

METHODS OF GENETIC TOXICOLOGY IN THE ASSESSMENT OF GENOMIC DAMAGE INDUCED BY ELECTROMAGNETIC IONIZING RADIATION

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Medical or occupational exposure of patients and healthcare personnel to ionizing radiation (IR) can be a cause of genetic disorders. In this article we discuss the efficiency of the following tests used to comprehensively assess the effects of ionizing radiation on the genetic apparatus of a cell: the Ames test, the micronucleus test and the FISH method. We provide examples of their use, outline their advantages and drawbacks, estimate the possibility of designing more advanced test systems and discuss requirements for their implementation.

Keywords: genetic toxicology, X-ray radiation, ionizing radiation, gamma rays, test system, Ames test, micronucleus test, FISH

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ИСПОЛЬЗОВАНИЕ МЕТОДОВ ГЕНЕТИЧЕСКОЙ ТОКСИКОЛОГИИ ДЛЯ ОЦЕНКИ ПОВРЕЖДАЮЩЕГО ДЕЙСТВИЯ ИОНИЗИРУЮЩИХ ИЗЛУЧЕНИЙ ЭЛЕКТРОМАГНИТНОЙ ПРИРОДЫ НА ГЕНОМЫ

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Пациенты и медицинский персонал регулярно подвергаются воздействию ионизирующих излучений (ИИ), которые могут быть причиной различных генетических нарушений в организме. В статье рассматриваются возможности тестов, используемых для интегральной оценки воздействия ИИ на генетический аппарат клетки: теста Эймса, микроядерного теста, метода FISH. Описываются примеры их использования, разбираются достоинства и недостатки каждого подхода, оцениваются перспективы разработки новых методов оценки и требования к ним.

Ключевые слова: генетическая токсикология, рентгеновское излучение, ионизирующее излучение, тест Эймса, микроядерный тест, метод FISH

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IR mutagenicity integrated assessment tests

The Ames test

The Ames test makes use of histidine auxotrophic strains of *Salmonella typhimurium*, which, when exposed to mutagens, can reverse to prototrophy. The improved version of the Ames test is now available: along with the well-studied mutations that cause the need for histidine, the salmonella genome has received a deletion in one of the repair genes (*uvrB-bio*), which has increased the sensitivity of these bacteria to mutagens. Also, an *rfa* mutation has been introduced into the genome of the test strains, blocking synthesis of the lipopolysaccharide capsule and thus increasing cell permeability. Some test strains of *Salmonella typhimurium* carry plasmid pKM 101 that contains genes increasing sensitivity of cells to agents enhancing DNA

recombination and inducing SOS mutagenesis. This plasmid also makes the cells of test strains resistant to ampicillin used as a marker for the presence of the plasmid [1].

In the study [2] researchers used the Ames test to assess the effect of X-ray computed tomography (at a standard dose range from 4.4 to 74.5 mGy) on bacterial viability, as well as genotoxic effects of irradiation. The mutant strain of *S. typhimurium TA 100 his G46, rfa, uvr-, pkm 101, bio* was used to evaluate mutagenicity. Toxicity was evaluated using the same mutant strain based on its survival rates observed in the experimental (irradiated) samples as compared to the controls. The irradiated samples had the number of colony-forming units (CFU) decreased approximately 5-fold.

The same bacterial model (*S. typhimurium TA 100*) was used to compare toxic and mutagenic effects of three different X-ray diagnostic procedures on X-ray machines [3]. The obtained

data suggest that, in the context of the Ames test, X-ray diagnostic imaging, except for the procedures performed on digital low-dose devices, produces toxic and weak mutagenic effects on the bacteria.

We believe that further search for the new test objects that can serve as a model system in toxicity and mutagenicity tests is a promising path from the viewpoint of assessing radiation safety of X-ray diagnostic regimes and methods.

