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5. RADIOBIOLOGY

Radiobiology is the science of the effects of ionizing radiation (IR) on biological tissue and living organisms. It is a combination of two scientific branches: radiation physics and biology. Ionizing radiation is defined as any type of electromagnetic or particulate radiation with sufficient energy to ionize atoms or molecules. That is, to eject one or more electrons from their outer orbitals. X-rays or γ-rays are two forms of electromagnetic radiation, while particulate radiations are α, β particles (electrons), protons, neutrons or heavy charged ions.

All living organisms consist of water and other inorganic and organic compounds. Water is the primary component of cells and accounts for about 2/3 of body mass. It is the environment of the majority of chemical reactions taking place in the body. Most of the water is in the lymph (95%), blood plasma (90%) and nervous tissue (approximately 90%), the least in the tooth enamel (0.2%). Non-organic compounds (minerals) are most commonly found in the form of water-soluble cations (e. g. K^+, Na^+, Cu^+, Cu^{2+}, Ca^{2+}, Fe^{2+}, Fe^{3+}, Mg^{2+}) and anions (Cl^-, NO_2^-, NO_3^-, SO_4^{2-}, CO_3^{2-}, PO_4^{3-}). Some of these are important elements in building bones and teeth (Ca, Mg, P). Carbon, oxygen, hydrogen and nitrogen comprise the main components of these biomolecules. Organic compounds are present in the form of small molecules (glucose, nucleotides, amino acids, fatty acids), and high-molecular weight macromolecules (nucleic acids, proteins, carbohydrates and lipids).

The cell is the smallest structural and functional unit of an organism capable of carrying out all basic life processes. The three main constituents of a cell are the nucleus, the cytoplasm with its organelles (i. a. mitochondria, endoplasmic reticulum) and the cell membrane (the cytoplasmic or plasma membrane), which is selectively permeable to ions and organic molecules. The nucleus contains the genetic information (DNA), the cytoplasm supports all cellular metabolic functions, and the plasma membrane is responsible for the movement of substances in and out of the cell; it is also involved in many cellular processes, such as cellular adhesion and cell signalling.

Cells use a variety of clearly defined signalling pathways to fulfil and regulate their activity. They respond to all physical and chemical changes in their environment. Intracellular signalling pathways are responsible for transmitting information within the cell. Signal transduction pathways control all vital cellular processes, such as cell growth, differentiation, motility and

metabolism, as well as cell survival and death. Cellular responses are triggered by the recognition of extracellular signals at the cell's surface (usually in the forms of chemical signals, such as hormones and growth factors), which result in the activation of complex cytoplasmic and nuclear biochemical pathways. Many cellular-specific proteins involved in these pathways undergo activation and/or inactivation processes caused by changes in protein phosphorylation and enzymatic activity, localization, or the formation of protein-protein complexes. Signals are received by receptors, specific proteins that function as molecular antennas (sensors) located in the plasma membrane. A receptor is the integral membrane protein capable of binding its specific signal molecules – so-called ligands – with a high affinity. Ligands bind to a specific part of the receptor in a similar manner as substrate binds to an enzyme. Dysregulation of normal signalling pathways can contribute to malignant transformations in human cells, and even cellular death.

5.1. Classification of radiation in radiobiology

When IR traverses through matter, it loses energy progressively through the various interactions along the length of its path. For a particular absorber, the rate of the energy loss depends on the energy, the type of radiation and the density of the material.

The density of energy deposition in tissue is called the Linear Energy Transfer (LET) of radiation. LET is a measure of energy deposition per unit length of the 'track' that the radiation creates as it proceeds through tissue ($-dE/dx$). The unit of measurement of LET is keV/μm. LET basically indicates the quality of different types of radiation and is important because the biological effect of radiation depends on its average LET. Charged particles generally have higher LET than electromagnetic radiation, due to the greater energy deposition along their track. Therefore, ionizing radiations are categorized into low and high LET radiations. X-rays and y-rays are considered low LET radiations because of their sparse ionization, and are usually less damaging than high LET (densely ionizing) radiation, such as neutrons or α-particles.

The main factors determining the effects of IR on living organisms are: dose size and type of radiation, radiation conditions, and the biological properties of the irradiated system. Dose is a measure of the energy transferred by the radiation unit to the absorbent mass (absorber). In radiobiology there are several types of dose units depending on how this energy transmission is described.

The **absorbed dose** is one of the most important dosimetric quantities in radiation protection. It is the radiation energy (Joules) absorbed by unit mass of material (tissues). The (S.I.) unit is known as the gray (Gy). Historically, the unit of measurement was Radiation Absorbed Dose [rad]; 1 Gy = 100 rad.

$$1\text{Gy} = 1\text{ J/kg} = 100\text{ rad}$$

To take into account the biological effects of radiation, radiation weighting factors (W_R) have been introduced (Table 1), which reflect the severity of the biological effects of different types and energies of radiation.

Table 1. Radiation weighting factors

Type and energy of radiation	W_R
Photons (X-rays, γ-rays), electrons (>5 keV)	1
Slow neutrons (<10 keV)	5
Intermediate neutrons (0.1-2 MeV)	20
Fast neutrons (2–20 MeV)	10
Protons (>2 MeV)	5
α-particles, (5 MeV); high energy ions	20

A biologically effective dose, termed **equivalent dose** (H_T) is defined as:

$$H_T\,[\text{Sv}] = \Sigma\, W_R \text{ x } D$$

where D is the absorbed dose averaged over a tissue or organ due to radiation; D is measured in units of grey. The equivalent dose is measured in sieverts (Sv); 1 Sv equals 1 J kg^{-1}. For X-rays and γ-rays, 1Sv equals 1Gy. The earlier unit was the **r**adiation **e**quivalent **m**an [rem]; 1Sv = 100 rem. The equivalent dose is expressed as a sum to include the effects of IR caused by more than one radiation type. This dose is used to compare the biological effectiveness of different kinds of radiation to the tissues.

The biological effect of radiation also depends on the type of tissue (organ) which has been irradiated. To quantify the total damage from the exposure of several tissues (organs), the concept of the **effective dose** (E) was introduced. This is the sum of the weighted equivalent doses in all the tissues (organs) of the human body, and is defined as:

$$E\,[\text{Sv}] = \Sigma\, W_R \text{ x } D \text{ x } W_T$$

where the so-called tissue weighting factor (W_T) reflects the relative contribution of this tissue (organ) to the total damage resulting from uniform irradiation of the whole body. The unit of measurement of the effective dose is Sv. Effective dose is used to estimate the risk of IR to humans.

There is also the concept of the **collective dose**, generally used for protection purposes and in calculations for population response, which is defined as the dose received per person (in sieverts) multiplied by the number of individuals exposed per year.

Table 2. Radiation weighting factors

Tissue (organ)	W_T
gonads (ovary, testis)	0.20
bone marrow, colon, lung, stomach	0.12
bladder, breast, liver, thyroid, oesophagus	0.05
skin, bone	0.01
all others	0.05
Whole body (total)	**1.00**

Radiation conditions differ, but mainly include dose rate, the mass of the irradiated tissues, irradiation of critical organs, and tissue oxygenation. The **dose rate** is the ratio of the absorbed dose to the time it was given (e.g. Gy/h). The influence of other radiation conditions on overall biological effects is discussed in the 'Radiosensitivity' section.

5.2. Mechanisms of radiation damage – direct and indirect radiation effects

When a high-energy photon or particle (e. g. γ-rays, α-particles) hits a human cell it produces a narrow 'track' (less than 1μm thick) as it proceeds through the material. Radiation absorbed by cells has the potential to affect a variety of critical cellular targets (nucleic acids, proteins, lipids, carbohydrates), causing ionisation and excitation. The radiation breaks one or more chemical bonds producing free radicals – atoms and molecules with unpaired electrons. Free radicals can easily react with other molecules leading to the chain of physical and chemical events that eventually produce the biological damage. The radical formation occurs in a matter of picoseconds after the passage of the photons.

The chemical and biological effects of ionizing radiation on living matter are the result of the deposition of radiation energy directly into the target macromolecule, which is a component of the cell, and of the indirect action resulting from the excitation and decay of water molecules – radiolysis of water. It is generally accepted that DNA is the critical radiation target in the cell. Thus, the interaction of radiation directly with DNA molecules is what is called a direct effect, and this is the dominant process in the interaction between high LET radiations with biological material. The interaction of radiation with water molecules, which is the major constituent of the cell, leads to production of free radicals that in turn can attack other critical molecules. This action of radiation through an attack by free radicals is known as the indirect effect. Free radicals are able to diffuse throughout the cell, so the initial ionization event does not even have to occur close to the DNA molecule in order to cause damage. It is

estimated that only approximately 1/3 of biological damage caused by low LET radiations (γ-rays) is caused by direct effects. Importantly, the indirect action of IR can be modified by radiation protectors and by chemical sensitizers. All chemicals (natural or synthetic) that are able to react with radicals and other reactive oxygen species (ROS) can inhibit the indirect action of radiation. These chemicals are called ROS scavengers.

