E.A. Domina

R.E.Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, Kyiv, Ukraine

Keywords: *cancer patients, radiotherapy, DNA repair, radiosensitivity, genetic instability.*

DOI: https://doi.org/10.15407/oncology.2024.03.222

THE ROLE OF REPAIR PROCESSES IN THE FORMATION OF RADIOSENSITIVITY OF HEALTHY AND TUMOUR CELLS IN HUMANS

Despite significant progress in the development and implementation of new radiotherapeutic techniques over the past decade and positive results of radiotherapy for cancer patients, overall and progression-free survival rates are still poor. The analysis of literature data and our own research in the field of clinical radiobiology and radiation oncology shows that cellular DNA repair systems increase the radioresistance of tumours and thus hinder the improvement of the effectiveness of patient therapy. The role of repair processes in shaping the radiosensitivity of tumours compared to healthy tissues is discussed in detail, and their differences are described. It is emphasized that the repair of irradiated DNA damage in tumour cells is more intensive than in healthy tissue cells due to increased expression of repair enzymes. Together with the instability of the tumour cell genome, this causes a large variation in their radiosensitivity and indicates the priority of research aimed at finding and developing radioprotectors to protect the genome of healthy cells from the environment of the irradiated tumour without affecting (reducing) its radiosensitivity. Such a way to optimize the results of therapeutic radiation will help minimize radiation complications that require additional therapy and worsen the quality of life of treated patients.

Radiobiological science has unequivocal evidence that DNA is the main target responsible for the fate of an irradiated cell. Cellular DNA repair systems are largely responsible for tumour radioresistance and thus hinder the optimization of the effectiveness of radiotherapy in cancer patients. The repair of damaged DNA in tumour cells is more intensive than in healthy tissue cells due to increased expression of repair enzymes [1–3]. In addition, tumour cells are inherently genetically unstable, which causes a large variation in their radiosensitivity. These differences in the radiosensitivity of the tumour and normal tissues in its vicinity contribute to radiation complications that require additional treatment and worsen the quality of life of patients after a course of therapeutic radiation [4].

Ukrainian scientists continue to make a significant contribution to the study of general and individual human radiosensitivity, which is largely determined by the efficiency of DNA repair systems. It has been established that the radiosensitivity of individuals is genetically determined, and the influence of endogenous and exogenous factors can modify it. Therefore, at the same radiation dose, radiation effects vary significantly [5–7]. This, in turn, creates the problem of genetic heterogeneity of the population, which must be taken into account when assessing the radiosensitivity of cells, critical tissues and organs, as well as the human body as a whole in normal and oncological conditions. In this direction, for the first time, a passport of individual radiosensitivity was developed for persons exposed to ionizing radiation in above-background doses, primarily for workers at nuclear power plants. The passport is based on the cytogenetic parameters of peripheral blood cells (T-lymphocytes) using G_0- and G_2-tests [8]. The implementation of the Passport of Individual Human Radiosensitivity by Cytogenetic Indicators approved by the Ministry of Health of Ukraine (Information Letter No. 322-2018) will help optimize radiation protection of professionals exposed to ionizing radiation.

Therefore, based on the current concepts and paradigms of clinical radiobiology and radiation oncology, it is relevant to review and analyse the role of repair processes in the formation of radiosensitivity of healthy and tumour cells (comparative aspects).

Repair processes and radiosensitivity of healthy human cells. The ability of cells to repair DNA is the most important indicator of their radiosensitivity. It is an extremely complex coordinated system, the efficiency of which determines the further viability of irradiated cells. In fact, it is a whole complex of interconnected signalling processes, each of which controls a certain link of intracellular metabolism. The repair system includes DNA damage sensors and effectors of reparative processes. Sensors are proteins that constantly survey the genome in search of damage. Once damage is detected, these proteins signal to effector groups that are responsible for cell fate: programmed cell death; processes that cause a block in the cell cycle progres-

