Chapter 3
Radiobiology for Radiation Protection

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3.1 Radiation Interactions and Production of Free Radicals

One of the most serious effects of radiation is that it induces changes in DNA molecules [1, 2]. Radiation can cause DNA damage either directly or indirectly; see Fig. 3.1. In the direct interaction, photons or charged particles directly interact with the DNA to create an ionization event. This ionization primarily targets hydrogen molecules, which leads to a loss of hydrogen in the DNA strand. This creates a free-radical site in the DNA. Free radicals are extremely reactive; they have high tendency for interacting with the surrounding molecules. When a free-radical site in DNA interacts with a molecule that can donate a hydrogen molecule, the DNA will return to its original, correct form. However, with oxygen present, the risk is high that an oxygen molecule will interact with the free-radical site, and this creates a single-strand break (SSB) in the DNA. Oxygen is the most potent molecule for DNA damage, but other molecules, e.g., proteins, can also interact with the free-radical site and cause DNA damage.

Water is the most abundant molecule inside cells. Radiation ionizes water molecules and thereby produces free radicals. The process is called radiolysis of water. In this way, radiation can indirectly cause DNA damage. Radiolysis of water occurs as follows: first, the water molecule is ionized; then, it is split into ionized hydrogen and a free-radical hydroxyl. Next, the ionized hydrogen can interact with a free electron, and this creates hydrogen, which is another free radical. These two free radicals (the hydroxyl and hydrogen) will diffuse into the surrounding media until they react with a molecule. Thus, several different secondary products may be created,

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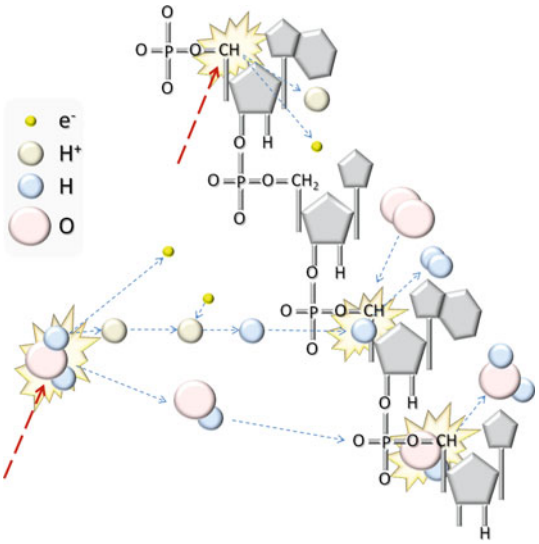

Fig. 3.1 Schematic illustration of the direct and indirect action. The *red arrow* at the top of the figure illustrates the direct interaction with the DNA molecule. The other *red arrow* illustrates the indirect interaction with the water molecule and the subsequent radiolysis

which are more or less harmful to DNA. When the induced free radicals diffuse into DNA, dehydrogenation occurs, and, as described above, a SSB may result.

The induction of SSBs depends on the oxygen level and ionization density. High oxygen pressure in tissues will increase the risk of DNA damage, either by direct or indirect action. This relationship between the oxygen level and DNA damage is not linear; however, in normal tissues, the oxygen level is nearly saturated; thus, small changes in the normal tissue oxygen pressure will cause only minor differences in DNA damage. In contrast, when the oxygen pressure is very low, small changes will cause marked changes in the number of DNA breaks. This situation occurs seldom in normal tissues, but it is frequent in tumors with poor vascularization; thus, tumors have abundant regions of poorly oxygenated cells that are less sensitive to radiation, due to the low oxygen pressure.

