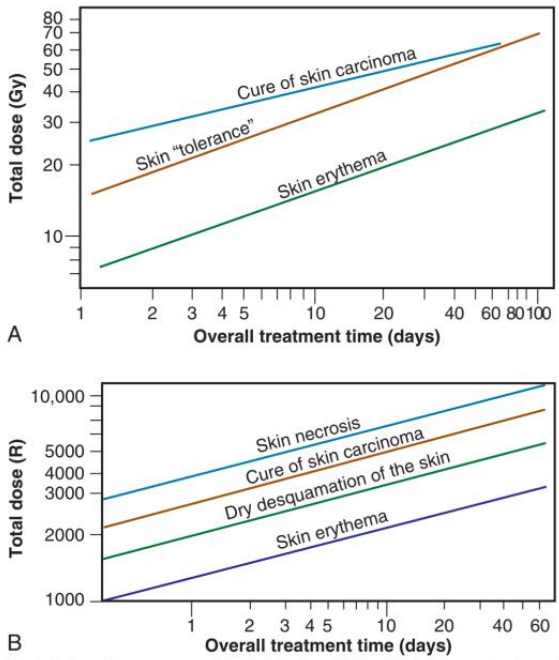

Fig. 1.3 Isoeffect curves relating the log of the total dose to the log of the overall treatment time for various levels of skin reaction, and the cure of skin cancer. (A) Isoeffect curves constructed by Cohen in 1966, based on a survey of earlier published data on radiotherapy "equivalents."[19–22] See text for details. The slope of the curves for skin complications was 0.33 and that for tumor control, 0.22. (B) Strandqvist's isoeffect curves, first published in 1944. All lines were drawn parallel and had a common slope of 0.33. (A, Modified from Cohen L. Radiation response and recovery: Radiobiological principles and their relation to clinical practice. In: Schwartz E, ed. *The Biological Basis of Radiation Therapy.* Philadelphia: J.B. Lippincott; 1966:208; B, modified from Strandqvist M. Studien uber die kumulative Wirkung der Roentgenstrahlen bei Fraktionierung. *Acta Radiol Suppl.* 1944;55:1.)

THE "GOLDEN AGE" OF RADIATION BIOLOGY AND THERAPY: THE SECOND 50 YEARS

Perhaps the defining event that ushered in the golden age of radiation biology was the publication of the first survival curve for mammalian cells exposed to graded doses of x-rays. This first report of a quantitative measure of intrinsic radiosensitivity of a human cell line (HeLa, derived from a cervical carcinoma[26]) was published by Puck and Marcus in 1956.[27] In order to put this seminal work in the proper perspective, it is first necessary to review the physicochemical basis for why ionizing radiation is toxic to biological materials.

The Interaction of Ionizing Radiation With Biological Materials

As mentioned in the introductory section of this chapter, ionizing radiation deposits energy as it traverses the absorbing medium through which it passes. The most important feature of the interaction of ionizing radiation with biological materials is the random and discrete nature of the energy deposition. Energy is deposited in increasingly energetic packets referred to as *spurs* (≤100 eV deposited), *blobs* (100–500 eV), or *short tracks* (500–5000 eV), each of which can leave from approximately three to several dozen ionized atoms in its wake. This is illustrated in Fig. 1.4, along with a segment of (interphase) chromatin shown to scale. The frequency distribution and density of the different types of energy deposition events along the track of the incident photon or particle are measures of the radiation's linear energy transfer (LET; see also the "Relative Biological Effectiveness" section to come). Because these energy deposition events are discrete, it follows that while the average energy deposited in a macroscopic volume of biological material is small, the distribution of this energy on a microscopic scale may be quite large. This explains why ionizing radiation is so efficient at producing biological damage; the total amount of energy deposited in a 70-kg human that