sion (checkpoints) and processes that repair DNA breaks. Let us dwell in more detail on the mechanisms of repair processes and formation of radiosensitivity of irradiated normal cells of the human organism. DNA double-strand breaks (DSBs) are recognized as critical radiation-induced damage because they cause genome instability, reproductive cell death, and are the earliest of pre-carcinogenic events [9]. A strong argument in favour of the leading role of DNA DSBs in the processes of chromosome aberrations formation is the similarity of DNA DSBs and chromosome aberrations output depending on the linear energy transfer of ionizing radiation (in both cases, a maximum in the region of 100–200 keV/μm is observed) and the stage of the cell cycle. Similar correlations are not observed for other types of DNA damage [10]. Two mechanisms, homologous recombination (HR) and non-homologous end joining (NHEJ), are involved in repair of this type of damage [11]. Both mechanisms differ from each other in the properties of the genes encoding the corresponding proteins, in their place in the cell cycle, as well as in the speed and error-free nature of repair. To implement HR, intact homologous DNA serves as a matrix. The HR mechanism is error-free and proceeds with the involvement of several genes: *RAD51B*, *RAD51C*, *RAD51D*, *XRCC2* and *XRCC3*. Absence of these genes or mutations in them block HR processes (*Fig. 1*). Along with these genes, two other gene families are involved in repair, the disruption of whose functions causes DNA repair defects in human cells: *BRCA1* and *BRCA2* [12].

Compared to HR, NHEJ repair, when DNA DSBs are reunited without homologous sequences, is a faster but less accurate process. Often deletions or base insertions are found in the repaired sites. Usually, unrepaired DNA DSBs are lethal due to loss of chromosome sections in subsequent mitosis, resulting in the loss of tens or hundreds of genes. A small part of genomic DNA includes coding genes or regulatory regions, and therefore the probability of breaks in them is low. In addition, these regions may turn out to be inactive (not expressed) and/or play an insignificant role in the functioning of the genome. NHEJ, according to the figurative expression [12], is a "fast and sloppy repair mechanism" that gives irradiated cells the maximum chance to survive (*Fig. 2*).

By affecting the repair processes of DSBs DNA, its radiosensitivity can be significantly enhanced. Thus, the key enzyme of NHEJ repair is DNA-dependent protein kinase. Compounds that inhibit the activity of this enzyme inhibit DNA repair processes and have a radiosensitising effect.

Note that DNA packaging and compact structure of chromosomes pose a problem for the implementation of repair processes. The corresponding proteins must be represented by a large number of copies and have sufficient mobility to detect damage within seconds or minutes after its appearance. The structure of chromatin changes becomes more relaxed to allow access of repair proteins.

Upon irradiation, one part of DSBs results from simultaneous breakage of two DNA strands at the

