KAPITEL 10 / *CHAPTER 10* [10]
PARTICULARITIES OF RADIATION CARCINOGENESIS: HISTORY AND FACTS

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Introduction.

Due to the Chernobyl catastrophe, the efforts of many radiobiologists are concentrated on clarification of patterns and mechanisms of biological action of ionizing radiation (IR) in the low dose range. Up to the middle of the last century, it was believed that a quantum of IR energy induced irreversible changes when interacting with chromosomes. These postulates essentially formed the basis of the linear threshold-free concept, which implies unconditional, including carcinogenic, danger of any levels of irradiation. However, in the middle of the last century, in the works of the famous scientist-radiobiologist N. Luchnik, an anomalous character of radiation reactions to low-dose exposure was discovered. It was shown that primary radiation-induced DNA damage can be repaired during repair processes. The most representative information on the response of cells to low-dose irradiation was obtained by cytogenetic study of human peripheral blood lymphocytes (PBL). The unique combination of properties has naturally put lymphocytes in the first place among other test systems designed to assess the genetic effects of low-dose irradiation. It is on this object that large-scale studies of the dose-effect relationship have been carried out [1-3]. The obtained facts indicate that the frequency of chromosomal aberrations (CA) in the low dose range linearly depends on the dose value. Another feature of the obtained dose curves is the presence of a dose-independent region (plateau) for the yield of radiation-induced CA. We have shown that such results are satisfactorily approximated using the model of piecewise linear splines [4].

Radiation carcinogenesis. Information about the occurrence of radiogenic cancer appeared 10 years after the discovery of X-rays. The first victims were medical workers who were unaware of the carcinogenic properties of IR. Later, it turned out that there is a long latency period between radiation exposure and cancer occurrence.

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At the same time, the issue of dose dependence of tumour occurrence and threshold of this phenomenon remained complicated and debatable.

The problem of radiation-induced carcinogenesis (RIC) has become especially urgent in connection with remote negative consequences of the Chernobyl catastrophe. According to WHO data, 90% of human malignant neoplasms (MNN) are caused by the impact of environmental factors, including radiation induced carcinogenesis (RIC). According to modern ideas, genome instability plays a determining role in the initiation of RIC. "Cancer is a genetically determined disease arising due to disruption of normal regulation of cell growth under the influence of a variety of mutations" [5]. As defined by [6] "Cancer is a disease of gene expression in which the normal functioning of the complex networks that control homoeostasis is disrupted, allowing cells to grow out of sync with the needs of the organism as a whole". Most tumours are induced solely by mutations in somatic cells, but some types can be caused by inherited mutations passed on by germ cells from one generation to the next. Hereditary tumours determined by a single gene account for only 5.0-7.0%, and the bulk are multifactorial tumours that develop under the influence of genetic and external environmental, including radiation, factors [7].

The process of malignant transformation begins with an initiating mutation that disrupts the regulatory mechanisms of cell multiplication. Depending on the cell type, the initiating mutation can be expressed immediately or after a latent period. In the latter case, for the expression of the initial oncogenic mutation, stimulation or promotion must occur, i.e. mitogenic signalling, which may be caused by the death of adjacent cells or exposure to agents capable of accelerating cell growth. The initiated (transformed) cell is morphologically indistinguishable from a normal cell. Elusive phenotypic changes are detected only after the formation of a cell clone: root growth, abnormalities in the shape of nuclei, etc. [8].

At the initiation stage, which proceeds latently, mutations - deletions of regulatory genes associated with DNA replication - occur [9].

Metabolic activation of carcinogens can be suppressed by antioxidants and therefore at this stage of carcinogenesis their detoxification (e.g. by glutathione-S-

transferases), as well as DNA repair and apoptosis of initiated cells are still possible. The changes arising during carcinogen exposure become irreversible (turn into mutations) only after replication and mitosis, i.e. during the next proliferative cycle, after which repair does not eliminate the changes in DNA structure [8].

The next stage of carcinogenesis - promotion - involves expression of the phenotype of initiated cells, evolution and selection of clones, clonal expansion, selective advance with the formation of proliferation foci, which in some cases can be considered as pre-tumour hyperplastic changes.

If at the initiation stage, there is activation of cellular oncogenes and/or inhibition of tumour-suppressor genes causing the appearance of DNA alterations, at the promotion stage there is activation of oxidative processes contributing to accelerated multiplication of initiated cells and irreversible process - formation of proliferate clone.