3.2 DNA Damage and Repair

Radiation-induced DNA damage primarily manifests as alterations in the nucleotide bases, base dimerization, base loss, hydrogen bond breaks, SSBs, and double-strand breaks (DSBs) [1, 2]. Late cellular effects are mainly caused by DSBs or more complex DNA breaks. A DSB can be caused directly by ionization events induced by a charged particle, or it can be created by two closely induced SSBs. The cell's DNA repair system can readily repair SSBs. This system comprises a panorama of enzymes. First, they recognize the damaged site; then, they unwind the DNA helix to

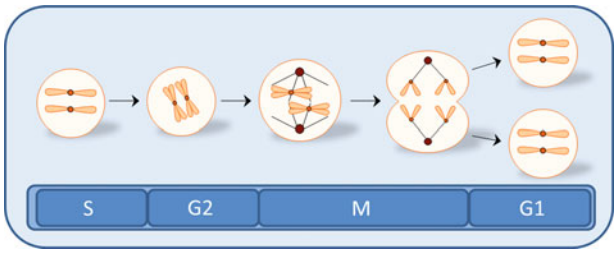

Fig. 3.2 Schematic illustration of the cell cycle ages; G1, S, G2, and mitosis (M). In the mitosis, it is demonstrated how the mitotic spindle fibers is guiding the chromosomes into different sites in the cell

provide access to the separate strands; then, they remove the damaged bases and rebuild the DNA strand, with the opposite strand for a template; finally, the DNA strand is correctly rewound to form the double-helical structure. This is a simple biological process that starts immediately after the SSB occurs, and it is finished within minutes. The repair rate depends on the specific tissue irradiated and the abundance of SSBs that are created per time unit. From a radiation protection perspective, it is worth noting that it might be more risky to receive a large dose delivered at once than to receive the same cumulative dose delivered over a long period of time. This is because, when SSBs are not repaired before a new SSB is created nearby, a DSB might form. This is important, because DSBs are much more complicated to restore; moreover, there is a risk that the DSB repair process might generate a mutation or deletion, which might later induce cancer.

There are two main repair processes for DSBs, *homologous recombination repair* (HRR), which acts after DNA duplication, and *nonhomologous end joining* (NHEJ), which acts throughout the cell cycle.

The different phases of the cell cycle are often referenced when describing cell age; see Fig. 3.2. Cell division is known as mitosis. In mitosis, the cell rearranges its components and divides into two cells. First, each chromosome pair is held together at the centromere, a molecular structure that binds sister chromosomes together while they are moved to the center of the cell. Then, chromosome pairs are separated by mitotic spindle fibers that guide one of each chromosome pair into different sides of the cell during division. After mitosis, the two daughter cells enter the cell cycle at the G1 (growth) phase in preparation for the S (DNA synthesis) phase. In the S phase, the single parent DNA is duplicated. Then, the cell enters the G2 phase to prepare for another round of mitosis. A nonproliferating cell is said to be in a G0 (resting) phase. From this phase, the cell can enter into the proliferating (G1) state when necessary. The HRR process acts when the cell has replicated its DNA. The HRR process uses the sister chromatin as a template, and DNA repair is highly accurate. The NHEJ process acts during all the other cell cycle stages and does not use the sister chromatin. For this reason, the NHEJ process is prone to making mistakes, which results in an incorrect DNA sequence.

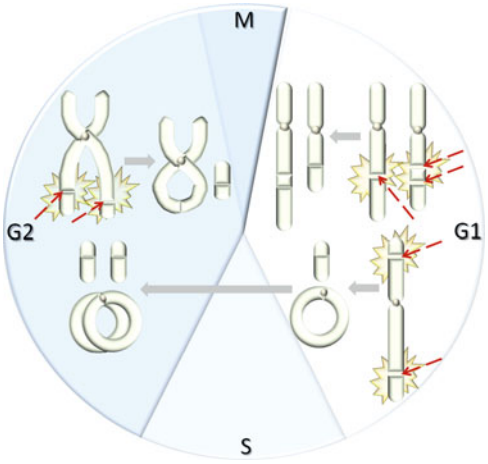

Fig. 3.3 Schematic illustration of some radiation-induced chromosome aberrations. The figure also illustrates the cell cycle ages: G1, S, G2, and mitosis (M). The small spherical structure in the chromosomes indicates the centromere. Acentric fragments are chromatids without centromere, which can be observed in the illustration, as well as ring structures, and chromosome exchanges

3.3 Chromosome Damage

Humans have a total of 46 chromosomes, which can be visualized with a light microscope. DNA damage can be observed with a light microscope only when the DNA is condensed into chromosomes, which occurs only during mitosis. However, radiation damage can sometimes be observed as a change in the chromosome structure. Deviations from the normal chromosome pattern may also indicate which stage the cell had occupied during irradiation. For example, irradiation in G1 can create dicentric chromosomes, acentric fragments, or overlapping rings [1, 2]; see Fig. 3.3.