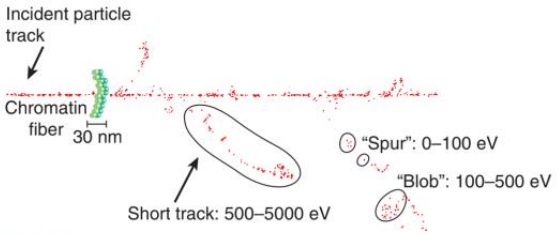

Fig. 1.4 Hypothetical α-particle track through an absorbing medium, illustrating the random and discrete energy deposition "events" along the track. Each event can be classified according to the amount of energy deposited locally, which, in turn, determines how many ionized atoms will be produced. A segment of chromatin is also shown, approximately to scale. (Modified from Goodhead DT. Physics of radiation action: microscopic features that determine biological consequences. In: Hagen U, Harder D, Jung H, et al., eds. *Radiation Research 1895-1995, Proceedings of the 10th International Congress of Radiation Research.* Volume 2: Congress Lectures. Wurzburg: Universitatsdruckerei H. Sturtz AG; 1995:43–48.)

will result in a 50% probability of death is only about 70 calories, about as much energy as is absorbed by drinking one sip of hot coffee.[28] The key difference is that the energy contained in the sip of coffee is uniformly distributed, not random and discrete.

Those biomolecules receiving a direct hit from a spur or blob receive, relatively speaking, a huge radiation dose, that is, a large energy deposition in a very small volume. For photons and charged particles, this energy deposition results in the ejection of orbital electrons from atoms, causing the target molecule to be converted first into an ion pair and then into a free radical. Further, the ejected electrons—themselves energetic charged particles—can go on to produce additional ionizations. For uncharged particles such as neutrons, the interaction is between the incident particles and the nuclei of the atoms in the absorbing medium, causing the ejection of recoil protons (charged) and lower-energy neutrons. The cycle of ionization, free radical production, and release of secondary charged particles continues until all of the energy of the incident photon or particle is expended. These interactions are complete within a picosecond after the initial energy transfer. After that time, the chemical reactions of the resulting free radicals predominate the radiation response (see later discussion).

Any and all cellular molecules are potential targets for the localized energy deposition events that occur in spurs, blobs, or short tracks. Whether the ionization of a particular biomolecule results in a measurable biological effect depends on a number of factors, including how probable a target the molecule represents from the point of view of the ionizing particle, how important the molecule is to the continued health of the cell, how many copies of the molecule are normally present in the cell and to what extent the cell can react to the loss of working copies, how important the cell is to the structure or function of its corresponding tissue or organ, and so on. DNA, for example, is obviously an important cellular macromolecule, and one that is present only as a single, double-stranded copy. On the other hand, other molecules in the cell may be less crucial to survival, yet are much more abundant than DNA and, therefore, have a much higher probability of being hit and ionized. By far, the most abundant molecule in the cell is water, comprising at least 70% to 80% of the cell on a per weight basis. The highly reactive free radicals formed by the radiolysis of water are capable of augmenting the DNA damage resulting from direct energy absorption by migrating to the DNA and damaging it indirectly. This mechanism is referred to as *indirect radiation action* to distinguish it from the aforementioned *direct radiation action*.[29] The direct and indirect action pathways for ionizing radiation are illustrated below.

Direct Effect

$$\mathrm{DNA} \xrightarrow[\text{(irradiate)}]{} [\mathrm{DNA}^{+} + \mathrm{e}^{-}] \rightarrow \underset{\text{(ion pair)}}{} \mathrm{DNA}^{\bullet}_{\text{(DNA free radical)}}$$

Indirect Effect

$$\mathrm{H_2O} \xrightarrow[\text{(irradiate)}]{} [\mathrm{H_2O}^{+} + \mathrm{e}^{-}] \xrightarrow[\text{(other radical reactions)}]{} {}^{\bullet}\mathrm{OH} + \mathrm{DNA} \rightarrow \mathrm{DNA}^{\bullet} + \mathrm{H_2O}$$

(ion pair) (DNA free radical and water)

The most highly reactive and damaging species produced by the radiolysis of water is the hydroxyl radical (˙OH), although other free radical species are also produced in varying yields.[30,31] Cell killing by indirect action constitutes some 70% of the total damage produced in DNA for low LET radiation.