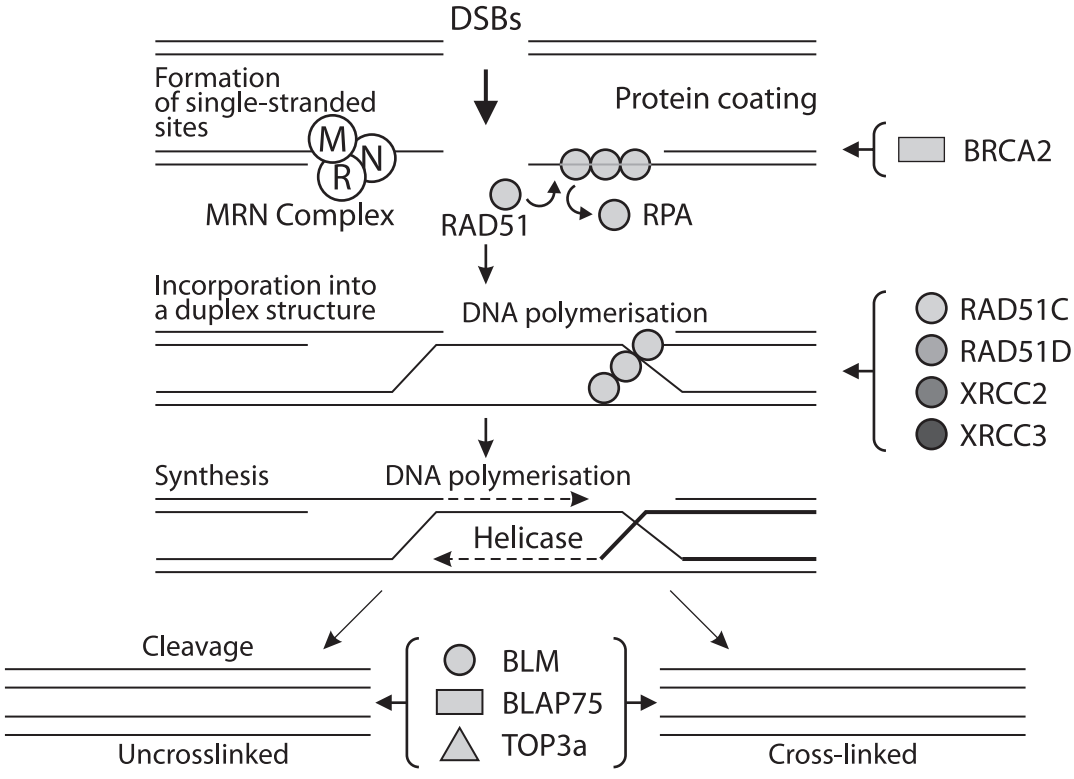

Fig. 1. Schematic of HR repair of DSBs DNA [12]

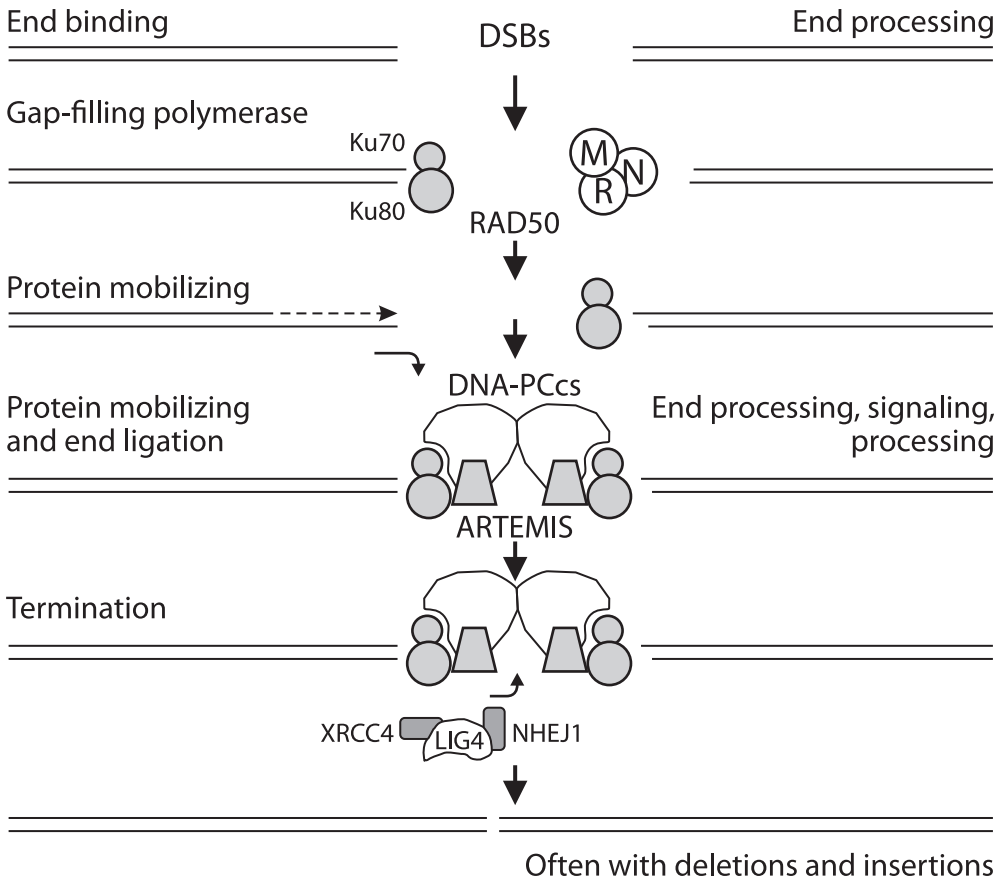

Fig. 2. Schematic of NHEJ repair of DSBs DNA [12]

same location and the occurrence of such breaks is proportional to the radiation dose. The formation of more than 90% of such DSBs is characteristic of irradiation in the low dose range. Another part of DSBs is formed as a result of two independent single breaks in a certain region of DNA; the number of breaks in this case increases in proportion to the square of the radiation dose and is characteristic of the action of large doses of radiation [13].