The dicentric chromosome is generated when radiation breaks the chromosome into two fractions: one fraction contains the centromere, and the other fraction is missing the centromere. Without the intact centromere, DNA may be lost during cell division, due to an incorrect reunion of the chromosomes. A dicentric chromosome is formed when two damaged chromosomes with centromeres are joined. This new chromosome now has now two centromeres, and when it enters S phase, both strands of DNA will be duplicated to form a dicentric chromosome. Acentric fragments are created when chromosome fractions are joined together without the centromere. This will also be duplicated during S phase, and the resulting chromosome contains an acentric fragment. Overlapping rings are created from a single chromosome when radiation induces two breaks in the two arms of the chromosome. In this case, the chromosome fraction with the centromere may form a ring by joining the two arms together at the break site. This ring will be duplicated in S phase to form

overlapping rings. In addition, chromosome fragments that are missing a centromere may be lost if they fail to join to another chromosome. This may represent the loss of a large amount of genetic material that might be essential for cellular control.

The loss of important genes involved in cell proliferation will cause an increase in the risk of sustaining late effects, like cancer. However, when a chromosome aberration is very large, it often leads to cell death during mitosis; the cell tries to divide but is hampered by the aberrant chromosome, and the mitotic spindle fibers are unable to guide the chromosome correctly. This unbalanced cell condition activates a protective cell signaling system that will force cell death. A well-functioning cell signaling system is crucial for assessing cellular damage and determining when to induce cellular repair and when to induce cell death. Thus, cell death can serve as an important defense mechanism for the organism to remove genetically damaged cells, and thus, it reduces the risk of cancer induction.

There are two main mechanisms for cellular death: natural, programmed cell death, called apoptosis, and forced cell death, called necrosis. In apoptosis, the signaling system informs the cell to condense its DNA content and then divide into multiple microspheres. These microspheres can enter the bloodstream and are removed by the liver. This occurs during normal cell turnover; it does not trigger inflammation processes, and the cell loss has a minor impact on the living organism. In contrast, cells that undergo mechanical or respiratory stress can die by necrosis. In necrosis, the cell swells until the cell membrane breaks, and the cellular contents are released into the surrounding tissue. This cellular debris triggers a cytokine reaction, and the organism will mount an inflammatory response.

Recent molecular biology techniques have offered insight into the molecular responses triggered by irradiation. For example, multiplex fluorescence in situ hybridization (M-FISH) allows specific labeling of the chromosomes to enhance visualization. This dramatically increased the ability to detect small chromosome aberrations. When irradiation breaks the chromosome into fragments, these fragments may rejoin correctly, they may be exchanged between chromosomes (chromosome exchanges), or they may be lost (chromosome deletions). The M-FISH technique allows detection of chromosome fragments that are not visible with standard light microscope techniques. In several studies, abundant exchanges between the chromosomes were observed; in some cases, chromosomes accumulated several attached chromosome fragments, and thus, severe aberrations were built into the chromosome. Accordingly, it is important to detect even small changes, deletions, and mutations in chromosomes to be able to accurately assess the damage.

3.4 Late Effects

The latest generation of techniques has enabled the detection of very small genetic aberrations and even radiation activation or inactivation of genes. Cellular studies have demonstrated that several hundreds to thousands of genes can be upregulated and downregulated after radiation exposure. The number and distributions of genes

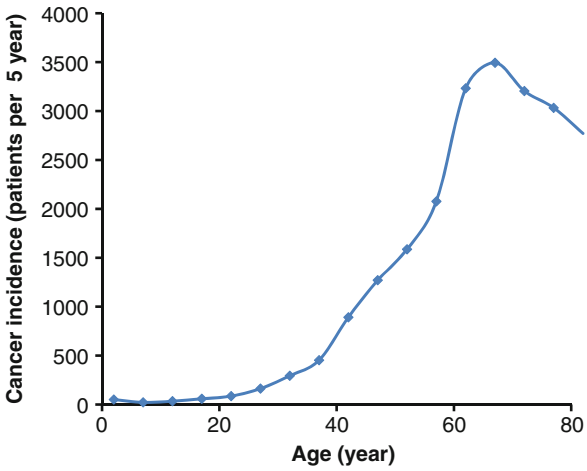

Fig. 3.4 The incidence of female cancers in the Swedish population during the year 2009 as a function of age