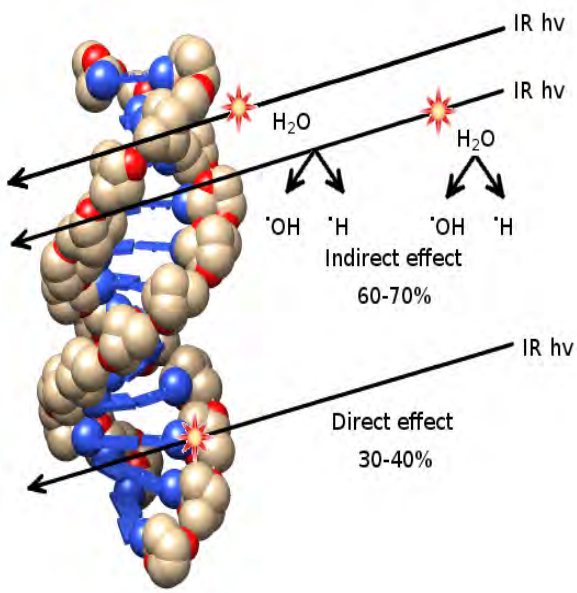

Figure 1. Direct and indirect radiation effects (Author: Michał Ponczek)

Together, the direct and indirect effects of radiation initiate a series of biochemical and molecular signalling events that can repair the damage or culminate in permanent physiological changes or cell death.

5.2.1. Radiolysis of water

Exposure of living matter (cells, tissues) to IR triggers a complex series of chemical reactions in water, which accounts for about 80% of cellular constituents. This process is called water radiolysis and leads to generation of specific radiolytic products. The radiation chemistry of water is a relatively well-known process described in several reviews. Ionization and excitation of water molecules are two basic primary processes resulting from electromagnetic interaction of IR with water. These processes produce short-lived H_2O^+ radical-cations, fast electrons and excited water molecules (H_2O^*). H_2O^+ radical cations and excited water,

which are very unstable, decompose within 10^{-13} s to form hydroxyl radical ($OH^{\cdot}$) and hydrogen radical ($H^{\cdot}$):

$$H_2O \rightarrow H_2O^+ + e^-, H_2O \rightarrow H_2O^*$$
$$H_2O + H_2O^+ \rightarrow + H_3O^+ + OH^{\cdot}$$
$$H_2O^* \rightarrow OH^{\cdot} + H^{\cdot}$$

The hydroxyl radical has an unpaired electron and is a highly reactive oxidizing agent. It can diffuse some distance and react with critical target molecules, producing another radical. The ejected electrons can interact with a water molecule to produce hydroxyl ions and a hydrogen radical (hydrogen atom):

$$e^- + H_2O \rightarrow H_2O^- \rightarrow OH^- + H^{\cdot}$$

Then, the electrons undergo solvation by dielectric interactions with neighbouring water molecules to form solvated (hydrated) electrons (e^-aq). A solvated electron reacts with a proton to give a hydrogen atom ($H^{\cdot}$):

$$e^-_{aq} + H^+ \rightarrow H^{\cdot}$$

The solvated electrons and hydrogen atoms are the strongest known reducing agents at pH 7.0. In oxygenated solutions, a solvated electron can be converted to the superoxide anion ($O_2^{\cdot -}$), which in turn is a powerful oxidizing species and a precursor of hydrogen peroxide (H_2O_2):

$$e^-_{aq} + O_2 \rightarrow O_2^{\cdot -} \text{ (superoxide anion)}$$

The virtual yields of water radiolysis products depend on the LET of the radiation and the pH. The concentration of these radicals is expressed as a G value (G is defined as the number of radicals or molecules produced per 100 eV of energy absorbed in the medium).

To summarise, at physiological pH in the aerobic cellular environment, the major primary ROS includes the superoxide anion, a hydroxyl radical and hydrogen peroxide. The high energy of photons (low LET radiations) absorbed by biological matter, and the passage of fast charged particles (high LET radiations) through the matter, initiate a complex series of events that are responsible for the final radiobiological effects. The timescale of these events, divided into four, subsequent temporal phases, is as follows:

– Physical phase – (duration up to 10^{-16}s) – ionization, energy deposition, excitation and formation of very active secondary electrons;

– Physico-chemical stage – (10^{-15}- 10^{-6}s) – electrons undergo fast reorganization to form primary radicals and other molecular products of water radiolysis;

– Chemical stage (10^{-6}s) – free radicals diffuse, interact with each other and with other molecules in the cellular environment, causing changes in life-critical molecules;

– Biological stage – (from several seconds to several years) – enzymatic reactions, recognition of damage and cellular signalling dysregulations, repair of the damage. This leads to death or mutation at the cellular level, and immune responses at the system level. Hormonal changes, carcinogenesis and even death are all possible final effects of these reactions in a human body.

5.3. The molecular radiobiology

5.3.1. Radiation-induced DNA damage

Ionizing radiation passing through living tissues generates reactive free radicals and other ROS. These ROS can interact with critical macromolecules, such as DNA, proteins and cellular membranes, and can induce cell damage and, potentially, cell dysfunction and death. It is believed that DNA is the most critical radiation target in the cell. The wide range of lesions in DNA that result from ionizing radiation include: (1) chemical alteration of the nitrogen bases or sugar moieties; (2) formation of the apurinic, and to a lesser extent, apyrimidinic sites; (3) single- and double-strand breaks (SSB and DSB) of the double helix molecule, and (4) cross-linking to DNA-related matrix proteins or nucleotides in the DNA molecule itself.

Oxidative modifications to the bases caused by the attack of the hydroxyl radicals have been mostly studied *in vitro* (irradiation of the free bases, nucleotides, DNA in aqueous solutions), and this chemistry is well understood. More than two hundred IR-induced oxidative modifications of the base are known. The most common is the attack of the hydroxyl radical on the bond between the C5 and C6 in the pyrimidines and C4 and C5 in the purines (Fig. 2).

Depending on the local oxygen conditions, the resulting intermediate products may undergo further oxidation or reduction. For example, the most common product of the deoxyguanine oxidation is 8-hydroxyguanine (8-oxoguanine), which is used as one of the biomarkers of oxidative stress in an organism (Fig. 3).

If not repaired, 8-oxoguanine can pair with adenine and cause a G:C to T:A transversion. Insertion of 8-oxoguanine during DNA replication can generate double-strand breaks. Treatment of DNA with the hydroxyl radical generating system can also lead to the formation of the imidazole-opened ring derivative of guanine, such as 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FaPy) as an

abundant lesion. The damage made by IR-induced hydroxyl radicals is similar to that produced by oxidative metabolism. Moreover, the DNA repair system, called a base excision repair (BER), efficiently repairs lethal lesions, so that in the repair-capable cells, isolated base damage may be irrelevant in radiation mutagenesis.

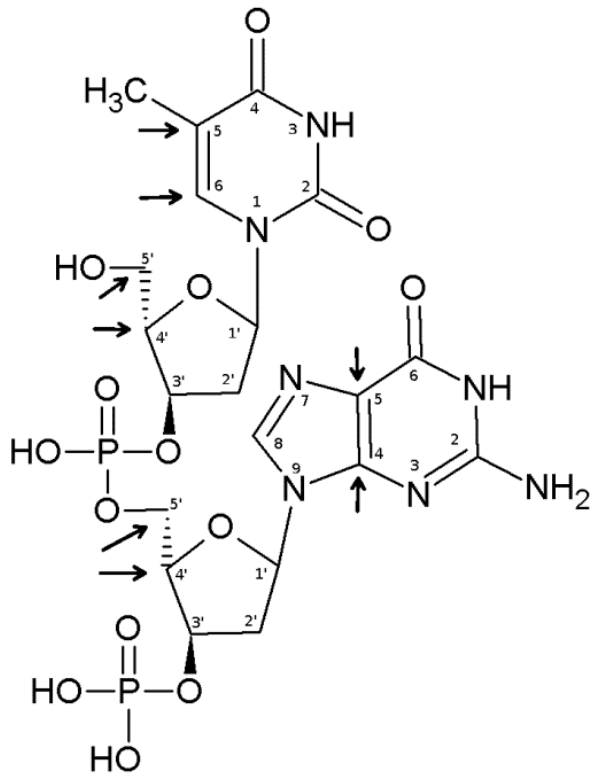

Figure 2. Positions of the hydroxyl radical attack in the DNA molecule (Author: Michał Ponczek)

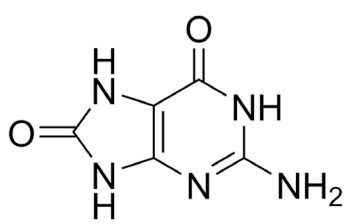

Figure 3. The product of guanine oxidation – 8-oxoguanine

The attack of the hydroxyl radical on the DNA sugar-phosphate backbone leads to the abstraction of the hydrogen atoms from the deoxyribose. As a result

of the hydrogen abstraction and subsequent reactions with oxygen molecules, several types of DNA damage can be formed: the cleavage of the phosphodiester bond and the formation of a single-strand DNA break, destabilization of the N-glycosidic bond and generation of the abasic deoxyribose residue or the opening of the deoxyribose ring, and the formation of a so-called alkali-labile site. Single-strand breaks are typically easily and rapidly repaired (DNA ligation). Double-strand breaks – that is, breaks that arise in both strands opposite to each other (or separated only a by few base pairs) – are less readily repaired and constitute much more critical IR lesions. The DSB that remains unrepaired or mis-repaired can induce chromosomal abnormalities and lead to mutations, genomic instability, and cell death. The aberrant chromosomes occur when broken ends re-join with other broken ends to form translocations, rings, dicentrics and others. Karyotyping, the micronuclei formation assay or fluorescent in-situ hybridization (FISH), can be applied to detect IR-induced unrepaired DNA damage in chromosomes. The majority of mutations do not affect cellular function as they do not alter the meaning of coded genetic information, or because they occur in differentiated cells that do not divide. Mutations in stem cells are harmful, for example haematopoietic stem cells leading to the development of blood cancers, and those that trigger divisions, such as mutations of the proto-oncogens leading to cancer.