How do the free radicals produced by the direct and indirect action of ionizing radiation go on to cause the myriad lesions that have been identified in irradiated DNA? Since they contain unpaired electrons, free radicals are highly reactive chemically and will undergo multiple reactions in an attempt to either acquire new electrons or rid themselves of remaining unpaired ones. These reactions are considered quite slow compared with the time scale of the initial ionization events but are still fast relative to normal enzymatic processes in a typical mammalian cell. For all intents and purposes, free radical reactions are complete within milliseconds of irradiation. The ˙OH radical is capable of both abstraction of hydrogen atoms from other molecules and addition across carbon-carbon or other double bonds. More complex macromolecules that have been converted to free radicals can undergo a series of transmutations in an attempt to rid themselves of unpaired electrons, many of which result in the breakage of nearby chemical bonds. In the case of DNA, these broken bonds may result in the loss of a base or an entire nucleotide, or a frank scission of the sugar phosphate backbone, involving either one or both DNA strands. In some cases, chemical bonds are broken initially but then rearranged, exchanged, or rejoined in inappropriate ways. Bases in DNA may be modified by the addition of one or more hydroxyl groups (e.g., the base thymine converted to thymine glycol), pyrimidines may become dimerized, and/or the DNA may become cross-linked to itself or to associated proteins. Again, because the initial energy deposition events are discrete, the free radicals produced also are clustered and, therefore, undergo their multiple chemical reactions and produce multiple damages in a highly localized area. This has been termed the *locally multiply damaged site*[32] or *cluster*[33] hypothesis. Examples of the types of damage found in irradiated DNA are shown in Fig. 1.5.

Biochemical Repair of DNA Damage

DNA is unique insofar as it is the only cellular macromolecule with its own repair system. Until as recently as 35 years ago, little was known about DNA repair processes in mammalian cells, particularly because of the complexities involved and the relative lack of spontaneously occurring mutants defective in genes involved with DNA repair. As a consequence, most studies of DNA repair were carried out either in bacteria or yeasts and usually employed UV radiation as the tool for producing DNA damage. Although these were rather simple and relatively clean systems in which to study DNA repair, their relevance to mammalian repair systems and to the broader spectrum of DNA damage produced by ionizing radiation ultimately limited their usefulness.

The study of DNA repair in mammalian cells received a significant boost during the late 1960s with publications by Cleaver[34,35] that identified the molecular defect responsible for the human disease xeroderma pigmentosum (XP). Patients with XP are exquisitely sensitive to sunlight and highly (skin) cancer prone. Cleaver showed that cells derived from such patients were likewise sensitive to UV radiation and defective in the nucleotide excision repair pathway (see later discussion). These cells were not especially sensitive to ionizing radiation, however. Several years later, Taylor et al.[36] reported that cells derived from patients with a second cancer-proneness disorder called ataxia telangiectasia (AT) were extremely sensitive to ionizing radiation and radiation-mimetic drugs, but not UV. In the years that followed, cell cultures derived from patients with these two conditions were used to help elucidate the complicated processes of DNA repair in mammalian cells. Today, dozens of other clinical syndromes associated with radiosensitivity, cancer proneness, or both have been identified.[37,38]

Today, many rodent and human genes involved in DNA repair have been cloned and extensively characterized.[39] Some 30 to 40 proteins participate in excision repair of base damage; about half that many are involved in the repair of strand breaks.[37] Many of these proteins function as component parts of larger repair complexes. Some are interchangeable and participate in other DNA repair and replication pathways as well. It is also noteworthy that some are not involved with the repair process per se, but rather link DNA repair to other cellular functions, including transcription, cell cycle arrest, chromatin remodeling, and apoptosis.[40]

