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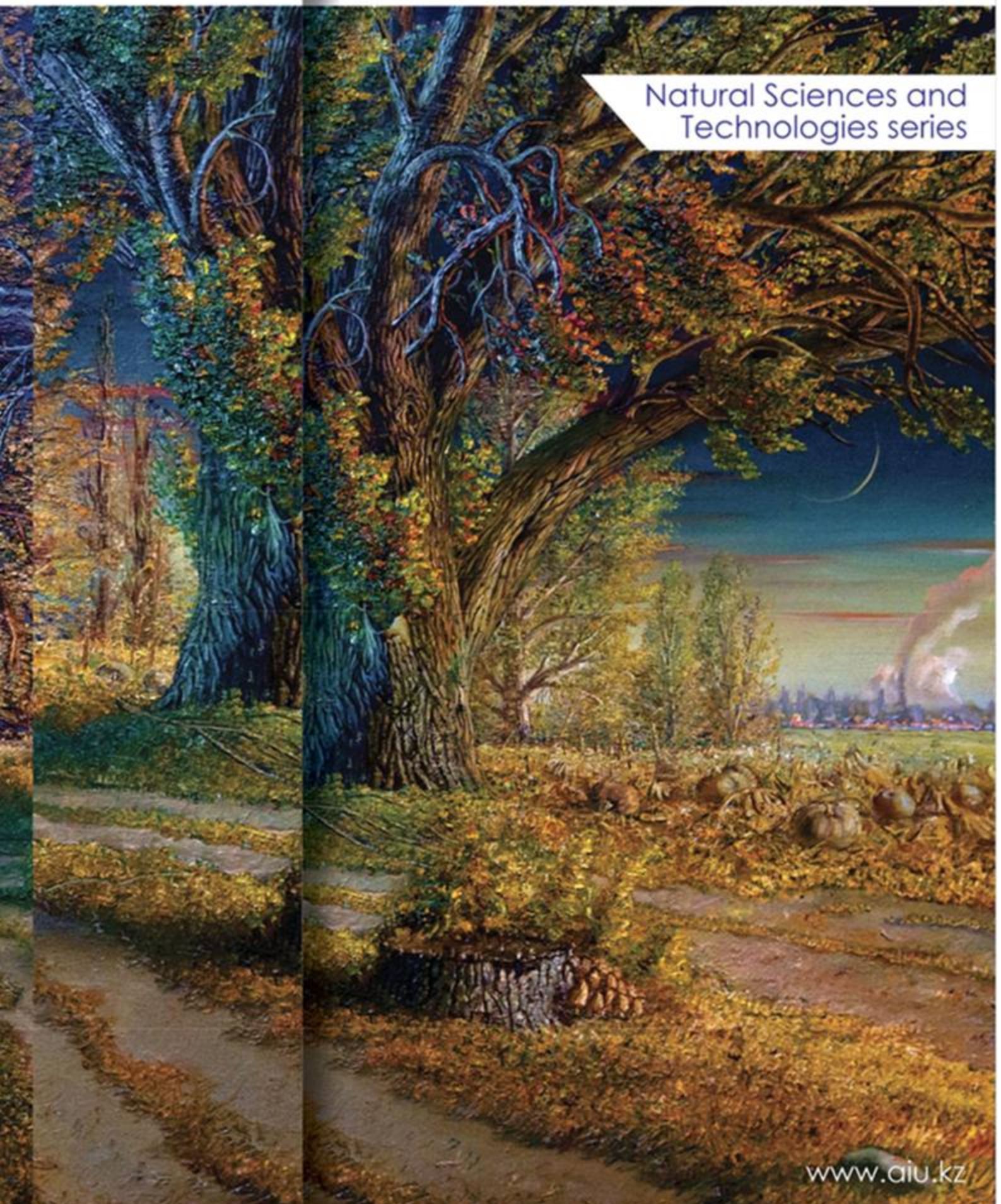

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CONTENT

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Blood mtDNA Copy Number as a Potential Indicator of X-ray Radiation Exposure in Animals