The most important feature of radiation toxicity is that IR induces not only isolated DNA lesions, but also clusters of lesions generated within a few tens of base pairs. The clustered DNA damage sites can include DSB and tandem lesions (DSB or SSB associated with modification of the bases, or much more complex lesions, such as multiple closely scattered DSB). Claster damage can arise from the combined indirect action of ROS, which are instantaneously produced in a very high yield during irradiation, and from direct effects induced by the track of the radiation. These processes are not yet well understood.

The broad variety of DNA lesion forms requires multiple, largely distinct DNA repair mechanisms. The majority of the DNA damage is repaired by a sequence of reactions involving specific enzymes mediated by multiple proteins. These typically include: the base excision repair (BER); nucleotide excision repair (NER); mismatch repair (MMR); repair through homological recombination (HR), and non-homologous end joining (NHEJ).

5.3.2. Radiation-induced oxidative damage to proteins and lipids

Protein oxidative modifications

Free amino acids and amino acid residues in proteins are highly susceptible to oxidation by ROS generated during exposure to IR. Exposure of proteins to these ROS can alter the physical and chemical structure of the target,

causing consequent oxidation of side-chain groups, protein scission, backbone fragmentation, cross-linking, unfolding, and/or formation of new reactive groups. The latter include oxidation of hydrophobic amino acyl residues to hydroxy and hydroperoxy derivatives, protein carbonylation, oxidation of -SH groups, dityrosine formation, and many others.

The hydroxyl radical generated during water radiolysis initiates the oxidation of the polypeptide backbone by abstraction of the hydrogen atom at the amino acid α-carbon. This results in generation of a carbon-centred radical (alkyl radical; R*) that rapidly reacts with the oxygen molecule to form the peroxyl radical (alkylperoxide radical) intermediate (ROO*), which can give rise to the (hydro)peroxide (alkylhydroperoxide; ROOH), followed by the formation of an alkoxyl radical (RO*). The resulting alkoxyl radical can be transformed into a hydroxylated (at the α-carbon) amino acid residue, or it can lead to the fragmentation of the polypeptide chain (Fig. 4, panel A). The alkyl, peroxyl and alkoxyl radicals can react with other amino acid residues of the same – or another – polypeptide chain of the protein, resulting in the formation of new, carbon-centred radicals (Fig. 4, panel Ba). In the absence of oxygen, when the formation of peroxyl radicals is hampered, the alkyl radicals can react with each other, within the same or different proteins, and this leads to cross-linkages between the polypeptide chains (Fig. 4, panel Bb). The alkoxyl radicals can also promote the reactions that lead to the breakdown of the polypeptide chain. Depending on the location of the cleavage at the α-carbon, two different types of breakdown products will be formed (Fig. 4, panel C) with one being the α-ketoacyl derivative (O=C=R) (Fig. 4, panel C, a – the α-amidation pathway).

The fragmentation of the polypeptide chain can also occur following an attack of ROS on the residues of glutamic acid, aspartic acid and proline, followed by reactions analogous to those described earlier (Fig. 4, panel A). This eventually leads to formation of a peptide fragment with the N-terminal amino acid existing as an N-pyruvyl derivative, with oxalic acid and hydrogen peroxide as the side reaction products. Abstraction of the hydrogen atom from the y-carbon atom of glutamic acid by the hydroxyl radical leading to chain fragmentation is shown in Fig. 4 (panel D).

As a result of the oxidation of proline residues, 2-pyrrolidone is formed, concomitant with the breakdown of the polypeptide chain (Fig. 5, panel A). In turn, the hydrolysis of 2-pyrrolidone in an acidic medium leads to the formation of 4-aminobutyric acid, whose presence in the hydrolysis products of proteins treated with oxidizing agents indicates the occurrence of the mechanism of protein fragmentation by proline oxidation.

All amino acid residues found in proteins are susceptible to oxidation. The most sensitive to IR-produced ROS are cysteine, methionine, tyrosine, and tryptophan. Cysteine and methionine residues in the polypeptide side-chain can be oxidized to disulphides and methionine sulfoxide residues, respectively (Fig. 5,

panel B). The majority of biological systems can repair these types of oxidative modifications because they possess enzymes such as disulphide reductases and methionine sulfoxide reductases that are able to convert the oxidized forms back to their unmodified forms.

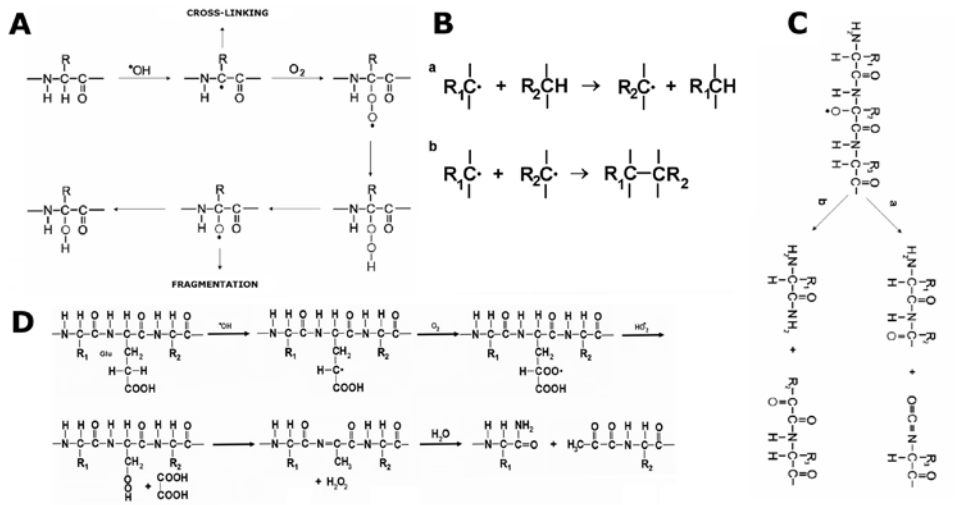

Figure 4. Oxidation of the polypeptide backbone by IR-generated hydroxyl radical: formation of the carbon-centred and peroxyl radicals, hydroperoxide, alkoxyl radical and a hydroxylated amino acid derivative (panel A); side reactions of radicals with other amino acid residues to form a new carbon-centred radical or formation of the protein-protein cross-linked derivative (panel B, a and b, respectively); peptide bond cleavage (panel C). Polypeptide fragmentation as a result of oxidation of the glutamyl residue (panel D) (Author: Michał Ponczek)

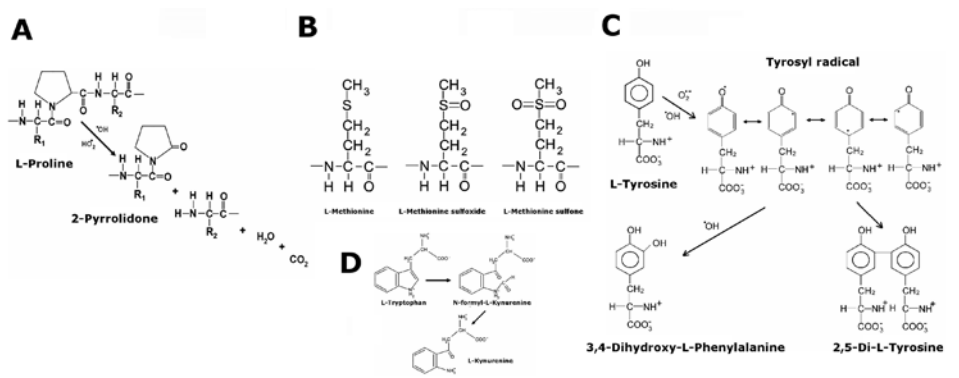

Figure 5. Oxidation of amino acid side chains: oxidation of the proline residue leading to peptide bond cleavage (panel A); products of the methionine residue oxidation (panel B); oxidation of the tyrosine residues that results in DOPA formation or cross-linking (panel C); products of the tryptophan residue oxidation (panel D) (Author: Michał Ponczek)

Oxidation of the tyrosine residues results in the generation of tyrosyl radicals, which can lead to the formation of 3,4-dihydroxyphenylalanine (DOPA) by incorporating an additional hydroxyl group into the aromatic ring. In contrast, the formation of 2,5-dithirosine leads to the cross-linking between the aromatic rings of the two molecules of the tyrosine (Fig. 5, panel C). Tryptophan residues can be oxidized to formylkynurenine and kynurenine (Fig. 5, panel D) while histidine to 2-oxohistine, asparagine and aspartic acid.

Oxidation of amino acid residues with free amine, amide or hydroxyl groups (especially arginine, lysine, threonine) leads to the formation of carbonyl derivatives. This also applies to proline, whose ring undergoes oxidation during fragmentation. Such amino acid derivatives can react with other free amino groups of lysine residues in the same or another protein molecule, to form cross-linking. This is another mechanism of cross-linking in polypeptide chains, in addition to the reactions of carbon-centred radicals and the formation of dityrosine. Carbonyl derivatives are also produced in the reactions of amino acid residues with lipid peroxidation products and reducing sugars. The presence of carbonyl groups in proteins have been used as a stable marker of ROS-mediated protein oxidation.