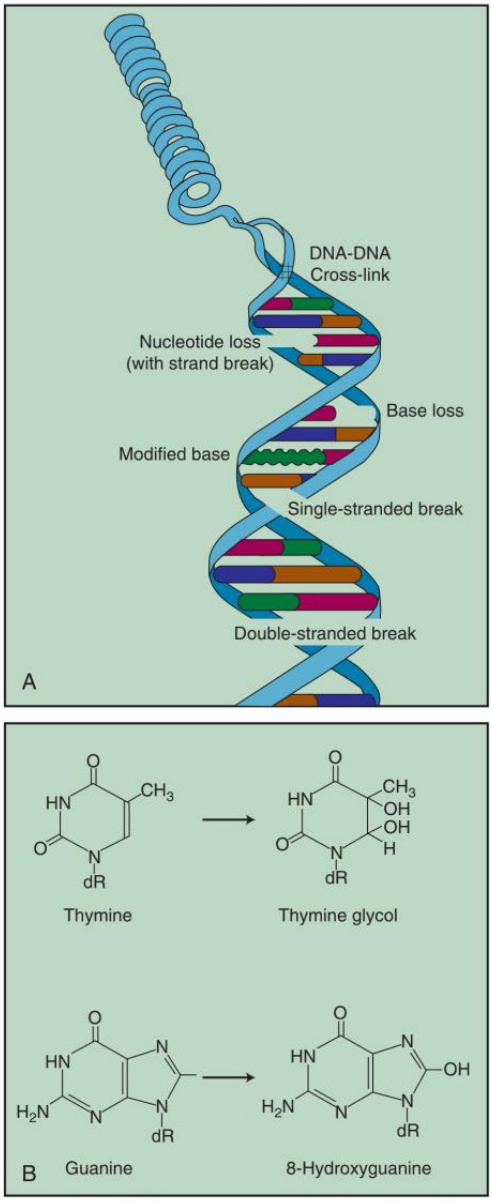

Fig. 1.5 Types of DNA damage produced by ionizing radiation. (A) Segment of irradiated DNA containing single- and double-stranded breaks, cross-links, and base damage. (B) Two types of modified bases observed in irradiated DNA include thymine glycol, which results from the addition of two hydroxyl (OH) groups across the carbon-carbon double bond of thymine, and 8-hydroxyguanine, produced by •OH radical addition to guanine.

This attests to the fact that the maintenance of genomic integrity results from a complex interplay between not only the repair proteins themselves but also others that serve as damage sensors, signaling mediators and transducers, and effectors. Collectively, this complex network of proteins that sense, initiate, and coordinate DNA damage signaling and repair with other cellular activities is termed the *DNA Damage Response* (DDR).[37,41] For example, the defect responsible for the disease AT is not in a gene that codes for a repair protein but rather in a gene that acts in part as a damage sensor and signal transducer but also participates in a related pathway that normally prevents cells from entering S phase and beginning DNA synthesis while residual DNA damage is present. This is termed the G_1 cell cycle checkpoint response.[42] Because of this genetic defect, AT cells do not experience the normal G_1 arrest after irradiation and enter S phase with residual DNA damage. This accounts both for the exquisite radiosensitivity of AT cells and the resulting genomic instability that can lead to cancer.

The molecular and biochemical intricacies of DNA repair in mammalian cells are described in detail in Chapter 2. A brief overview is also presented next.

Base Excision Repair

The repair of base damage is initiated by DNA repair enzymes called *glycosylases*, which recognize specific types of damaged bases and excise them without otherwise disturbing the DNA strand.[43] The action of the glycosylase results in the formation of another type of damage observed in irradiated DNA—an apurinic or apyrimidinic (AP) site. The AP site is then recognized by another repair enzyme, an endonuclease that nicks the DNA adjacent to the lesion, in effect creating a DNA single-stranded break. This break then becomes the substrate for an exonuclease, which removes the abasic site, along with a few additional bases. The small gap that results is patched by DNA polymerase using the opposite, hopefully undamaged, DNA strand as a template. Finally, DNA ligase seals the patch in place.

Nucleotide Excision Repair

The DNA glycosylases that begin the process of base excision repair do not recognize all known forms of base damage, however, particularly bulky or complex lesions.[43] In such cases, another group of enzymes, termed *structure-specific endonucleases*, initiate the excision repair process. These repair proteins do not recognize the specific lesion but rather the structural distortions in DNA that necessarily accompany a complex base lesion. The structure-specific endonucleases incise the affected DNA strand on both sides of the lesion, releasing an oligonucleotide fragment made up of the damage site and several bases on either side of it. After this step, the remainder of the nucleotide excision repair process is similar to that of base excision repair. The gap is then filled by DNA polymerase and sealed by DNA ligase.

For both types of excision repair, active genes in the process of transcription are repaired preferentially and more quickly. This has been termed *transcription-coupled repair*.[44]

Single-Strand Break Repair

Single-strand breaks (SSBs) in the DNA backbone are common lesions, produced in the tens of thousands per cell per day as part of normal metabolism and respiration[45] on top of any additional breaks introduced by radiation exposure. These are repaired using the machinery of excision repair, that is, gap filling by DNA polymerase and sealing by DNA ligase.