up- or downregulated depend on the cell type studied. An alteration in the expression of one gene can affect multiple cell signaling cascades throughout the cell. Because cell signaling is tremendously complex and diverse, alterations in many pathways may activate the signaling pathway that leads to cell death. In normal tissues, induced cell death stimulates the neighboring cells to divide. The new cells fill in the tissue gap left by the removal of dead cells. Thus, normal tissue can preserve its architecture and function. However, with each division, the cells in normal tissues experience a shortening of the telomeres at the end of the chromosomes. This limits the life of a cell, because, when all the telomeres are consumed, the cell can no longer divide. At that point, tissue gaps due to cell death can no longer be replaced by the neighboring cells. Instead, other tissue components, like collagen, will replace the missing cells. This reduces the function and plasticity of the organ, and the organism manifests signs of aging.

The aging process is most active when genetic damage stimulates successive cell divisions. Moreover, multiple genetic aberrations can induce the conversion of a normal cell into a cancerous cell. At first, the cell may retain its normal DNA with accurate repair of the damage. When this fails, the cell must detect the damage and stop cell division before it loses control and becomes a cancerous cell. Cell division can be stopped by cell death or cell senescence. Senescent cells can no longer divide. They retain function, and genetic damage can still be induced, but they no longer have the capacity to multiply. Thus, a senescent cell is not regarded as a cancer cell, and it is not fatal for the organism. When a precancerous cell is allowed to survive, it can accumulate genetic damage, and conversion into a cancerous state increases with time.

Of course, radiation is not the only factor that causes genetic damage, but it contributes to the overall risk for cancer induction. Therefore, radiation exposure should be as low as reasonably achievable whenever possible. For most cancers, the incidence increases with the age of an individual; see Fig. 3.4. This is a reflection of the fact that multiple genetic changes are required for a normal cell to be converted into a cancerous cell. The features that identify a cancerous cell include: resistance

to cell death, sustained proliferation, evasion from growth suppressors, induction of angiogenesis, replicative immortality, the ability to reprogram energy metabolism, evasion from immune destruction, and the ability to invade and metastasize [3].

3.5 Radiosensitivity and Cell Survival Curves

Cell death mechanisms have been extensively studied [1, 2]. In the field of radiobiology, in vitro systems are commonly used, because radiation is easily applied and then cells can be followed in culture for successive periods of time. In these experiments, cells are cultured in conditions similar to the physiological conditions of normal tissues. Depending on cell type, cells may attach to the bottom of the culture dish, or they may float in the medium for cell growth.

In a typical cell survival experiment, the cells are seeded in culture dishes at a cell concentration sufficient to ensure a detectable number of cells at the end of the experiment. The classical quantification of cell survival involves counting cells that are capable of dividing several times to form a cluster of cells. The number of cell divisions depends on the cell line, but normally, 5–6 divisions occur within about 2 weeks. Therefore, when around 100 nonirradiated cells are seeded, after 2 weeks of culturing, spheroid clusters of cells can be noted. The number of clusters is typically less than the number of seeded cells. The plating efficiency is determined as the number of clusters divided by the number of seeded cells, times 100%. The plating efficiency of nonirradiated cells is typically between 50% and 100%.

In a typical experiment, cells are irradiated with a stepwise increase in the absorbed dose, with a maximum absorbed dose of about 10 Gy. The cells are then cultured for a few weeks, and subsequently, the survival rate is determined. The survival curve plots the logarithm of the survival fraction (because the survival fraction is frequently $<1\%$) against the mean absorbed dose. When the first cell survival experiments were performed, it was noted that the resulting survival plots often produced rounded curves. After several attempts, the mathematical model that best fit this phenomenon was the linear quadric model; see Fig. 3.5. The linear quadric model is the most common model used currently, because it is the simplest mathematical model with a reasonable fit to the data from most cell survival experiments. However, it should be noted that it is a simplified model, and caution should be taken when the model is used for extrapolation and interpolation outside the data set. Nevertheless, from a biological standpoint, the model appears to be relevant, even though it may not be exact.