HR has been found to occur in the late S/G_2-periods of the cell cycle, while NHEJ occurs in the G_1-period [14, 15], and according to [12] — in all periods of the cell cycle. Since NHEJ is often accompanied by shortening of nucleotide chain ends and other repair errors, while HR provides error-free repair of double-stranded DNA, the genotoxic effect of ionizing radiation is most pronounced in cells that were in the radiosensitive G_1-period of the mitotic cycle at the time of irradiation.

The choice of the mechanism of DSBs repair in DNA is determined by several factors. According to [16] sensor proteins compete for binding to DNA ends, which partly determines the choice of repair mechanisms. This is the so-called passive competition. There is an insight into the role of matrix accessibility. For homologous recombination to occur, a homologous DNA site must be available, which is located on the sister chromatid in the S- or G_2-period of the cell cycle.

It is known that cells exhibit increased radioresistance in the late S-period [9]. Knockout or reduction of HR gene activity removes the phenomenon of radioresistance at this point of the cell cycle [17]. Based on this, it is concluded that inhibition of the NHEJ system has more severe consequences for cells than suppression of the HR system.

Due to the impeded repair, "direct" DNA DSBs are more important in the development of mutagenesis than "oblique" ones [18]. It is concluded that slow and unrepairable DSBs represent "straight" DNA chain breaks. Unrepaired or incorrectly repaired DNA DSBs are triggers of events leading to the formation of such biological effects as cell death, mutations, malignant transformation, etc. Loss or alterations in DNA genetic information are directly related to changes in the clonogenic potential of somatic cells. One of the main types of effects of radiation on DNA is structural rearrangements of chromosomes, which are visualized at the metaphase stage. Therefore, to manifest radiation damage to the genome, a cell must retain the ability to divide, i.e. have clonogenic potential.

Repair processes and radiosensitivity of human tumour cells. Compared to healthy tissues, tumours are characterized by intensive proliferation, which partially

determines their specificity. As noted above, DNA repair processes in tumour cells are more intensive than in healthy cells [1–3]. The difference in radiosensitivity of tumour and healthy cells constitutes the radiotherapeutic interval. Tumours are classified as radiosensitive if they regress after irradiation without necrosis of the surrounding connective tissue and as radioresistant — without regression at doses that destroy the surrounding healthy tissue. Although this division is rather conventional [19].

The radiosensitivity of a tumour depends on its histological structure, the degree of differentiation of cellular elements, the ratio of the stroma and parenchyma. Radiosensitivity of a tumour also depends on its size. Small tumours with a well-developed blood network are characterized by more intensive cell division and increased radiosensitivity. The localization of the tumour also affects its radiosensitivity. For example, squamous cell cancer of the root of the tongue is more radiosensitive than tongue tip cancer. This depends on the different degree of blood supply and oxygenation of the irradiated area.

The formation of radiosensitivity of tumour cells is influenced, first of all, by the processes of DNA repair, regeneration, repopulation, and adaptation, which provide homoeostasis and protection of molecular and cellular structures; as well as prolonged hypoxia, glucose deficiency, and cells in the resting stage (G_0).

Currently, the increase in the effectiveness of radiation therapy of cancer patients is associated with the consideration of individual radiosensitivity of tumours and tumour bearers [20]. Individual radiosensitivity is polygenic and multifactorial in nature, as it depends on the effective work of many components of this system. Those tumour cells responsible for radiosensitivity are, firstly, those tumour cells that are in a state of hypoxia; secondly, those in the quiescent stage and in the DNA synthesis stage of the cell cycle; thirdly, cells with high reparative potential. The ratio of these cell subpopulations in tumours of different types is not the same, which is reflected in individual radiosensitivity. Therefore, identification of radiosensitive patients using informative radiobiological methods (G_2-assay) taking into account their individual radiosensitivity will allow effective therapeutic irradiation at high doses or with the use of radio-modifiers.