Double-Strand Break Repair

Despite the fact that unrepaired or misrejoined double-strand breaks (DSBs) often have the most catastrophic consequences for the cell in terms of loss of reproductive integrity,[46] how mammalian cells repair these lesions has been more difficult to elucidate than how they repair base damage. Much of what was originally discovered about these repair processes is derived from studies of x-ray-sensitive rodent cells that were later discovered to harbor specific defects in strand break repair.[47] Since then, dozens of other rodent and human cells characterized

by DDR defects have been identified and are also used to help probe these fundamental processes.

With respect to the repair of DSBs, the situation is more complicated in that the damage on each strand of DNA may be different and, therefore, no intact template would be available to guide the repair process. Under these circumstances, cells must rely on a somewhat error-prone process that rejoins the break(s) regardless of the loss of intervening base pairs for which there is no template (nonhomologous end joining [NHEJ]) or depend on genetic recombination in which a template for presumably error-free repair is obtained from recently replicated DNA of a sister chromatid (homologous recombination [HR][48]) to cope with the damage. NHEJ occurs throughout the cell cycle, but predominates in cells that have not yet replicated their DNA, that is, cells in the G_1 or G_0 phases of the cell cycle. NHEJ involves a heterodimeric enzyme complex consisting of the proteins Ku-70 and Ku-80, the catalytic subunit of DNA protein kinase (DNA-PK_{CS}), and DNA ligase IV. Cells that have already replicated most or all of their DNA—in the late S or G_2 phases of the cell cycle—depend on HR to repair DSBs. HR involves the assembly of a nucleoprotein filament that contains, among others, the proteins Rad51 and Rad52. This filament then invades the homologous DNA sequence of a sister chromatid, which becomes the template for repair. The BRCA2 protein is also implicated in HR as it interacts with the Rad51 protein.[38] Defects in either the *BRCA1* (which helps determine which DSB repair pathway will be used in a particular situation) or *BRCA2* genes are associated with hereditary breast and ovarian cancer.[49]

Mismatch Repair

The primary role of mismatch repair (MMR) is to eliminate from newly synthesized DNA errors such as base/base mismatches and insertion/deletion loops caused by DNA polymerase.[50] This process consists of three steps: mismatch recognition and assembly of the repair complex, degradation of the error-containing strand, and repair synthesis. In humans, MMR involves at least five proteins, including hMSH2 and hMLH1, as well as other members of the DNA repair and replication machinery.

Radiation-induced DNA lesions are not targets for mismatch repair per se. However, one manifestation of a defect in mismatch repair is germane to any study of oncogenesis: genomic instability,[51] which renders affected cells hypermutable. This "mutator phenotype" is associated with several cancer predisposition syndromes, in particular, hereditary nonpolyposis colon cancer (HNPCC, a.k.a. Lynch syndrome).[52,53] Genomic instability is considered one of the main enablers of normal cells to accumulate cancer-causing mutations and also drives tumor progression to more aggressive and potentially treatment-resistant phenotypes.

The DDR as a Clinical Target

Historically, attempts to inhibit the repair of radiation-induced DNA damage were of interest to researchers probing these fundamental processes. However, clinical translation was typically lacking, mostly out of concern that normal tissues would also be affected in an adverse way. More recently, it has become clear that the cells of many tumors harbor one or more defects in the DDR (as a consequence of genomic instability) that are not present in normal cells and that this difference might be exploitable clinically.

One approach along these lines is the use of inhibitors of the protein poly(ADP-ribose) polymerase (PARP).[54,55] As of 2018, dozens of trials were underway using PARP inhibitors in combination with chemo- and immunotherapies.[56,57] PARP is a damage sensor involved in both base excision and SSB repair that, if inhibited, leads to the persistence of SSBs. If left unrejoined, these breaks can cause the collapse of replication forks in DNA that then impede DNA replication, transcription, and HR repair,[55] leading to radiosensitization and, ultimately, cell death.[58]

In normal cells, little or no toxicity caused by PARP inhibition would be expected, as all DDR pathways are intact and salvage repair pathways to bypass PARP inhibition are active. In tumor cells already harboring defects in HR, however, PARP inhibition would be preferentially toxic. One clinical example is the targeting of breast cancers harboring cellular defects in the BRCA1/2 proteins—which either orchestrate or are directly involved in HR—for PARP inhibition. This overall approach of using the combined lethal effect of two genetic defects (one inherent HR defect plus one synthetic one induced by PARP inhibition) that are otherwise nonlethal singly is termed *synthetic lethality*.[54,55,58] Synthetic lethality approaches targeting DDR proteins (including those other than PARP) likely will play increasingly important roles in the future.