The progression stage is the acquisition of invasiveness, i.e. the ability of tumour cells to invade the surrounding normal tissue, disrupting its function. The acquisition of invasiveness requires additional mutations. The stage of progression means "essentially going beyond the limits of proper carcinogenesis, transition of the process into blastomogenesis, generalisation and metastasis" [8]. The last stage in tumour progression is metastasis - migration of primary tumour cells to other tissues and organs, formation of secondary tumours. Tumour progression is a multistage process and the accumulation of several somatic mutations is necessary for the development of metastases. Most tumour types are of clonal origin, that is, they develop from a single atypical cell. This mechanism is intrinsic to the initiating mutation. As genetic instability develops in the primary tumour, which is clonal in origin, secondary mutations can occur in different tumour cells. Secondary mutations are the result of genetic instability arising from loss of control of DNA replication or DNA repair. The results of cytogenetic study of secondary tumours arising after radiation therapy of cancer patients showed that the most common karyotype of cells of such tumours is chromosomes with multiple deletions [10]. Mutations in genes controlling DNA repair processes may also be involved in the process of tumour initiation or progression.

Mutations occur in the genome of tumour cells at a much higher rate than in

normal cells. Changes in the conditions of genome functioning, decrease in the efficiency of repair processes correspondingly increase the level of genetic instability, the manifestations of which vary from increased mutability of individual genes to disorders of organism development as a whole. The accumulation of mutations in genes controlling cell growth and cell death, the increased frequency of chromosomal rearrangements in tumour cells are associated with the increase in genome instability [11]. Thus, tumour development is accompanied by accumulation of genetic changes, the early sign of which is genome instability. Genomic disorders determine and model malignant phenotype, metastatic potential, and tumour progression rate.

IR is among the most potent immunosuppressants and complete carcinogens, which are able to achieve their neoplastic potential at all stages of the tumour process. The participation of IR may also be expressed in the implementation of one of the stages of carcinogenesis. Malignant transformation of cells is detected already at doses less than 0.3 cGy, which insignificantly exceeds the levels of radiation background. Thus, IR can initiate and cause the emergence of new tumours and/or accelerate the processes of malignant transformation of cells, the development of which was not initially associated with irradiation.

There are two main classes of genes responsible for the initiation of carcinogenesis processes, but the mechanisms of their involvement in malignant transformation of cells are directly opposite. The first are oncogenes or growth acceleration genes, mutations in which are phenotypically manifested as dominant. This means that the mutant oncogene stimulates continuous cell growth despite the presence of the normal alley. The second are tumour growth suppressor genes, which prevent cells from proliferating. Mutations in these genes behave as recessive alleys, meaning that the presence of one normal alley is sufficient to regulate cell growth. Thus, oncogenes cause malignant cell degeneration when their expression is increased, and tumour growth suppressor genes when their expression is decreased or completely switched off. When proto-oncogenes are converted into oncogenes, new genetic information appears, while inactivation of suppressor genes is the result of loss of genetic information. Radiation-induced inactivation of suppressor genes is largely due

to deletions, and activation of proto-oncogenes is due to point mutations or chromosomal rearrangements. CA, which are formed due to DNA double-strand breaks, play a more important role in the development of RIC than point mutations [12]. Transduction has been established in RIC: radiation-induced DNA fragments are released from a damaged cell after its death and are incorporated into the genome of a neighbouring normal cell, thus transforming it [13].

Until recently, the prevailing paradigm in radiobiology was that IR causes oncogenic transformation of cells through the genetic mechanism of formation of radiation-induced DNA damage, which is realized in point and chromosomal mutations. The data on the mechanisms of RIC formation have been obtained, which allow to reasonably search for markers of increased risk of tumour development of radiation genesis. Especially promising are cancer-specific DNA markers, some of which are formed at early, preclinical stages of carcinogenesis. At present, this paradigm has been supplemented by extremely important facts, according to which epigenetic mechanisms aimed at altering gene function rather than structure are also involved in the occurrence of RICs [14-16]. Intensive accumulation of knowledge about epigenetic regulation of genome functioning highlights the necessity to study new aspects of genotoxic and carcinogenic effects of IR on the human organism. The obtained evidence indicates that inherited changes regulated by epigenetic modifications may play a crucial role in cancer evolution [17,18]. The process of DNA methylation provides structural and functional organization of the genome, but there are no changes in the genetic code. Epigenetic modification of DNA not only increases the level of spontaneous mutagenesis, but also affects the nature of interaction between DNA and carcinogens. While genotoxic carcinogens induce mutations, epigenetic carcinogens modulate the expression of DNA damage, acting as initiators and promoters of carcinogenesis. Cells have defence mechanisms (suppressor genes, their protein products, enzymes) that "counteract carcinogens and oncogenes" [8]. However, they may be susceptible to mutation, epigenetic inactivation, genotoxic and oxidative stress, which, in turn, increases carcinogenic risk.