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Abstract. The dangerous effects of ionizing radiation on living organisms are a key problem for many countries, including Kazakhstan. The relevance of the study is due to the fact that in some regions of Kazakhstan, there is an increased level of background radiation, primarily associated with rich uranium deposits, as well as the presence of long-lived radioactive isotopes on the territory of the former Semipalatinsk nuclear test site. Moreover, occupational exposure to medical radiation may result in major consequences to human health. Since physical dosimeters have limitations in accurately assessing the extent of individual damage, one of the main tasks in radiobiology is the search for biomarkers of individual radioresistance. The aim of the study was to investigate the impact of ionizing radiation (X-ray) on the level of free circulating mitochondrial DNA. Blood plasma samples were collected from experimental rats following exposure to X-ray radiation. Quantitative real-time polymerase chain reaction was used to detect the copy number of mitochondrial DNA. On average, the copy number of free-circulating mitochondrial DNA in the blood plasma of irradiated rats (4.51x107copies/mL) was 2.7 times higher compared to the control group (1.67x107copies/mL). The obtained results indicate the potential role of free-circulating mitochondrial DNA as a biomarker for assessing X-ray exposure.

Keywords: ionizing radiation, X-ray, free circulating mitochondrial DNA (cf mtDNA), biomarker**.**

Introduction

Ionizing radiation is a type of radiation that has enough energy to remove tightly bound electrons from atoms, causing the atoms to become charged or ionized. Living organisms are exposed to natural radiation from the Earth's crust and space on a daily basis. Additionally, since the discovery of X-rays, various practical applications of ionizing radiation have emerged in fields such as radiation therapy and X-ray diagnostics. On the other hand, the discovery of X-rays has also led to the introduction of artificial radiation into everyday life [1]. Special attention should be paid to occupational exposure. Medical workers who operate or are in close proximity to the Electron-Optical Converter (EOC) for intraoperative X-ray imaging may be at risk of exposure to ionizing radiation. Prolonged or repeated exposure to X-rays can increase the risk of radiation-related health issues. It has been found that medical personnel who work directly with X-ray equipment are more susceptible to the risk of carcinogenesis than those who do not encounter ionizing radiation at work [2]. For example, Chou et al. demonstrated in their study that female orthopedic surgeons are at a higher risk of developing breast cancer compared to women who do not work with X-rays [3]. According to 2022 United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) estimates, the number of occupationally exposed workers totals 24 million, and 9 million of these are medical workers [UNSCEAR Report, 2021/22, https://www.unscear.org/unscear/publications/2020_2021_4.html]. Although UNSCEAR recognizes that the number of monitored workers is underestimated for both categories.

Due to the high energy levels of ionizing radiation, it can easily penetrate into the irradiated object, initiating radiolysis of H_2O inside cells, oxidative damage, and functional impairment of mitochondria [4]. Interestingly, ionizing radiation affects not only target cells but also neighboring cells, thereby demonstrating a "bystander effect" [5]. Furthermore, the consequences of radiation can be traced over generations in daughter cells, which, similar to the targeted parent cells, exhibit increased genomic instability, oxidative stress, and neoplastic transformation [4, 6].

Ionizing radiation affects cellular integrity in two main ways. Either directly, by causing single-strand (SSB) or double-strand (DSB) breaks in DNA structure [7]. In the case of SSB, repair occurs with less mutation rate, due to the presence of an untouched second strand, while DSBs are more problematic in this regard. Non-homologous end joining (NHEJ) is the most commonly used repair pathway of the organism to combat DSBs. However, NHEJ more often leads to mutations, which are likely to induce apoptosis [8]. Alternatively, ionizing radiation can take an indirect path by increasing the level of reactive oxygen species (ROS). In normal conditions, ROS are secondary messengers participating in various physiological processes of the organism [9]. But when the level of ROS increases, oxidative stress occurs, which leads to a disturbance in the integrity of nucleic acids [10]. Excessive ROS production is also associated with radiation-induced radiolysis of H_2O. Additionally, ionizing radiation initiates the leakage of electrons from the electron transport chain (ETC), which also leads to excessive generation of ROS [11]. According to the study conducted by Kim et al., ionizing radiation indirectly enhances mitochondrial biogenesis and expression of mitochondrial genes through its destructive effect. Most likely, the reason is that cells, in an attempt to compensate for radiation-induced mitochondrial dysfunction and increase their energy reserves, begin to increase the number of copies of mitochondrial DNA [12].