The rounded cell survival curve can be divided into linear and quadric components. The linear component represents the “one target, one hit” model. With the DSB as the target, the hit is the sum of direct and indirect damage to DNA by charged particles that traverse the DNA region of interest. As mentioned above, many DSBs are repaired, and only a fraction of all DSBs lead to cell death. The production of DSBs is assumed to be correlated to the mean absorbed dose, D, and the fraction of these DSBs that lead to cell death is represented by the

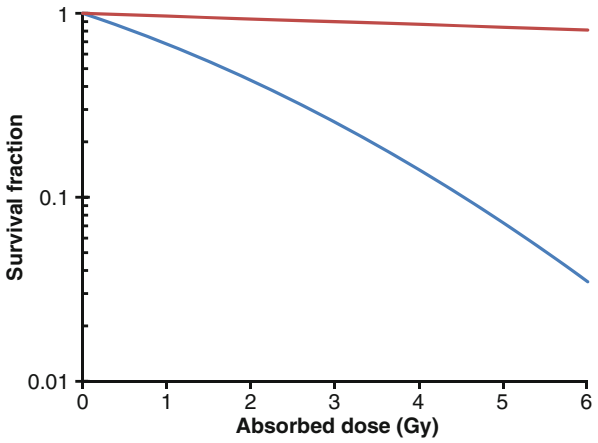

Fig. 3.5 Plot of the linear quadric (LQ) model. The *red line* is a result of the LQ model when the quadric term is missing. The survival fraction is then a pure exponential function with absorbed dose. Inclusion of the quadric term (*blue curve*) will bend the curve more rapidly with increasing absorbed doses

parameter α, which is a measure of the sensitivity of the cells to DSBs. Thus, the equation shows that the survival fraction is equal to an exponential function of absorbed dose times the radiosensitivity parameter. This component forms a straight line on the log-linear survival plot. The quadric component represents a "two hits, one target" model. Here, the target is the DSB, but the damage requires two SSBs. Again, each hit is the sum of direct and indirect damage to DNA by charged particles that traverse the DNA region of interest. In this model, a single SSB will not induce cell death; hence, another SSB must be created close to the former SSB to make a DSB. Because SSBs are rapidly repaired by the cell, the time frame between the two SSBs must be shorter than the time to repair the first SSB. Each radiation hit has a certain probability, β', for creating a SSB. This probability is correlated to the mean absorbed dose of that hit. Thus, the total probability that two hits will interact is the product of the two individual probabilities. Therefore, the survival fraction will be equal to $\beta' \times D'$ times $\beta'' \times D''$, which can be reduced to its more common form, $\beta \times D^2$. The parameter β is the radiosensitivity for inducing two SSBs that cause a lethal DSB. This component will form a curve on the log-linear survival plot. The linear quadric model is a combination of these two phenomena; thus, the survival fraction is a function of the natural logarithm with an exponent of $(-\alpha D - \beta D^2)$.

The radiosensitivity of a cell depends on the apoptotic potential, cell age, repair capacity, oxygen tension, linear energy transfer, dose rate, and absorbed dose [1, 2]. Different cell lines have exhibited widely different radiosensitivities. In particular, cells with hampered apoptotic signaling will be less sensitive to DNA damage, and the cell type will tolerate irradiation well. This type of cell displays a survival curve with a broad shoulder. In contrast, cells with highly active apoptotic signaling will

be sensitive to small damages, and the cell type will readily commit apoptosis. This type of cell displays a survival curve with a small shoulder and a rapid, nearly straight decline in cell survival.

Cell radiosensitivity also varies during different cell cycle phases. To determine the radiosensitivity experimentally, cells must be synchronized in the cell cycle. Distinct G1 and G2 checkpoints allow the cell to control the cellular machinery and decide whether to continue to the next phases. The cell cycle can be stopped experimentally at the checkpoints by adding different chemicals. For example, when a chemical is added to block the cells in G1, all cells will progress through the cell cycle until they reach G1, and then, they will pause at this checkpoint. This synchronizes all the cells to the G1 phase. When the chemical is removed, the cells enter the cycle again. When the cells are irradiated at different time points, the effects on different phases can be clearly distinguished. These types of studies revealed that cells are most sensitive to radiation during mitosis, late G1, G2, and early S phase. Cells in G0 phase are often less sensitive to irradiation. This might explain why nonproliferating cells appeared to be less radiosensitive than proliferating cells.