IR integrated assessment tests based on quantification of stable and unstable chromosome aberrations

*Unstable aberrations of lymphocyte chromosomes.
Micronuclear test*

The micronuclear test performed using peripheral blood cells (erythrocytes, lymphocytes, buccal cells) allows detection of structural changes (aberrations) in chromosomes. Chromosome aberrations result from DNA rupture. Micronuclei are small DNA-containing formations that consist of acentric fragments of chromosomes devoid of centromeres or chromosomes stagnating at the anaphase or telophase stages. At the telophase stage, these fragments can join the nuclei of daughter cells or form single or multiple micronuclei in the cells' cytoplasm [4].

Petrashova et al. [5] studied samples of peripheral blood lymphocytes and buccal epithelium of miners working underground where radon concentration is high. Using the micronuclear test, the researchers revealed an increased (1.6–1.7 times) number of binucleated lymphocytes with micronuclei in the blood samples of miners who had been working for 20–40 years as compared to those with less work experience. Analysis of buccal epithelial cells showed that there were almost 2 times as many karyolysed cells and 20 times as many as binucleated cells in the experimental group as opposed to the control group. These results may point to the effect ionizing radiation produces on cytokinesis, which, when disrupted, can lead to the appearance of multinucleated cells.

Unstable aberrations of chromosomes of peripheral blood lymphocytes (formation of dicentric, acentric fragments and centric rings) help to estimate the level of ionizing radiation during radiological examinations of patients and screening of people who have been exposed to radiation [6]. Quantitative indicators of unstable aberrations, the so-called "biological" doses, provide information about the effect of radiation on the human body and reveal individual radiosensitivity of a person. This allows a more accurate assessment of possible early and long-term effects of irradiation. Even when exposed to the lowest doses of ionizing radiation (1 mGy or less, typical for X-ray examinations of the chest, esophagus and stomach), peripheral blood lymphocytes of the examined individuals show an increase in the level of chromosome aberrations [7]. Indicators of the "biological" dose reflecting low-dose irradiation are the subject of active discussions [8, 9]. A higher level of chromosome aberrations in peripheral blood lymphocytes may signal pathological processes in the human body even in the absence of clinical manifestations.

E. A. Demina [10] believes it expedient to apply biological (cytogenetic) dosimetry methods to estimate radiation doses received during X-ray screening. The researcher proposes registering radiation-induced chromosome aberrations in the peripheral blood lymphocytes *in vivo* and *in vitro* following an X-ray examination; the *in vitro* analysis can be performed in flasks with donor blood placed on the patient over the exposed areas. Such a control setting allows getting dosimetry

data by modeling irradiation conditions and using a tissue equivalent. In their studies [11], the researchers demonstrated the effectiveness of such modeling applied to chest X-ray and mammography.

The idea of using such control techniques seems interesting, but we believe it is not the optimal solution to reduce the risk of overexposure. Firstly, the control procedure takes place simultaneously with the radiographic examination of the patient. Data obtained during simultaneous irradiation of patient's and donor's blood cells may indicate overexposure (given a dose-effect calibration curve is available), but the patient will already have received the high dose. Secondly, the radiosensitivity of the control sample may differ from that of the irradiated patient, being either more radiosensitive or radioresistant. Thirdly, such control of X-ray diagnostic equipment is absolutely important for assessing the degree of radiation hazard that X-ray examinations expose patients to. However, we believe this problem requires models with a standard response to IR. Moreover, tests of X-ray diagnostic equipment that make use of these models should be conducted before the actual examination of the patient. Such an approach can stimulate solutions aimed at minimizing the radiation dose received during maintenance of X-ray machines and, more importantly, support the development of low-dose X-ray techniques, prompt introduction of technical innovations enabling further modernization of X-ray machines, elaborated to reduce radiation doses received by patients and personnel.

Cytogenetic methods based on the analysis of unstable aberrations frequency (dicentric, acentric and centric rings) are used for estimating the effect of irradiation on biological tissue and IR dosimetry [12]. Being a classical method, quantification of unstable aberrations of peripheral blood lymphocytes still has a number of limitations; however, it is often used in the examination of people exposed to IR.