The conformational changes that result from this complex of reactions lead to the decrease or loss of protein biological function.

Lipid peroxidation

The hydroxyl radical and other highly oxidizing agents produced during water radiolysis may interact with unsaturated fatty acids in biological membranes, resulting in their peroxidation. This process is initiated by the abstraction of hydrogen from the unsaturated fatty acid leading to formation a carbon-centred lipid radical (L*), which is stabilized by a molecular rearrangement of the double bonds to produce a conjugated diene which then combines with oxygen to form a peroxyl radical (LOO*) (Fig. 6).

The peroxyl radical is itself capable of abstracting a hydrogen atom from another polyunsaturated fatty acid, thus starting a chain reaction. As a result, fatty acid peroxide (lipid peroxide; LOOH) and another alkyl radical (L*) are formed, which can contribute to the next peroxidation reaction. These series of reactions are termed the propagation phase, which imply that one initiating 'hit' can result in the alteration of many unsaturated fatty acids to lipid peroxides. For metals such as Fe^{+2}, in the presence of the transition, the re-initiation process can occur, in which the ROOH formed can undergo a reductive cleavage producing alkoxyl radical.

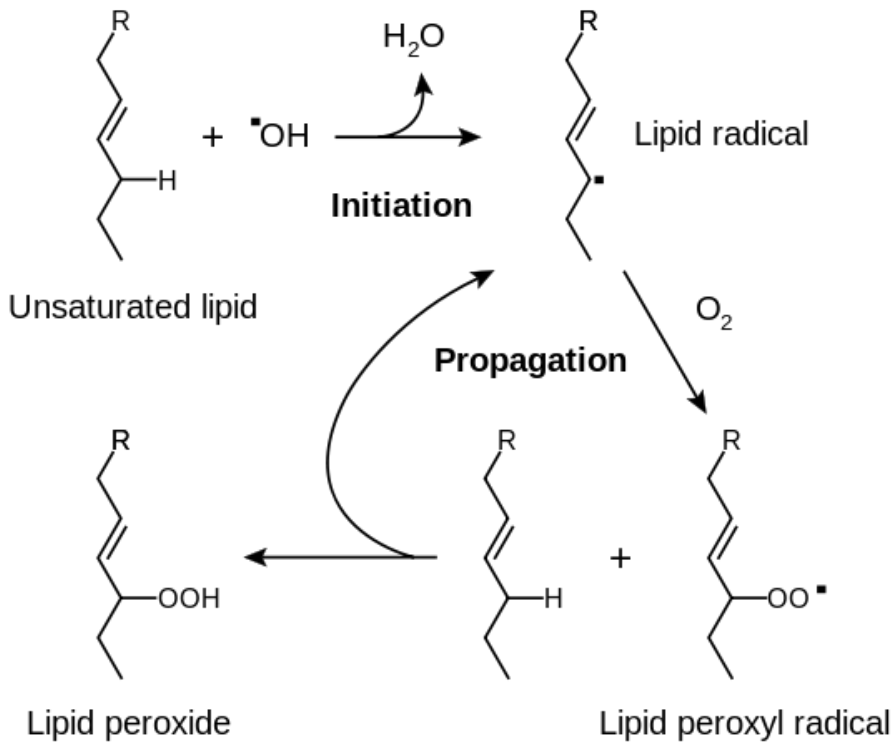

Figure 6. The initiation and propagation phase of lipid peroxidation

During the termination phase of lipid peroxidation, the reactions between radicals that lead to non-radical products dominate. Termination reactions (recombination of free radicals) can include the reaction between two alkyl radicals or two peroxyl radicals or two different radicals, including:

$$L^* + L^* \rightarrow L\text{-}L$$
$$LOO^* + LOO^* \rightarrow L{=}O + LOH + O_2$$
$$LOO^* + L^* \rightarrow L{=}O + LOH$$

The products of termination reactions are fatty acid dimers (in membranes – phospholipid dimers), as well as oxo and hydroxy fatty acids (these are the modified, damaged lipid molecules), which eventually undergo a breakdown (including through a β-elimination reaction) to produce many products. The most common is malondialdehyde (MDA) which is a major bioactive marker of lipid peroxidation. In addition, other aldehydes and hydroxyaldehydes are

generated, such as 4-hydroxynonenal (4-HNE), 2-propenal (acrolein), hepta-2,4-dienal, hydroxyoctanal, hydrocarbons (ethane, pentane), and many others. Increased plasma MDA has been found in persons occupationally exposed to low radiation doses. The aldehydes, MDA and 4-HNE, can cause DNA strand breaks, and are cytotoxic, mutagenic and carcinogenic agents.

5.4. Cellular responses to ionizing radiation

The molecular mechanisms of IR-induced cellular injury depend on many factors that primarily include radiation dosage as well as the cell type, cycle phase, and its transformed status. Taking into account the chemical processes occurring during irradiation, the extent of the cellular damage that results from the specific type of IR is similar in terms of the dose and the amount of DNA. However, the final IR effect is determined by the post-radiation processes such as DNA damage repair and the proliferative activity of the cell. There are somatic cells and germ cells in the human body. Cells can propagate through division; somatic cells undergo mitosis, whereas germ cells divide through meiosis. Somatic cells are usually classified into the following three groups:

1. Stem cells – self-renewing cells that exist to produce cells for a differentiated cell population (e.g. stem cells of the haematopoietic system, epidermis and mucosal lining of the intestine),
2. Transit cells – cells in movement to another population (e.g. a reticulocyte that is an immature cell, differentiating to become an erythrocyte)
3. Mature cells – entirely differentiated, and do not undergo mitosis (e.g. neural or muscle cells).

The cellular death of non-proliferating (static) mature cells can be defined as the loss of a specific function, while for stem cells and other cells capable of many divisions it is defined as the loss of reproductive integrity (reproductive death). A surviving cell that maintains its reproductive integrity and is able to proliferate is supposed to be clonogenic. Accordingly, in radiation biology, IR-induced cell death has been functionally classified into interphase or reproductive (mitotic) death. The former, observed early post-irradiation, is the death of irradiated cells before they enter mitosis. The latter, which is observed after several cycles of cell division, is the loss of the proliferative ability of the cell.

5.4.1. Radiation-induced cell cycle arrest

The mammalian cell cycle is divided into two major phases: the interphase and the mitotic phase (M) (Fig. 7).

During the M phase the cell divides its copied DNA and the cytoplasm to make two new cells. The M phase comprises two distinct division-related processes, mitosis and cytokinesis (C). The G_1 is the first gap in cellular activity

between mitosis and the S phase, in which DNA synthesis takes place, and G_2 the second gap in activity between the S phase and the next mitosis.

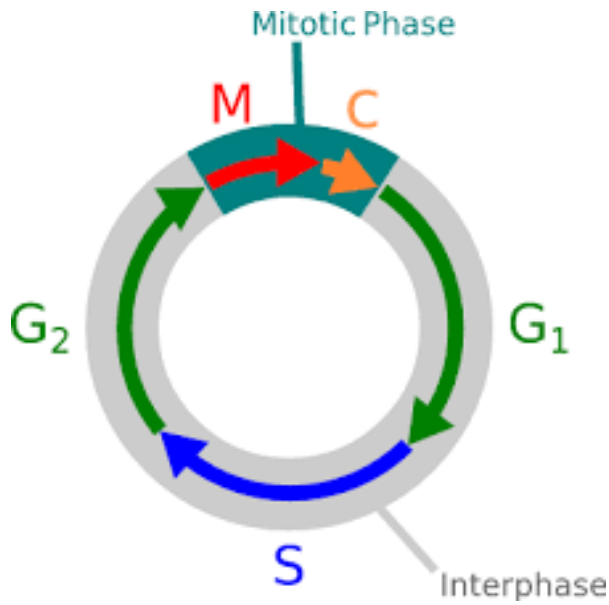

Figure. 7. The overview of the cell cycle (*figure used with permission under Creative Commons license*)

There are types of cells (e.g. early embryo, stem cells, cancer cells) that divide rapidly, and their daughter cells can undergo another round of cell division without delay. Other types of cells divide slowly, or do not divide; when cells stop progressing through the cycle, they can enter the resting state (G_0 phase). This can be a permanent state for some cells, while others can re-start division in response to the right signal. Big variations in radiosensitivity in different phases have been shown. Although the mechanisms responsible for this phenomenon are not fully understood, it could be related to some of the changes in chromatin organization, and with the different amounts of cell cycle phase-dependent thiols in the cell. Commonly, cells are the most radiosensitive in the M and late G_2 phases, and the most resistant in the late S phase. At the beginning of the G_1 phase, cells are relatively resistant to IR, followed by an increase in their sensitivity.

IR damages cells by altering the protein expression that affects the signalling pathways involved in damage/repair mechanisms. Several defence mechanisms exist to restore DNA integrity (Fig. 8). Damage of the cellular DNA (SSB, DSB) activates the expression of two specific kinases – ATM (ataxia-telangiectasia mutated) kinase, and ATR (ataxia-telangiectasia and Rad3 related) kinase. These are involved in the DNA damage response (DDR) pathway, which in turn

induces cell cycle arrest and activates a range of downstream targets involved in DNA repair. DDR is a network of cellular pathways that sense, signal and repair DNA lesions. The ATM gene is mutated in the autosomal recessive disease ataxia telangiectasia (AT), which is characterized by a pleiotropic phenotype including neuronal degeneration, oculocutaneous telangiectasias, immune dysfunction, and cancer predisposition.