Radiosensitivity also depends on a cell's capacity for DNA damage repair. The time required for this repair has been measured in split-dose experiments. In those studies, cells were irradiated with two doses, separated by a certain time interval. Often, cells were irradiated at low temperature to block proliferation effects, that is, cell number will not change due to cell division during the experiments. These experiments have shown that all reparable damages were repaired within a few hours; in fact, most DNA damage was repaired during the first hour. The capacity for DNA damage repair has also been tested in dose-rate experiments. There, the same cumulative dose was delivered over different times; for example, 2 Gy over 10 min is delivered four times faster than 2 Gy over 40 min. In those studies, the number of cells killed decreased as the dose rate decreased. With a slow dose rate, all reparable DNA damage could be repaired before the next DNA damage occurred. This resulted in low accumulated DNA damage, and the apoptotic signaling cascade was not triggered.

Cell radiosensitivity increases with increased oxygen pressure [4]. Experiments with different oxygen pressures have shown that non-oxygenated cells were almost 300% less radiosensitive than well-oxygenated cells; see Fig. 3.6. But, as mentioned above, in normal tissues, the oxygen level is often sufficiently high that no large differences in radiosensitivity have been observed due to variable oxygen pressure.

Linear energy transfer (LET) has a pronounced effect on the radiosensitivity of normal tissue. Low ionization, that is, low LET, is produced by gamma and electron irradiation. This radiation causes a shoulder to appear in the cell survival curve. With high-LET radiation, the shoulder seems to disappear, and the slope of the curve is considerably increased. High LET in nuclear medicine arises with alpha decay, e.g., ^{223}Ra, which is used in phase 3 trials for treating disseminated prostate cancer. The ionization around the alpha particle creates LET values around 100 keV/μm. This high-level LET will produce multiple DSBs, which increases the complexity of cellular repair. An LET value of 100 keV/μm was found to be optimal for killing cancerous cells. Higher LET values caused a reduction in the

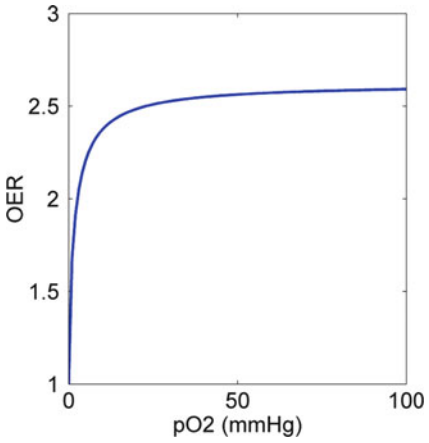

Fig. 3.6 The oxygen enhancement ratio (OER) for cell survival as a function of oxygen pressure (pO_2). The *blue line* is derived from the equation proposed by Kirkpatrick et al. In normal tissue, the pO_2 is around 40 mmHg, while tumor tissue might consist of large regions with low oxygen concentrations, making these tumor cells less radiosensitive

effect per absorbed dose. At an ionization density of 100 keV/μm, the mean distance between ionization events is around 2 nm. This is the distance between the two strands of DNA, and theoretically, maximal DNA damage will occur at this ionization level. Increasing the LET will reduce the distance between ionization events, and the DNA will be overionized, that is, further ionization will not contribute to the existing DSBs. This is consistent with the observation that high-LET radiation is insensitive to the oxygen level. In other words, the main damage to DNA is from a direct hit, and the induced free radicals create an environment that conserves damage without the need for oxygen.

Most cell survival experiments have shown that alpha emitters are five times more potent for killing cells than gamma and electron irradiation. For radiation protection purposes, the corresponding weighting factors are 20 for high- and 1 for low-LET irradiation in their potency for inducing late effects. Thus, the factor for inducing late effects is considerably higher than that for inducing acute effects. The biological reasons behind this difference between LETs are not well characterized. However, high-LET irradiation causes more complex DNA damage. Thus, we can speculate that complex DNA damage may be a more potent inducer of cancer throughout a cell's life than the DNA damage caused by low-LET irradiation.