For a long time since the discovery of circulating nucleic acids, they have been attracting more and more attention. However, the mechanisms of release of circulating molecules into the bloodstream are not fully understood. According to some data, circulating extracellular DNA is released into the bloodstream in both normal and pathological conditions through various physiological and pathological mechanisms, for example, during apoptotic cell death. [13]. Moreover, in recent years, the focus has shifted from nuclear DNA (nDNA) to mitochondrial DNA (mtDNA). For example, the number of studies on changes in the level of free-circulating mtDNA (cf mtDNA) in response to various triggers continues to grow. Regarding the question of the impact of ionizing radiation on mtDNA, it is believed that mtDNA is more susceptible to radiation than nDNA, as mtDNA lacks histones and therefore is not protected by them, and it lacks an efficient repair system. In addition, mtDNA is located in close proximity to the ETC, which makes it more sensitive to ROS [10, 14]. Moreover, mtDNA can be released into the bloodstream during apoptosis and necrosis resulting from radiation exposure. In summary, it is evident that cf mtDNA could be a promising biomarker for assessing radiation exposure.

Materials and Methods

Animals. The study included 12-week-old male Wistar rats *(Rattus norvegicus albinus).* The rats were kept under standard conditions with a regular light/dark cycle in a temperature-controlled room and provided with a standard diet. Two groups were formed: Group 1 (n = 18), the experimental group, was exposed to X-ray with a total dose of 1 Gy using linear accelerator “Clinac 600C” (Varian Medical System, USA); Group 2 (n = 9), the control group, underwent simulated radiation without receiving the corresponding dose. Euthanasia of rats was performed using isoflurane on the following day after the last animal irradiation.

The study fully complied with all ethical principles and requirements of the Helsinki Declaration and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. The research was approved by the ethics committee of Nazarbayev University (protocol #03-2022).

Plasma extraction. Blood was collected in BD Vacutainer tubes (#367525, Becton Dickinson and Company, United Kingdom) containing EDTA-K2. To separate the plasma from whole blood, centrifugation was performed at 9000 rpm for 15 minutes. The plasma was aliquoted into 0.5 mL tubes and frozen at -80°C.

DNA extraction. The extraction of total extracellular DNA from blood plasma samples was performed using the "PROBA-NK/PROBA-NK-PLUS" reagent kit (#D07-2, DNA-Technology, Russia) following the manufacturer's protocol. The measurement of extracellular DNA concentration

in the samples was performed using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, USA).

PCR analysis. The determination of the level of cf mtDNA was performed using qPCR. All samples were analyzed in duplicate using the QuantStudio™ 3 Real-Time PCR System (Thermo Fisher Scientific, USA).

As a target for the detection and quantification of fc mtDNA, the mt-specific 16S rRNA (230 bp) was selected. The primer sequence and PCR program are presented in Table 4. The primers were synthesized by Syntol LLC (#2774, Russia).

The reaction mixture, with a total volume of 25 μl, consisted of 12.5 μl of Thermo Scientific Maxima SYBR Green/ROX qPCR Master Mix (2X) (#K0222, Thermo Fisher Scientific, USA), 20 pmol of forward/reverse primers, 100 ng of DNA sample, and was brought to the final volume with nuclease-free deionized water.

Statistical analysis. Data on the level of cf mtDNA in rat blood plasma are presented as the mean ± standard deviation.The t-test (Student's t-test) was utilized to identify significant differences in the level of cf mtDNA between the experimental and control groups of rats. The normality of the distribution was assessed using the Kolmogorov-Smirnov test. A significance level of $p < 0.05$ was considered statistically significant. Statistical analysis of the data was performed using GraphPad Prism 9 software (GraphPad Software Inc., La Jolla, California, USA).

Results and Discussion

Animals. In the control group of rats, the mean value of cf mtDNA was 1.67×10^7 copies/mL. In the blood plasma of rats exposed to X ray the mean value of cf mtDNA was 4.51×10^7 copies/mL (Fig.1).