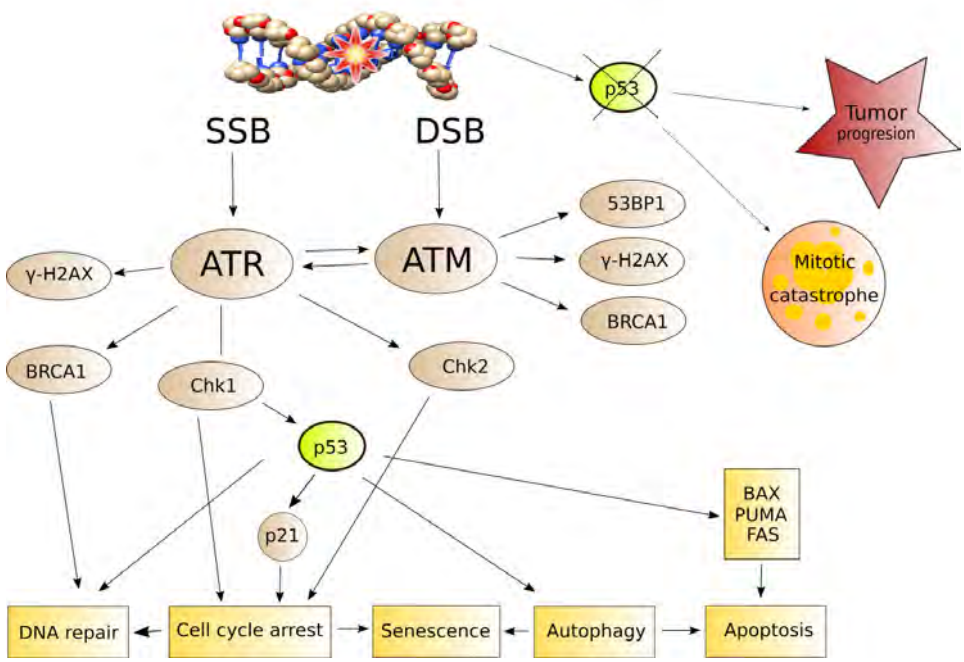

Figure 8. The DNA damage response (DDR) pathway and cellular responses to ionizing radiation (Author: Michał Ponczek)

The ability or inability of the repair mechanisms to fix the IR-induced DNA damage decides the fate of the cell – survival or death. One of the primary substrates of the ATR and ATM kinases is histon H2AX; its phosphorylation (to form γH2AX) induces the recruitment and attachment of the subsequent proteins in DDR signalling. The most well-studied ATM/ATR targets are the protein kinases CHK1 and CHK2, which together with ATM and ATR act to reduce the activity of the cyclin-dependent kinases (CDKs) through various mechanisms. Some of these mechanisms are mediated by activation of the p53 transcription factor. Inhibition of the CDKs leads to deceleration or arrest of the cell-cycle progression. Therefore, DNA damage can lead to arrest at the cell

cycle checkpoints that exist at the boundaries between adjacent phases G1/S and G2/M. There is also an intra-S checkpoint. Cell cycle arrest is believed to increase the time available for DNA repair before the subsequent replication or mitosis. In parallel, ATM/ATR signalling enhances the repair mechanisms by inducing DNA-repair-proteins, transcriptionally or post-transcriptionally, by recruiting repair factors to the damage and by activating DNA repair proteins. The main factor involved in modulating the IR-induced cellular response is a p53 protein, which is a transcription factor and tumour suppressor gene. P53 is often denoted as the 'guardian of the genome' as it controls many target genes that can induce either arrest of the cell cycle and DNA repair, or trigger apoptosis.

To summarize, irradiation of a cell can result in one of a variety of possible outcomes (Fig. 8), such as: no effect; cell cycle arrest; DNA repair; cell death; genomic instability; mutation (wherein a cell survives but the DNA was not repaired, or was improperly repaired); transformation and tumour progression (wherein a cell survives but the mutation leads to a transformed phenotype and possibly carcinogenesis), and bystander effects (in which the irradiated cell sends signals to neighbouring un-irradiated cells and induces genetic damage in these cells). DNA damage that is hard to repair, and/or more severe damage, will induce cell death, either in the form of apoptosis, which is a p53-dependent pathway, or p53-independent mitotic catastrophe.

5.4.2. Mechanisms of cellular death after irradiation

Cells that fail to repair DNA damage caused by IR are killed. The detailed mechanisms of cellular death are still not fully understood. Cellular death, however can be caused through different molecular pathways, each representing a different mode. Several types of cellular death have been demonstrated to occur *in vivo* and *in vitro*, in response to IR. Depending on the radiation dose, type and radiosensitivity of the exposed cell, IR can induce apoptosis, necrosis, senescence, autophagy or mitotic catastrophe. Apoptosis is a major control mechanism by which cells undergo self-destruction. It is a programmed cell death, an evolutionarily maintained and highly regulated process that is required to remove damaged, infected, extraneous or transformed cells from normal tissues. The morphological signs of apoptosis include cell shrinkage, membrane blebbing, condensation of chromatin, DNA fragmentation and eventually disintegration of the cell into the membrane-bound microvesicles, or so-called apoptotic bodies (Fig. 9).

These apoptotic bodies are removed by a phagocytic system *in vivo*, which prevents the inflammation associated with cellular death. Apoptosis remains the main IR-induced death mechanism in cells from the lymphoid and myeloid lineages, while significantly less apoptosis is seen in cells of the epithelial origin. Execution of apoptosis is intimately associated with the activation of caspases, a family of cysteine-aspartic proteases. Caspases are present in cells as non-active

zymogens and become activated when the cell comes across external or internal stimuli. Caspases are divided into the initiator and effector (executioner) caspases. IR-induced apoptosis can activate a cascade of caspases *via* the extrinsic and/or intrinsic pathway (Fig. 10). Overall, these apoptotic pathways converge to activate the effector caspase-3, caspase-6 and caspase 7. Caspases cleave the vital cellular proteins and dismantle the cell.

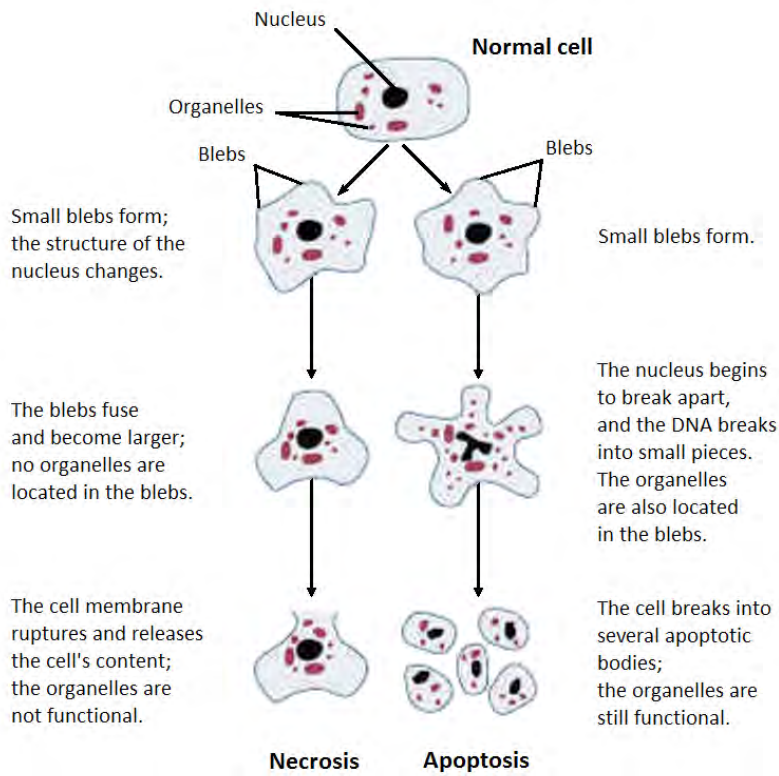

Figure 9. Major differences between apoptosis and necrosis (*figure used with permission under Creative Commons license*)

The intrinsic pathway, also referred to as the mitochondrial pathway, can be triggered by a signal from the inside of the cell, such as cytotoxic DNA damage or elevated intracellular ROS.

The mitochondrial pathway is firmly controlled by the opposite actions of the members of the Bcl-2 family. These proteins are divided into functionally distinct groups: inhibitors of apoptosis (including Bcl-2, Bcl-XL), which inhibit their pro-apoptotic counterparts that promote apoptosis. The pro-apoptotic

proteins include BAX, BAK, BID, PUMA and many others. Upon detection of cytotoxic internal stimuli, two pro-apoptotic proteins, BAX and BAK, undergo structural changes that lead to their activation. Both BAX and BAK migrate to the mitochondrion, where they undergo homodimerization and introduce pores in the mitochondrial outer membrane. This results in Mitochondrial Outer Membrane Permeabilization (MOMP), and disrupts the mitochondrial function. The release of cytochrome c, which interacts with Apoptotic Protease Activating Factor 1 (APAF-1) and procaspase-9, leads to apoptosome formation – a complex structure in which procaspase-9 dimerizes and undergoes auto-activation. The principal function of the apoptosome is to trigger a cascade of caspase activation beginning with the effector caspases (3 and 7) being responsible for cell death.