Tumour development is also associated with increased genome instability [21, 22], which affects error-free DNA repair processes.

When cells are exposed to hypoxia, the expression of DNA repair genes decreases, which leads to disruption of homologous recombination (HR repair). Researchers note that hypoxia can act as a factor in the selection of cells with mutations [12].

Concluding remarks. The analysis of literature data and our own research in the field of clinical radiobiology and radiation oncology proves that cellular DNA repair systems in a number of the above cases can increase the radioresistance of tumours and thus inhibit the effectiveness of therapy in these patients. The noted functional differences in the repair processes in tumour cells compared to healthy tissues outline a clear path to improving the effectiveness of radiation treatment of cancer patients: the development and implementation of protectors to protect the genome of healthy cells from the environment of the irradiated tumour without affecting (reducing) its radiosensitivity. This, in turn, will help to minimize radiation complications that require additional therapy and worsen the quality of life of treated patients.

REFERENCES

1. **Ciszewski WM, Tavecchio M, Dastych J, Curtin NJ.** DNAPK inhibition by NU7441 sensitizes breast cancer cells to ionizing radiation and doxorubicin. Breast Cancer Res Treat 2014; **143** (1): 47–55. doi: 10.1007/s10549-013-2785-6.
2. **Chow JP, Man WY, Mao M, *et al.*** PARP1 is overexpressed in nasopharyngeal carcinoma and its inhibition enhances radiotherapy. Mol Cancer Ther 2013; **12** (11): 2517–28. doi: 10.1158/1535-7163.MCT-13-0010.
3. **Domina EA, Dumanskyi YuV, Makovetska L.I, et *al.*** Radiation therapy of cervical cancer patients: view of radiobiologists. Oncology 2024; **26** (2): 85–94. doi: 10.15407/oncology.2024.02.085. (in Ukrainian)
4. **Ivankova VS, Domina EA, Khrulenko TV, *et al.*** Radiotherapy of oncogynecological patients in the conditions of modern technologies. Clin oncol 2021; **1** (1): 1–5. doi: 10.32471/clinicaloncology.2663-466X.43-3.28535. (in Ukrainian)
5. **Domina EA, Druzhyna MO, Ryabchenko NM.** Individual human radio sensitivity. K: Logos, 2006. 126 p. (in Ukrainian)
6. **Kovalev EE, Smirnova OA.** Estimation of radiation risk based on concept of individual variability of radiaosensitivity. AFRRI Contact Report 96-1. Glen I. Reeves (eds.) Armed Forces Radiobiology Research Institute Bethesda, Maryland, USA, 1996. 202 p.
7. **Domina EA.** Radiogenic cancer: epidemiology and primary prevention. K: Naukova Dumka, 2016. 196 p. (in Ukrainian)
8. **Domina EA, Drugina MO.** Passport of individual radiosensitivity of humans for cytogenetic indicators. Information sheet No. 322-2018. Kiev, Ministry of Education and Science of Ukraine, 2018. 4 p. (in Ukrainian)
9. **Grinevich SA, Domina EA.** Immune and cytogenetic effects of dense- and rare-ionizing radiations. K: Avicenna, 2021. 384 p. (in Ukrainian)
10. **Obaturov GM, Filimonov AS.** Role of DNA double-strand breaks in the processes of radiation structural mutagenesis in eukaryotic cells. Rad Biol Radioecol 1993; **33** (2 (5)). 717–23.
11. **Lieber MR.** The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. Ann Rev Biochem 2010; **79** (1): 181–211.
12. **Joiner MC, van der Kogel A.** Basic Clinical Radiobiology (4th ed). London: CRC Press, 2009. 375 p. https://doi.org/10.1201/b15450.
13. **Yarmonenko SP, Vainson AA.** Radiobiology of man and animals. M: Vysshaya Shkola, 2004. 549 c.
14. **Rothkamm K, Kruger I, Thompson LH, Lobrich M.** Pathways of DNA double-strand break repair during the mammalian cell cycle. Mol Cell Biol 2003; **23** (16): 5706–15. doi: 10.1128/MCB.23.16.5706-5715.2003.