Cytogenetic Effects of Ionizing Radiation

When cells divide following radiation exposure, chromosomes frequently contain visible structural aberrations that are the result of any unrepaired or misrejoined DNA damage that persists from the time of irradiation. Most chromosome aberrations are lethal to the cell. In some cases, these aberrations physically interfere with the processes of mitosis and cytokinesis, resulting in prompt cell death. In other cases, cell division can occur but the loss or uneven distribution of genetic material between the cell's progeny is ultimately lethal as well, although the affected cells may linger for several days before they die, with some even be able to go through a few more cell divisions in the interim.

Most chromosome aberrations result from an interaction between two damage sites; therefore, they can be grouped into three different types of "exchange" categories. A fourth category is reserved for those chromosome aberrations that are thought to result from a single damage site.[59] These categories are described here; representative types of aberrations from each category are shown in Fig. 1.6:

1. Intra-arm Exchanges: An interaction between lesions on the same arm of a single chromosome (example: interstitial deletion).
2. Inter-arm Exchanges: An interaction between lesions on opposite arms of the same chromosome (example: centric ring).
3. Interchanges: An interaction between lesions on different chromosomes (example: dicentric).
4. "Single Hit" Breaks: The complete severance of part of one arm of a single chromosome not obviously associated with any more than a single lesion (example: terminal deletion).

These four categories can be further subdivided according to whether the initial radiation damage occurred before or after the DNA is replicated (a chromosome- vs. chromatid-type aberration, respectively) and, for the three exchange categories, whether the lesion interaction is symmetrical or asymmetrical. Asymmetrical exchanges always lead to the formation of acentric fragments that are usually lost in subsequent cell divisions and, therefore, are nearly always fatal to the cell. These fragments may be retained transiently in the cell's progeny as extranuclear chromatin bodies called *micronuclei*. Symmetrical exchanges are more insidious in that they do not lead to the formation of acentric fragments and the accompanying loss of genetic material at the next cell division; thus, they do not always kill the cell. As such, they will be transmitted to all progeny of the original cell. Some types of symmetrical exchanges (e.g., a reciprocal translocation) have been implicated in radiation carcinogenesis insofar as they have the net effect of either bringing new combinations of genes together or separating preexisting groups of genes.[28] Depending on where in the genome the translocation takes place, genes normally active could be turned off or vice versa, potentially with adverse consequences.

Quantitation of the types and frequencies of chromosome aberrations in irradiated cells can be used to probe dose-response relationships for ionizing radiation and, to a first approximation, also can serve as a radiation dosimeter. For example, the dose-response curve for the