Genetic and epigenetic abnormalities can cause inherited disturbances of

homoeostasis pathways through two different mechanisms. As mentioned above, there is activation of oncogenes, mainly through point mutations, or through inactivation of tumour suppressor genes. The idea that both copies of a tumour suppressor gene must be disabled in a malignant cell line was proposed by Knudson in 2001 and has been widely accepted by the scientific community. Direct mutations in the coding sequence, loss of parts or whole copies of genes, or epigenetic silencing can interact with each other to drive key control genes into an "off" state [19].

Thus, the potential for epigenetic and genetic events to interact to direct sequential cellular abnormalities throughout neoplastic progression has been discovered. According to Knudson's "two-hit theory", neoplastic transformation is "a complex, multi-step process involving random activation of oncogenes and/or silencing of tumour suppressor genes and is accomplished through genetic and epigenetic events". Unlike genetic alterations, the changes that arise from epigenetic modifications are potentially reversible. This creates a real possibility to modify epigenetic changes at the initial stages of carcinogenesis, preventing the development of malignant tumours.

The main criterion for the development of distant stochastic (carcinogenic) consequences of irradiation is considered to be the probability of unrepaired genome damage in the surviving cell. Through apoptosis in a multicellular organism genetically defective cells are eliminated, including those with mutations responsible for malignant transformation. The processes of repair and apoptosis are effective at different periods of time. The period when the processes of repair and apoptosis are simultaneously effective is short. At the same time, the key role in prevention and development of the effects of low doses of IR belongs to repair processes.

It is now recognized that genome stability is ensured by the efficient functioning of the so-called DNA damage response system (DDR) in the cell. DDR is a universal system for repairing cell genome damage resulting both from endogenous processes (e.g. replication errors) and external stress factors, including IR. DNA damage repair (DDR) is a whole group of signalling processes closely related to each other, each of which controls a certain link of intracellular metabolism. The repair system includes two sets of components: DNA damage sensors and effectors of reparative processes.

Sensors consist of groups of proteins that constantly "survey" the genome in search of damage. Once damage is detected, the proteins signal the three major effector groups responsible for the fate of the irradiated cell. These are programmed death processes, which discard damaged cells; DNA repair processes, which repair defects (breaks); and checkpoints, which cause a block in the cell's progression through the cycle [20].

Unrepaired or erroneously repaired two-strand DNA breaks are triggers of events leading to the formation of such biological effects as cell death, mutations, malignant transformation, etc. Loss or changes 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 are structural changes in chromosomes, which are visualized at the metaphase stage. 30-40% of DNA breaks in the cell are caused by the indirect action of free radicals - products of water radiolysis. Free radicals containing unpaired electrons are characterized by extremely high reactivity and are able to diffuse over a distance of 1 nm. Mutational damage to DNA leads to genome instability in the early stages of carcinogenesis. Genetic instability drifts towards the progression of the cancer process and the formation of new mutations in oncogenes and tumour-suppressor genes that lead cells to selectively accelerate growth and clonal selection [8].

Irradiation induces a wider range of genetic damage compared to the action of chemical agents. Density-ionizing radiation induces a greater yield of difficult-to-repair two-strand DNA breaks compared to rare-ionizing radiation and is therefore characterized as a stronger initiator and promoter of radiogenic cancer [7, 21].

Radiation-induced damage to the genetic material of a cell is the leading cause of its reproductive death and the appearance of inherited disorders. It is especially manifested at high doses and high power of IR, when reparative capabilities of a cell are insufficient for elimination of a large amount of formed DNA damages. As the dose rate decreases, the significance of genetic damages due to repair processes decreases. Damages of membrane structures, which fulfil an important role in maintaining cell functioning and viability, begin to prevail. Increase of dose rate and value (up to certain limits) leads to decrease of promoter and increase of initiating function of irradiation in RIC development.

The genetic predisposition to radiation-induced damage in combination with reduced reparative potential of cells, cell cycle dysregulation, abnormalities in cellular and humoral immunity indicators contributes to an increase in carcinogenic risk by about 10 times [22].

Assessment of the risk of radiogenic cancer development in real environmental conditions is associated with significant difficulties due to a multistage of carcinogenesis and the impact of additional factors on the irradiated cell genome. Sublethal and potentially lethal damage initiated by radiation persists in the cell for a long time until the subsequent exposure to the promoter [23]. The influence of the dose rate factor is related to the intensity of DNA repair processes, especially at fractionated irradiation regime. The frequency of carcinogenic effects is sharply reduced during irradiation with low dose rate (up to 15 mGy/min).