15. **West SC.** Molecular views of recombination proteins and their control. Nat Rev Mol Cell Biol 2003; **4** (6): 435–45. doi: 10.1038/nrm1127.
16. **Brugmans L, Kanaar R, Essers J.** Analysis of DNA double-strand break repair pathways in mice. Mutat Res 2007; **614:** 95–108. doi:10.1016/J.MRFMMM.2006.01.022/.
17. **Tamulevicius P, Wang M, Jliakis G.** Homology-directed repair is required for the development of radioresistance during S-phase: interplay between double-strand break repair and checkpoint response. Radiat Res 2007; **167** (1): 1–11. doi: 10.1667/RR0751.1.
18. **Cramer P, Verhoeven EE, Filon AR, *et al.*** Damage in cockaine syndrome. Cells Radiat Res 2011; **175** (4): 432–43. doi: 10.1667/RR1972.1.
19. **Ivankova VS, Domina EA.** Problems of tumor sensitivity in radiation oncology. K: Zdorov'ya, 2012. 192 p. (in Ukrainian)
20. **Domina EA, Ivankova VS.** Individual radiosensitivity of tumours (radiobiological and clinical aspects). Problems of ecological and medical genetics and clinical immunology 2009; **4** (91): 33–42. (in Ukrainian)
21. **Bristov RG, Hill RP.** Hypoxia and metabolism. Hypoxia DNA repair and genetic instability. Nat Rev Cancer 2008; **8** (3): 180–92. doi: 10.1038/nrc2344.
22. **Domina EA.** Radiation-induced instability of the human genome during low-dose irradiation. Chapter 8. Heritage of European science '2024. Karlsruhe: ScientificWorldNetAkhatAV, 2024: 88–101.

РОЛЬ ПРОЦЕСІВ РЕПАРАЦІЇ У ФОРМУВАННІ РАДІОЧУТЛИВОСТІ ЗДОРОВИХ ТА ПУХЛИННИХ КЛІТИН ЛЮДИНИ

Е.А. Дьоміна

Інститут експериментальної патології, онкології і радіобіології ім. Р.Є. Кавецького НАН України, Київ, Україна

Резюме. *Незважаючи на суттєвий прогрес у створенні та впровадженні нової радіотерапевтичної техніки упродовж останнього десятиріччя та позитивні результати променевої терапії онкологічних хворих показники загальної та безрецидивної їх виживаності в цілому залишають бажати кращого. Аналіз даних літератури та власних досліджень в галузі клінічної радіобіології і радіаційної онкології доводить, що клітинні системи репарації ДНК підвищують радіорезистентність пухлин і таким чином стримують підвищення ефективності терапії хворих. Детально розглянута роль процесів репарації у формуванні радіочутливості пухлин порівняно із здоровими тканинами, описані їх відмінності. Акцентується увага на тому, що репарація пошкоджень опроміненої ДНК у пухлинних клітинах відбувається більш інтенсивно, ніж у клітинах здорової тканини через підвищення експресії ферментів репарації. Разом з нестабільністю геному пухлинних клітин це обумовлює велику варіабельність їх радіочутливості та вказує на пріоритетність досліджень, спрямованих на пошук та розробку радіопротекторів для захисту геному здоро вих клітин із оточення опромінюваної пухлини, не впливаючи (не знижуючи) її радіочутливість. Такий шлях оптимізації результатів терапевтичного опромінення сприятиме мінімізації променевих ускладнень, що потребують додаткової терапії та погіршують якість життя пролікованих хворих.*

Ключові слова: онкологічні хворі, променева терапія, репарація ДНК, радіочутливість, генетична нестабільність.

Адреса для листування:

Дьоміна Е.А.
3022, Київ, вул. Васильківська, 45
Інститут експериментальної патології, онкології і радіобіології ім. Р.Є. Кавецького НАН України
E-mail: edjomina@ukr.net

Одержано: 10.09.2024