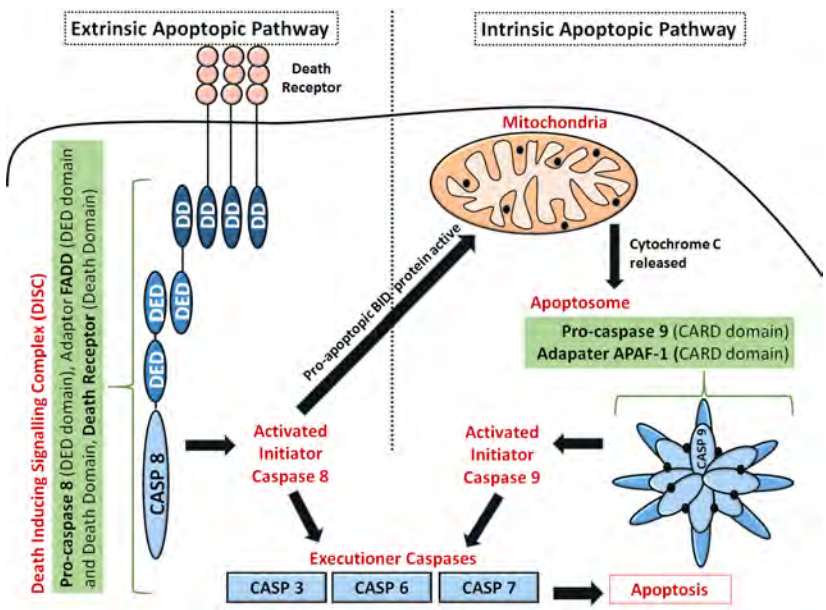

Figure 10. The extrinsic and intrinsic apoptotic signalling pathways (*figure used with permission under Creative Commons license*)

The extrinsic apoptotic pathway is also involved in IR-induced apoptosis. This pathway is known as the receptor-mediated pathway because it requires a ligand-dependent activation of the specific transmembrane proteins, the so-called death receptors. Death receptors comprise a subset of the tumour necrosis factor (TNF) receptor superfamily (which includes Fas and TNFα), characterized by distinct protein motifs – namely Death Domains (DD) and Death Effector Domains (DED). The death receptor (for example, Fas) is stimulated by the Fas ligand (FasL) which attracts the DD-containing molecule, the adapter protein – Fas-Associated protein with Death Domain (FADD). Recruitment of FADD attracts other DD/DED-

containing proteins, such as pro-caspase-8 (and -10), to promote the formation of the so-called Death Inducing Signalling Complex (DISC) in the cytoplasmic compartment. DISC formation is necessary for the cleavage and activation of procaspase 8 to form caspase 8, which goes on to cleave and activate other caspases such as procaspase 3 initiating the caspase cascade, which leads to cellular death.

IR-induced necrosis is regarded as a passive, pathological process, characterized by cellular swelling, rapture of the plasma membrane and uncontrolled release of cytoplasmic content into the intercellular space. It is associated with increased inflammation of the surrounding tissues (Fig. 9). In necrotic cells, the level of ATP dramatically decreases as a result of depolarization of the mitochondrial membrane, followed by impairment of the electron transport. Not only mitochondria, but also other cytoplasmic organelles undergo destruction (nucleus, lysosomes, endoplasmic reticulum, ER). The influx of calcium ions to the cytosol from the ER is a typical sign of necrosis. Elevation of the calcium concentration activates nucleases that cleave the DNA. Necrosis in general results from more severe stress compared to apoptosis and senescence. For example, high IR exposure (≥30–50 Gy), was shown to induce necrosis in neurons. Although necrosis has long been considered an accidental cell death, recent studies suggest that there are several genetically regulated forms of necrosis, including necroptosis.

Mitotic catastrophe is also one of the major forms of IR-induced cell death, which denotes a mechanism of delayed mitotic-linked cellular death. Mitotic catastrophe (also called mitotic death), involves a sequence of events that are caused by premature or inappropriate entry of cells into mitosis and aberrant chromosome segregation due to severe DNA damage. Mitotic catastrophe leads to the formation of giant cells with aberrant nuclear morphology or multiple nuclei, and/or several micronuclei. It is noteworthy that this type of death is believed to be the p53-independent pathway, and is executed by rapidly dividing cells (stem cells, epithelial cells), and by most non-haematopoietic tumour (fast proliferating) cells in response to IR. Aberrant mitosis in response to DNA damage (also caused by IR) and mitotic death can also be associated with the aberrant duplication of centrosomes, the structures responsible for spindle microtubule formation. The characteristic feature of the cells that follow mitotic catastrophe is that the death is delayed, occurring 2–6 days post-irradiation. Mitotic catastrophe in apoptosis-competent cells is frequently followed by a delayed type of apoptotic death, or in other cells, by delayed necrosis.

Autophagy is an important catabolic process in which the cell digests itself *via* degradation of intracellular components such as proteins and organelles, to gain energy and nutrients (metabolic precursors). Autophagy mainly contributes to cell survival under adverse conditions, but when a stress factor persists for longer, autophagy can lead to so-called Type II programmed cell death.

Additionally, IR can induce a permanent cell cycle arrest, called senescence, a state in which the cell remains viable but with altered functions, and is no longer

competent for proliferation. Two major types of senescence have been identified: replicative senescence, which is a consequence of telomere shortening, and accelerated cellular senescence, in which the cell functions in the state of persistent, chronic DDR signalling. Senescent cells display characteristic phenotypic traits – they become enlarged and flattened with increased granularity. Senescent cells can be identified by their positive staining for the senescence-associated-β-galactosidase. In some cases, it has been demonstrated that increasing radiation doses shift the cellular response from senescence to apoptosis and/or autophagy, with higher doses leading to necrosis. However, there is no absolute response of all cells to a given dose of radiation exposure. Some cell types rapidly undergo apoptosis in response to the same level of IR that induces senescence in other cell types.

5.4.3. Cell survival curves

Cell survival curves describe the relationship between the surviving fraction of cells and the absorbed dose. Cell survival as a function of radiation dose can be graphically presented by plotting the surviving fraction (using a logarithmic scale) on the ordinate against dose (a linear scale) on the abscissa (Fig. 11).

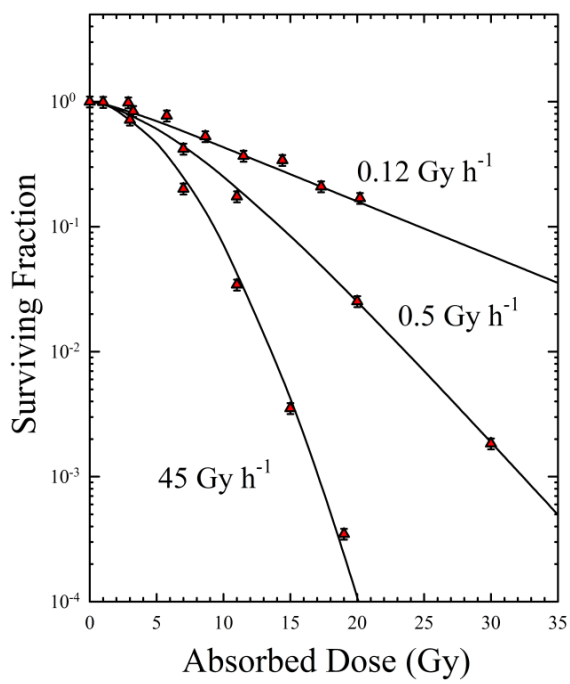

Figure 11. An example of a cell survival curve (*figure used with permission under Creative Commons license*)

The shape of the cell survival curve is influenced by the type of radiation. High LET (densely) IR exhibits cell survival curves that are almost exponential functions of the dose, shown by an nearly straight line on the log–linear plot. For low LET (sparsely) IR, however, the curves show an initial slope followed by a shoulder region that then turns nearly straight at higher doses.

5.5. The effects of ionizing radiation on the human organism

Throughout their lifetime, humans are exposed to low doses of IR from natural sources. The man-made sources which also contribute to IR exposure include industrial (nuclear power plant workers), and medical (radiotherapy, diagnostic X-rays) sources. Radiation accidents and incidents (nuclear weapon explosions, terrorist acts with radioactive materials) have led to much higher exposure doses. Despite the many practical applications of IR, exposure to high radiation doses has fatal consequences. The major sources of data on the health risks of IR exposure are the epidemiological studies of the Japanese survivors of atomic bombing, the workers who cleaned up after the Chernobyl and Fukushima nuclear plant accidents, and other populations accidentally exposed to high (even lethal) radiation doses. Secondary, also important sources of information on the effects of IR exposure on humans, are studies of oncological patients undergoing radiotherapy. Radiation harm can occur from external irradiation (outside the body), external or internal contamination with radioactive materials (the latter include inhalation, digestion or absorption through the skin), as well as from combinations of all of these exposure types. Injury from a nuclear detonation depends on the location of the victim relative to the hypocentre and the resulting exposure to heat, bomb blast, and radiation. Heat and light cause thermal injury, such as skin and/or retinal burns and blindness (due to the temporary depletion of photopigment from retinal receptors). The blast wave can result in fractures, wounds, rupture of internal organs and pulmonary haemorrhage and oedema. Acute overexposure to IR results in Acute Radiation Syndrome (ARS). Radiation also causes cutaneous injury and scarring, chorioretinal damage (from the exposure to infrared energy), and depending on the dose and dose rate, a variety of long-term effects (late toxicity). Cutaneous injury is characterized by loss of epidermis, sometimes dermis. Skin damage can cover small areas but it may extend deeply into the soft tissues, reaching the underlying muscle and bone.