*Stable chromosome aberrations in blood lymphocytes.
The FISH method*

Stable chromosome aberrations include symmetrical translocations, insertions and inversions. Their frequency remains almost the same for a long time after irradiation: months or even years. Cell proliferation does not lead to elimination of aberrations of this type, cells do not die and, therefore, continue to divide. The frequency of translocations can be analyzed using G-banding. To increase the informative value of the method, each chromosome is analyzed. However, it is a time-consuming process that requires highly-qualified specialists, even when automatic karyotyping is available. That is why FISH (fluorescent in situ hybridization) is the method of choice when it comes to analyzing the frequency of translocations. It is believed that FISH allows quick and reliable detection of aberrations frequency and can be used to construct dose-effect calibration curves for symmetric translocations.

The analysis of stable aberrations observed in patients who had undergone radiotherapy and in those who had suffered the atomic bombardment in Hiroshima [13] proved that stable translocations persist for long periods of time. The frequency of symmetrical translocations in such patients is about 90–95 %, it has not changed for decades and correlates with the received doses of radiation. Thus, the analysis of translocations is a viable way to perform retrospective assessments of IR doses.

Advantages and disadvantages of tests

To sum up, currently all types of integrated assessment are used of the effects produced by IR on living beings. Speaking

of humans, the methods also allow determining the severity of exposure. However, none of the tests is perfect. For example, it takes time for a phenotype to fix in the species' offspring; therefore, mutations that trigger phenotypical changes cannot be assessed immediately. Nevertheless, integrated assessment of the IR effect is performed on plants and small animals. Methods based on the unstable chromosome aberrations, in particular, the micronuclear test, are non-specific, since not only IR but also various toxins induce formation of micronuclei, although the number of micronuclei may be indicative of the severity of radiation damage after the exposure. Methods that estimate the number of dicentric, acentric and centric rings have flaws of their own, which we discussed above and can be used for early assessment in the case of a single acute exposure event given that irradiation was relatively uniform. The most common assessment method is based on stable aberrations. It is convenient due to the prolonged persistence of aberrations. Both G-banding and FISH are used to identify stable aberrations. However, differential staining is incapable of detecting additional threads attached to the chromosome, the absence of a thread or its malposition if the length of the thread is less than 3-5% of the chromosome's length [14]. Besides, G-banding is labor-intensive and requires highly-qualified specialists. The FISH method is used more widely, has a high resolution, but is labor-intensive and requires special equipment and reagents.

Relevance of application of genetic toxicology methods to X-ray diagnostic imaging and endovascular treatment using modern dose-contributing radiation technologies

In our opinion, a number of important points related to the use of X-rays in medical diagnosis should be noted. The number of medical X-ray procedures is growing [15], the average individual and collective radiation doses received during chest and

other X-ray examinations and fluoroscopic procedures have decreased slightly [16]. Above, we mentioned that attention should be paid to radiation safety measures in the context of X-ray-guided interventions [17], as well as to the major contribution of computed tomography (CT) to the treatment-related annual collective effective dose [18].

Indeed, methods of endovascular X-ray diagnosis and treatment are highly effective, but the radiation doses that come with them are significant [15]. The same is true for CT scans [18]. Research paper [19] shows that the dose-dependence of the amount of radiation-induced foci of DNA repair proteins is characterized by an increased effect in the range of 12–32 mGy. Other studies centered on IR, including X-ray (close dose range), mention the same effect [19]. A number of CT procedures imply radiation doses of such magnitude. In this light, development and improvement of methods for integrated assessment of IR effect produced on the genome of cells of critical organs is urgent and promising.

CONCLUSIONS

New methods are necessary for integrated assessment of the effect produced by electromagnetic IR, including X-rays, on the genome of cells of irradiated organs, that would allow accurate identification of individual radiosensitivity of patients and personnel performing X-ray-guided interventions and could be safely used in medical diagnosis. The requirements for such methods are determined by the object and objectives of the study. These methods should be easily accessible, not labor-intensive or time-consuming and cheap. Unfortunately, no method currently available meets all the listed requirements. However, we believe that the search should continue. Such methods could be employed by test systems aimed to monitor technical condition of the so-called "heavy" equipment enabling X-ray diagnosis and endovascular treatment.

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