3.6 Acute Effects

As mentioned above, the induction of cell death is a way for the organism to protect itself from potential cancerous cell growth [1, 2]. With low-dose radiation, this is probably an important step for reducing the risk of cancer induction. However, with

higher absorbed doses, organs may become compromised. With abundant cell loss, organ architecture becomes rearranged, and organ function is reduced. This process progresses with accumulated damage. The first signs of organ damage are fatigue and nausea. This biological response begins at a threshold dose of 500 mGy, which is far above the achieved absorbed doses in routine nuclear medicine practice. Fatigue and nausea occur in response to the loss of cells in the crypts in the intestine, which causes intestinal bleeding. An increase in the absorbed dose to around 3 Gy will cause vomiting and diarrhea. At higher absorbed doses, damage to the intestine might elicit a gastrointestinal syndrome, which leads to death.

When the whole body is exposed to radiation, the bone marrow will set the limit for the maximal tolerated dose. Bone marrow cells are highly radiosensitive; an absorbed dose of 2 Gy or higher will dramatically reduce the number of immune system cells, e.g., granulocytes. After a high bone marrow dose, the individual will become highly sensitive to infections, but with early hospital care, bone marrow density can slowly recover, and it is nearly restored after a few months. With 5 Gy and higher exposures, the bone marrow will be completely damaged, and the only rescue option is bone marrow transplantation. At absorbed doses higher than 12 Gy, the patient will die due to cerebrovascular syndrome. The absorbed doses mentioned here are far beyond those used in nuclear medical practice. Typical absorbed doses are around 1–10 mSv/year, and additional effective doses will not impact cell number; thus, permanent organ damage is uncommon. The few cells that might be lost in protective mechanisms are effectively replaced by the growth of neighboring cells.

3.7 Summary

The human body has a built-in system for protecting against radiation exposure [1, 2]. At low exposures, the biological outcome is highly variable. The large variation in late effects stems from physical, chemical, and biological factors. A yearly effective radiation dose of around 4 mSv per year is caused by a few charged particles to traverse a single cell per year. Poisson statistics on that low number will display large variability in the number of traverses per cell; some cells will receive none at all, and others might receive ten per year. Moreover, each traverse has a variable effect in the number of ionizations that are capable of producing free radicals and direct hits to the genome. This creates a wide variation in the number of SSBs and DSBs created. The repair mechanism for the induced DNA damage also varies in sensitivity between cell types and between individuals. Apoptosis also varies in its capacity for sensing the degree of genetic damage. Taken together, these and other variations involved in transforming a normal cell into a cancerous cell make it unfeasible to accurately predict an individual's risk of developing cancer. Consequently, risk figures refer to population studies. However, increased absorbed doses will result in more complex biological damage, and cancer induction is well documented at high absorbed doses. There is evidence that at exposures to doses of ionizing radiation drawn to 100 mSv, there is an

increased risk for cancer development. In some specific situations like induction of childhood cancer after irradiation of the fetus in the late stage at pregnancy, there are statistical significant increases down to around 10 mSv. According to ICRP, there is a risk of developing a lethal cancer of about 5% per Sv (0.5% per 100 mSv, 0.05% per 10 mSv, etc.) as a mean for the population, with around three times higher risk for newborn and 3–10 times lower for elderly people. In conclusion, it is our responsibility to ensure that the absorbed dose to personal and patients is as low as reasonably achievable.

References

1. Hall EJ, Giaccia A (2011) Radiobiology for the radiologist. Lippincott Williams, Philadelphia, PA. ISBN 10: 1608311937
2. Saha GB (2010) Physics and radiobiology of nuclear medicine. Springer, New York. ISBN 10: 1441921508
3. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144(5):646–674
4. Kirkpatrick JP, Cardenas-Navia LI, Dewhirst MW (2004) Predicting the effect of temporal variations in pO_2 on tumor radiosensitivity. Int J Radiat Oncol Biol Phys 59(3):822–833