The effects of IR on the human population are commonly divided into somatic and genetic. Somatic effects include harm that individuals exposed to IR suffer during their lifetime, such as the increased risk of IR-induced cancers (carcinogenesis), opacification of the eye lens, infertility, and shortened lifespan. Genetic effects (also called hereditary effects) include IR-induced changes in genomic DNA of the exposed individual's (chromosome aberrations, mutations),

which can contribute to the birth of defective descendants. Radiation is a known teratogenic agent; it disturbs the development of the embryo or foetus. Depending on the dose and the stage of development at the time of exposure, the main effects of radiation on a foetus include: foetal or neonatal death, malformations, growth retardation, congenital defects and cancer induction.

The biological effects of ionizing radiation on the human body depend on the nature and energy of the radiation, the time and mode of interaction and radiosensitivity of the exposed cells.

5.5.1. Radiosensitivity

Radiosensitivity is a broad term which can be applied to cells, tissues, organs and individuals. Cellular radiosensitivity defines the degree of response of the cells to IR. It refers to a wide range of events (endpoints) measured at the cellular level, such as cell viability, DNA damage and repair, markers of the cell cycle, apoptosis (or other cell death), chromosomal damage, etc. The response to IR can vary by cell type. The vulnerability of tissue to radiation injury depends on the degree of differentiation of the cells in the tissue and their proliferative activity. In 1906, French radiologists Bergeron and Tribondeau noted that radiosensitivity of a mammalian cell is proportional to the cell division rate and inversely proportional to the degree of their differentiation. This observation is called the law (or principles) of Bergon and Tribondeau. Tissues of the human body are well-organized structures composed of cells that have an epithelial and connective tissue origin. The epithelial cells (e.g. in the intestinal epithelium) and haematopoietic bone marrow cells continuously self-regenerate, they are cells renewing within a few days. Conversely, the endothelial vascular cells and fibroblasts that compose an underlying stroma are slowly renewing (proliferating). Therefore, the most radiosensitive are bone marrow and lymphoid tissue, germ cells and intestinal epithelial cells. Less sensitive are muscle cells, parenchymal organs (such as the liver), the nervous tissue, and the connective tissue. There is also a concept of 'relative radiosensitivity', which takes into account the importance of tissue to the body and the effects of its radiation damage. Taking this approach, the concept of the critical organ has been introduced, defined as the most important organ to the body that is the most damaged by radiation. For example, to X-rays and γ-rays, the critical organs are the bone marrow, the gonads and the eye lens. Generally, the radiation's effects can be modified by different factors, such as the type of radiation, dose, dose rate, dose fractionation (in radiotherapy), the mass of the irradiated tissue, tissue oxygenation, the organ irradiated, and the addition of radical scavengers. Rich vascularization, and thus a good supply of oxygen to tissues, increases their radiosensitivity.

At the organ level, IR-induced injury results from the direct destruction of highly radiosensitive cells, such as the stem cells in the bone marrow

(a rapidly renewable system). Damage may also result from the constriction of microcirculation, from oedema and inflammation of the basement membrane, and this injury can progress to fibrosis. The IR may have little effect on the parenchymal cells (slowly renewable/non-renewable system), but ultimate parenchymal atrophy and death over several months can result from fibrosis and occlusion of the microcirculation.

The individual radiosensitivity of various representatives of the population is, of course, relatively diverse. It can also vary within the same individual, for example, with age and with health conditions. For the description of radiosensitivity of the given population the concept of lethal dose (LD) has been introduced. All variants of this dose assume a single irradiation in a short time (up to several hours) of the whole body and no medical assistance after irradiation. The most useful for comparison is the mean lethal dose; it can be measured by assays such as $LD_{50/30}$ ($LD_{50/60}$) which defines the radiation dose required to kill 50% of a given population within 30 days (60 days) of exposure. Minimal lethal dose (LD_{min}) refers to the smallest radiation dose at which deaths can occur due to irradiation of a given population, while the maximal lethal dose (LD_{max}) defines the minimal radiation dose that causes death of all individuals of the irradiated population.

As an example, detonation of the nuclear device over Hiroshima, in 1945, resulted in approximately 150,000 casualties and 75,000 fatalities, in all survivors the estimated exposure was below 3 Gy. It has been suggested that the mean lethal dose of radiation (of the whole-body radiation) that kills 50% of human population within 60 days ($LD_{50/60}$) is at the range of 3.25–4 Gy in persons that have no supportive care, and 6–7 Gy in those having transfusion and antibiotics support.

5.5.2. Acute and late radiation toxicity, stochastic and deterministic effects

The timescale involved between the breakage of the chemical bonds in the vital macromolecules and the biological effect may be hours to years, depending on the type of damage. When a cell death is the result, it may happen in hours to days. If the damage is cancer induction, then its expression may be delayed for years. Based on the timescale as well as functional and histopathological endpoints, habitually, the effects of IR on tissues (or organs) has been divided into acute (early) responses and chronic (late) responses. Acute radiation toxicity are manifested soon after exposure to radiation (mostly within a few weeks) and are characterized by death of the critical cell populations. Especially damage is more evident in the haematopoietic and epithelial cells. Radiation at doses higher than those applied radiotherapy may completely destroy these cells. Early responses also involve gene activation resulting in tissue dysfunction followed by

the increased vascular permeability, tissue oedema, production of cytokines and growth factors, chemoattraction of macrophages and other white cells, leading to inflammation. These early responses to the stromal cells can persist of weeks or even months until have been settled. Late effects are delayed (may occur after 12 months) and may be consequential to acute damage in the overlying tissues, such as mucosa or the epidermis. Late effects can include, for example fibrosis, atrophy, ulceration, stenosis or obstruction of the intestine. Late effects may also be genetic and caused by absorption of radiation directly in the target tissue (mutations, chromosomal aberrations, carcinogenesis).

The acute/subacute effects of IR are known as so called deterministic effects (non-stochastic effects). They have a specific threshold dose. A deterministic effect (tissue reaction) is defined as a one that increases in severity with increasing dose, usually above a threshold dose, in affected individuals. Skin erythema, organ dysfunction, fibrosis, cataract, blood changes, decrease in sperm count are all examples of the deterministic effects. For example, the total body irradiation at the dose >5 Gy results in the bone marrow suppression, but this suppression is not observed for the dose <5 Gy. A stochastic effect is defined as a one in which the probability of occurrence increases with an increasing dose but the severity in affected individuals does not depend on the dose. These are statistically measurable effects. There is no threshold dose for the effects that are truly stochastic, because these effects arise in single cells and it is assumed that there is always some small probability of the event occurring even at very small doses. Stochastic effects (usually chronic effects) are for example IR-induced genetic mutations, chromosome aberrations, carcinogenesis.

5.5.3. Whole body irradiation

The response of animals, used for *in vivo* studies, to a single dose of whole body irradiation (WBI) can be characterized by four overlapping syndromes, including prodromal, haematological, gastrointestinal, and neurovascular syndrome, which are manifested following different doses, at different post-radiation time. The similar syndromes also apply to human victims of radiation accidents; the dose ranges after which the particular syndrome is seen vary between rodents and human. The neurovascular syndrome occurs following large IR doses (more than 20 Gy) and usually causes a rapid death (within hours to days) due to dysfunction of cardiovascular and nervous system. The gastrointestinal syndrome occurs following exposure to doses above 8–12 Gy, and in rodents the upper doses of this range generally result in death (within a week) which is mainly caused by a severe damage of the gastrointestinal tract mucosa. A subsequent loss of the protective barrier results in infection, loss of electrolytes and the fluid volume imbalance. In human victims, intensive treatment with antibiotics, replacement of fluids and electrolytes can prevent early death from this syndrome. However, these

patients can die due to the injury of other organs. The haematopoietic syndrome in rodents occurs at doses in the range of 3–10 Gy (2–8 Gy in human), which is caused by a severe depletion of blood morphotic elements, such as red blood cells (RBCs), white blood cells (WBCs) and blood platelets (PL), due to killing of the precursor cells in the bone marrow. As a result, the animals die usually between 12–30 days after irradiation, and in case of human a death happens fairly later. Treatment of human victims may include bone marrow transplantation to prevent death, provided that the radiation dose was not too high. There are considerable differences in radiation doses required to induce death due to the haematopoietic syndrome (for example, in LD_{50} values) between different animal species, even between strains of the same species. For human the mean lethal dose (LD_{50}) has been estimated at 4 Gy (4–7 Gy depending on the supportive care).

In relation to human, high instantaneous doses (>10 Gy) can occur accidentally (explosions of nuclear weapons, nuclear power plant accidents, handling unshielded radiation sources or radioactive waste). Data on some post-radiation symptoms were also collected based on the medical human exposure to TBI (a routine procedure before the bone marrow transplantation). Health effects after an individual is exposed to low or high doses of gamma irradiation have been described in several publications. In general, doses of IR below 0.15 Gy (15 rad) produce no noticeable symptoms or signs. This range includes, for example, lifetime radiation exposure from natural background radiation, the majority of nuclear diagnostic tests or nuclear power plant functioning. Increased radiation doses (0.15 to less than 0.5 Gy; 15 to <50 rad) result in subclinical responses, characterized by very few, if any, clinical or haematological symptoms. This level of IR exposure produce no visible manifestations, with the probability of chromosomal breaks occuring. At radiation doses from 0.5 to 30 Gy (50 to 3,000 rad) or more, clinical responses do occur. Acute radiation syndrome (ARS) is seen in individuals following acute whole body irradiation with doses of 1 or more Gy (≥100 rad).