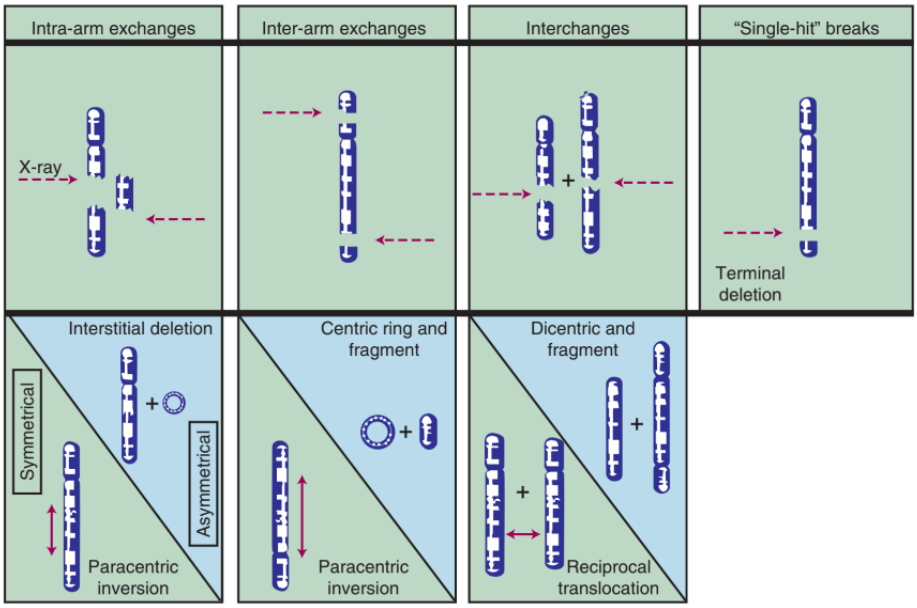

Fig. 1.6 Types of radiation-induced chromosome aberrations that are the result of unrepaired or misrejoined DNA damage. Aberrations are classified according to whether they involve a single or multiple chromosomes, whether the damage is thought to be caused by the passage of a single charged particle track ("one-hit" aberration), or by the interaction of damages produced by two different tracks ("two-hit" aberration), and whether the irradiation occurred prior to or after the chromosomes had replicated (chromosome- vs. chromatid-type aberrations, respectively; only chromosome-type aberrations are shown). The aberrations can be further subdivided according to whether broken pieces of the chromosome rearrange themselves symmetrically (with no net loss of genetic material) or asymmetrically (acentric fragments produced).

induction of exchange-type aberrations after exposure to low-LET radiation tends to be linear-quadratic in shape, whereas that for single-hit aberrations tends to be linear. In mathematical terms, the incidence, I, of a particular aberration as a function of radiation dose, D, can be expressed as

$$I = \alpha D + \beta D^2 + c \quad \text{for exchange-type aberrations}$$
$$I = \alpha D + c \quad \text{for single-hit aberrations,}$$

where α and β are proportionality constants related to the yields of the particular type of aberration and c is the spontaneous frequency of that aberration in unirradiated cells. For fractionated doses or continuous low dose rates of low-LET radiation, the yield of exchange-type aberrations decreases relative to that for acute doses, and the dose-response curve becomes more linear. For high-LET radiations, dose-response curves become steeper (higher aberration yields per unit dose) and more linear compared with those for low-LET radiations.

Cell Survival Curves and Survival Curve Theory

What Is Meant by "Cell Death"?

The traditional definition of death as a permanent, irreversible cessation of vital functions is not the same as what constitutes "death" to the radiation biologist or oncologist. For proliferating cells—including those maintained in vitro, the stem cells of normal tissues, and tumor clonogens—cell death in the radiobiological sense refers to a loss of reproductive integrity, that is, an inability to sustain proliferation indefinitely. This type of "reproductive" or "clonogenic" death does not preclude the possibility that a cell may remain physically intact, metabolically active, and continue its tissue-specific functions for some time after irradiation.[60]

Compared with nearly 65 years ago, when the term *clonogenic death* was first coined and used as an endpoint in assays of cellular radiosensitivity,[27,61] by today's standards it is clearly an operationally defined term that encompasses several distinct mechanisms by which cells die, all of which result in a cell losing its ability to divide indefinitely. These modes of cell death include mitotic catastrophe, apoptosis, necrosis, senescence, and autophagy. Strictly speaking, differentiation is included as well, because differentiated cells lose their ability to divide.[62,63]

Mitotic catastrophe is the major mode of radiation-induced death for most mammalian cells, occurring secondary to chromosome aberrations and/or spindle defects that interfere with the cell division process.[64,65] Accordingly, this type of cell death occurs during or soon after an attempted cell division postirradiation (although not necessarily during the very first division attempt), leaving in its wake large, flattened, and multinucleated cells that are typically aneuploid. Apoptosis, or programmed cell death, is a type of nonmitotic or interphase death commonly associated with embryonic development and normal tissue remodeling and homeostasis.[66] However, certain normal tissue and tumor cells also undergo apoptosis following irradiation, including normal cells of hematopoietic or lymphoid origin, crypt cells of the small intestine, salivary gland cells, plus a few tumor cell lines of gynecological and hematological origin.[67] Cells undergoing apoptosis exhibit a number of characteristic morphological (nuclear condensation and fragmentation, membrane blebbing, etc.) and biochemical (DNA degradation) changes that culminate in the fragmentation of the cell, typically within 12 to