The problem of radiogenic cancer largely lies in understanding of the mechanisms of action of low doses of radiation on the genome, tissues, organs and the human body as a whole. Close attention to this problem is due to the fact that, firstly, the effects of low doses of radiation are promoters of carcinogenesis [24]; secondly, the effect of radiation on the processes of oncogenesis in the range of low doses may be greater per unit dose compared to the effects of high doses. This is explained by the fact that in the first case cell death is less pronounced and therefore repair processes are not stimulated by cell death; induced genome instability changes the sensitivity of irradiated cells to the action of other damaging factors.

The classical microdosimetric definition of a "low dose" of IR is a dose, at the action of which one ionizing track falls on one sensitive cell volume on average. The definition of "low dose" is strictly tied to the concept of "sensitive volume". If the cell nucleus is taken as a sensitive volume, then the dose per one act of energy absorption under rare ionizing irradiation is 0.2-0.3 cGy; if DNA is considered as a target, then the doses are in the range of 20-30 cGy.

At present, the problem of carcinogenic effects of low doses of irradiation, including molecular mechanisms of reactions to irradiation at high and low doses, continues to be actively discussed. Our radiation-epidemiological and cytogenetic

studies on a representative sample of participants of the liquidation of consequences of the Chernobyl accident (more than 17 thousand people) proved that "low doses of absorbed ionizing radiation are statistically significant risk factors for cancer and preservation of the dose-effect relationship for radiation cytogenetic markers (dicentric and ring chromosomes) in somatic cells of irradiated persons with malignant neoplasms in remote terms after Chernobyl confirm the radiogenic character of these diseases" [23,25].

One of the intensively developed problems associated with the aetiology of radiation carcinogenesis is radiation-induced genomic instability (RING). One of the main phenotypic manifestations of RING and malignant transformation of cells is chromosomal instability. The chromatid-type aberrations characteristic of the malignant phenotype are more likely to persist in a series of irradiated cell generations.

It has been established that RING is an effect of low-dose radiation exposure, the consequences of which can manifest themselves in 50 and more cell generations at doses less than 50 m3v for rare-ionizing radiation and 10 m3v for dense-ionizing radiation [26]. The study of the dose-effect relationship for RING induction indicates the saturation of the effect of irradiation in the low dose range. In somatic cells of liquidators exposed in the dose range up to 0.25 Gy, an increased frequency of mutations was found in 10 years, which is associated with the development of RING [27].

One of the important mechanisms of RING is the "bystander effect" phenomenon, in which the disorders similar to radiation-induced ones may occur in unirradiated cells located in close proximity to irradiated cells. In contrast to irradiated cells, in which mutations are represented by gene deletions, in bystander cells these are predominantly point mutations. "Bystander effect" plays an important role in the realization of remote radiation-induced carcinogenic effects. Formation of "bystander effect" increases carcinogenic risk in the area of low-dose radiation [28].

At low-dose irradiation in vitro and in vivo, the "bystander effect" is limited to the irradiation zone, i.e. it is formed within the irradiated volume, while at high doses of IR the produced clastogenic factors get into the bloodstream and thus cause distant

tissue damage [28].

The results of radiobiological studies of the science-intensive problem of RING indicate its key role in the development of RIC [29, 30].

Experimental and theoretical research in radiation biology contributes to the improvement of radiation therapy (RT) for cancer patients at different levels [31]. Theoretical insights - the conceptual basis of RT. This allows to study in depth the mechanisms and processes underlying the formation of radiation reactions of tumour and healthy tissues. These are primarily DNA repair mechanisms. Problem of reducing the frequency and severity of late complications in critical tissues and organs, including the occurrence of secondary cancer of radiation genesis as a consequence of RT remains important. Achievement of success in this direction is possible due to the development and use of radioprotectors that selectively increase the activity of repair enzymes and, thus, the radioresistance of healthy tissues surrounding the tumour [32].

Conclusion.

Genome instability plays a determining role in the initiation of radiation carcinogenesis. In contrast to genetic changes, changes arising due to epigenetic modifications are potentially reversible. Increasing the rate and magnitude of the radiation dose leads to a decrease in the promoter function and increase in the initiating function of radiation in the development of RIC. Low doses of absorbed IR are statistically significant carcinogenic risk factors. Preservation of dose-effect relationship for radiation chromosome markers in the blood of "liquidators" with oncological pathology in remote terms after the Chernobyl accident confirms the radiogenic nature of these diseas**es.**