Acute Radiation Syndrome (radiation sickness)

Acute Radiation Syndrome (ARS), is defined as 'an acute illness caused by irradiation of the entire body (or most of the body) by a high dose of penetrating ionizing radiation in a very short period of time (usually a matter of minutes)'. ARS is also known as radiation sickness, and can be seen after exposures to doses >1 Gy. The degree of ARS may be classified by the absorbed dose and the time over which the energy from the radiation has been deposited in the tissues. As mentioned earlier, clinical components of the ARS include haematopoietic, gastrointestinal and cerebrovascular syndromes. However, the clinical phase of ARS can also be divided into four overlapping stages:

1) a mild phase (0.5–1 Gy, 50–100 rad);
2) the haematopoietic (or the bone marrow) syndrome (1–8 Gy, 100–800 rad);

3) the gastrointestinal syndrome (8–30 Gy, 800–3,000 rad);

4) the central nervous system (or cerebrovascular) syndrome (>30 Gy, >3,000 rad).

Each syndrome can be divided into four stages: the initial (prodrone), latent, and manifesting illness stages. The last of these is recovery or death. Depending on the dose absorbed, symptoms can appear at different times. The initial phase of ARS usually occurs in the first two days, but it can develop up to six days after exposure. The latent phase is a short period, lasting from several days to a month, when the symptoms transiently improve. Then, the manifest illness symptoms appear, characterized by strong immunosuppression. This period can last for weeks and is the most difficult to treat. If the individual survives this third stage, recovery is possible. Victims exposed to supralethal doses of IR can experience all these stages in just a period of several hours, leading to early death. If the energy is deposited over more than a few days (i.e. at a lower dose rate), the severity of the effects can be greatly reduced and the time of onset delayed. The phases of ARS as a function of the exposed dose, at high exposure rates, are summarized in Table 3. Clinical responses to radiation at doses in a range of 0.5 to above 30 Gy will be discussed below.

The **mild phase** of ARS (0.5–1 Gy) is characterized by mild, non-specific signs of toxicity. At a dose of 1 Gy, the majority of persons express temporary haematopoietic manifestations. The first clinical symptoms, nausea and vomiting, usually appear within 4–8 hours. Within 7–15 days after exposure, a moderate leukopenia (low WBC count) appears. However, blood cell counts finally go back to normal within 4–6 weeks after exposure.

The **haematopoietic syndrome** (1–8 Gy) can be seen after WBI exposures that exceed 1 Gy; it is hardly ever clinically relevant below this radiation dose. The dividing capability of the haematopoietic progenitor cells significantly decreases after a whole-body radiation dose of 2–3 Gy, leading to haematological crisis. This crisis is characterized by hypoplasia or aplasia, and bone marrow failure syndromes that result in development of pancytopenia – a condition in which the number of all blood cells dramatically falls. However, small subpopulations of stem and/or progenitor cells are selectively more radio-resistant, and these are responsible for recovery from haematopoiesis after exposure to doses even as high as 6 Gy. Pancytopenia, which includes leukopenia (low counts of lymphocytes and granulocytes; WBC counts), thrombocytopenia (low PL count) and anaemia (low RBC count), is a condition that predisposes to infection, bleeding and poor wound healing. Lymphopenia is the most common, occurring before manifestation of other cytopenia. Since the rate of the lymphocyte depletion is predictable (a 50% decline in total lymphocyte count occurs within the first 24 h after exposure, and becomes more severe after 48 h), the lymphocyte depletion kinetics can be used as an important component of biodosimetry (Waselenko et al. 2004). Individuals with additional injuries, such as burns and trauma – very

common after detonation of a nuclear device (60–70% of victims) – can develop lymphopenia as a result of these injuries alone.

Table 3. Dose dependent-injury of humans exposed to high doses of IR

Radiation dose (Gy)	severity	symptoms	Prognosis (without treatment)	Radiation syndrome
0.5–1	mild	minor decline of blood cell counts	survival –almost certain	-
1–2	mild/ moderate	early signs of BM damage	>90% victims survive	BMs
2–3.5	moderate	moderate/severe BM damage	survival – probable	BMs
3.5–5.5	severe	severe BM and minor GI damage	death within approx. 6 weeks (50% victims)	BMs
5.5–7.5	severe	pancytopenia, moderate GI damage	death – probable within 2–3 weeks	BMs/GIs
7.5–10	severe	hypotension, noticeable GI and BM damage	death – probable within 1-2.5 weeks	GIs
10–20	severe	Severe GI damage, pneumonitis, mental status, cognitive dysfunction	death-certain within 5–12 days	GIs/CNSs
20–30; >30	severe	fever, cerebrovascular collapse, shock	certain death within 2–5 days	CNSs

BM – bone marrow; BMs – bone marrow syndrome; GI – gastrointestinal; GIs – gastrointestinal syndrome; CNS – central nervous system; CNSs – central nervous system syndrome

The onset of other cytopenia differs and is much more dose- and dose rate-dependent. Although red blood cell precursors are affected at these doses, because of the lifespan of a peripheral red blood cell (90–120 days), anaemia may not become clinically evident for several days or weeks after exposure. Cells that proliferate more slowly (e.g. the cells of the central nervous system, connective tissues, etc) are largely unaffected.

Different clinical manifestations of the haematopoietic syndrome of ARS, depending on the phase, have been described. The prodromal phase, which

typically lasts up to 2–3 days, is characterized by fatigue, sleeplessness and lethargy that progress to headache, anorexia, nausea, and vomiting within – depending on the dose – approximately 8 hours after initial exposure. Varying alterations in the peripheral blood can be seen, with the earliest changes demonstrated as a marked lymphopenia. The latent phase lasts from 3–4 days to 3 weeks. The marked signs of this phase include a progressive decrease in total blood leukocyte counts and hair follicle death. The third phase, the symptomatic or bone marrow depression phase (18–21 days after exposure), is characterized by a plethora of symptoms, such as chills, fever, depression, a swollen throat, inflammation of the gums (gingivitis), bleeding gums, small blood blisters, and bruises. The leukopenia and thrombocytopenia weaken the body's natural defences against disease and haemorrhage causing anaemia and acute infectious diseases. Coagulopathies begin to appear due to blood platelet anomalies (purpura, haemorrhage), as well as hair loss (hair epilation). Depending on the dose and intensity of the treatment protocols, the clinical image can vary from serious to fatal. The recovery phase is marked by a general improvement of the patient over a 3–6 month period. There is a good prognosis for recovery if the doses received do not exceed 6 Gy (1–6 Gy). For doses of 6–8 Gy the prognosis is rather poor. Nevertheless, some victims are expected to survive if they receive intense medical treatment. The $LD_{50/30}$ for whole-body irradiation is estimated to be between 3.5–4.5 Gy for those who receive minimal or no medical treatment.

Gastrointestinal syndrome (8–30 Gy) is most often a result of the loss of intestinal crypts and the breakdown of the mucosal barrier. The prodromal phase of this syndrome is very rapid in onset and characterized by nausea and diarrhoea, which typically decreases after several days, followed by a short latent period. The symptoms then return, and these include WBC depletion as seen in haematopoietic ARS syndrome, nausea, vomiting, diarrhoea (sometimes bloody), abdominal pain, fever, and massive electrolyte imbalances, which ultimately then result in death. Other systemic effects can include malabsorption, malnutrition, small-bowel obstruction, dehydration and hypotension. Damage of the intestine mucosa and microcirculation leads to gastrointestinal bleeding, that increases anaemia and the risk of sepsis and/or acute renal failure. At these doses the mortality rate of the gastrointestinal syndrome exceeds that of the haematopoietic syndrome. Treatments are palliative. People exposed to absorbed doses of ≥10 Gy are expected to die, although aggressive medical intervention can improve the survival rate. It's worth noting that there is an exception – if the dose is fractionated, as with the bone marrow transplantation patients who receive a standard whole-body dose of 15.75 Gy and are well managed, with fluids, antibiotics, and a sterile environment, the individual has a reasonable chance of survival.

The central nervous system syndrome (>30 Gy). Symptoms of this syndrome typically have an immediate onset, and include violent nausea and vomiting, diarrhoea, headache, irrational behaviour, circulatory system collapse,

and lack of neuromuscular coordination, occurring within a few minutes after irradiation. This syndrome is less well defined than other syndromes. Note that the signs and symptoms of different organ systems significantly overlap at each radiation dose; cerebrovascular symptoms do not appear until exposure to a high whole-body dose. Individuals exposed to supralethal exposures (>20–30 Gy) can present fever, hypotension and major impairment of cognitive function. The prodromal stage of the cerebrovascular syndrome is characterized by disorientation, confusion and prostration, and can also be accompanied by a loss of balance and seizures. A physical examination can show papilledema (optic nerve swelling) due to increased intracranial pressure, ataxia (a neurological sign consisting of lack of voluntary coordination of muscle movements), reduced or absent deep tendon and corneal reflexes. Diarrhoea, respiratory suffering, fever, and cardiovascular shock can occur within 5–6 hours post-exposure. This phase mimics the clinical symptoms of acute sepsis and septic shock, both of which should be considered. Convulsions, coma, and death follow within 48 hours after irradiation.

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