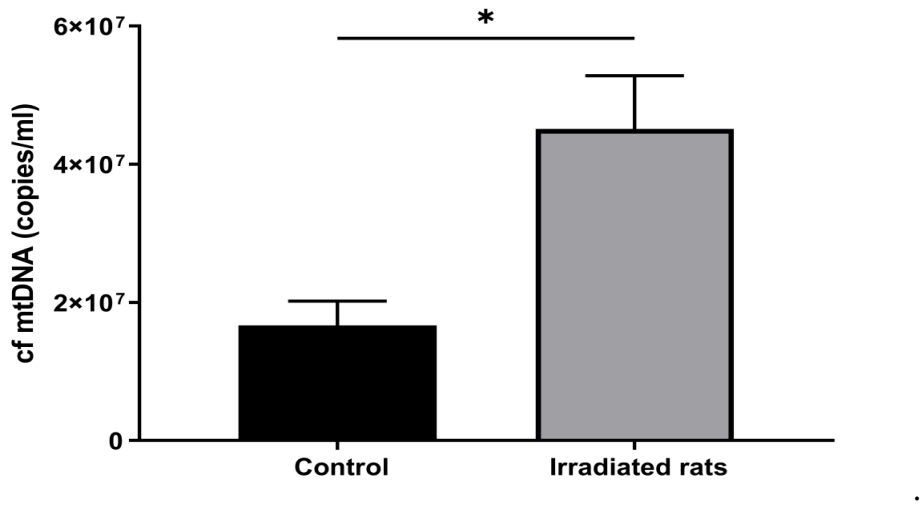

Figure 1. The copy number of cf mtDNA in the plasma of irradiated rats and control ($p < 0.05$).

The role of radiation in public health is a critical topic that encompasses various aspects of radiation exposure, its effects on human health, and the measures taken to protect the public.

Research in this field aims to understand the potential health risks associated with radiation exposure, develop strategies for radiation protection, and inform public health policies and guidelines.

The topic of the impact of radiation on people is particularly important for Kazakhstan due to the country's historical association with nuclear testing. Kazakhstan was the site of extensive nuclear testing during the Soviet era, primarily at the Semipalatinsk Test Site. The consequences of these

tests have had a profound and lasting impact on the health and well-being of the Kazakh population [15,16].

Kazakhstan also is known to have elevated levels of radon gas in certain regions. Radon is a naturally occurring radioactive gas that is produced by the decay of uranium in soil, rock, and water. The geological composition of Kazakhstan, including the presence of uranium-rich deposits, contributes to the higher radon levels observed in some areas.

Several studies and monitoring programs have been conducted in Kazakhstan to assess radon levels. These studies have found that certain regions, particularly those with uranium mining or geological formations associated with higher radon emissions, have elevated radon concentrations. These regions include parts of the East and North Kazakhstan, and Akmola region [17].

Occupational exposure to various hazards can contribute to adverse health effects in workers and potentially impact public health. Currently, the identification of biomarkers for the response to ionizing radiation is a priority scientific task. The use of biomarkers allows for a more accurate assessment of the effects of radiation on the human body and provides valuable information for diagnosis and prediction of consequences. Research in this field is actively conducted in the fields of radiobiology and medicine. It is focused on the search and investigation of various biomarkers that can indicate the presence of radiation, the extent of its impact on the body, and individual sensitivity to radiation.

Free-circulating nucleic acids have emerged as potential biomarkers of radiation exposure [18]. These nucleic acids, including free-circulating DNA and RNA, can be released into the bloodstream and other body fluids in response to cellular damage caused by ionizing radiation. The quantification and analysis of these biomarkers can provide valuable information about the extent of radiation exposure, its effects on the body, and potential health risks.

Mitochondria have their own DNA, known as mitochondrial DNA (mtDNA). Due to their proximity to radiation sources and their involvement in metabolic processes, mitochondria can be sensitive to the effects of ionizing radiation. Ionizing radiation can cause damage to mtDNA, leading to impaired mitochondrial function and disruption of cellular energy metabolism. This makes mitochondria an important subject of study in radiobiology and allows the use of mitochondrial DNA as a potential biomarker of radiation exposure [19].

Thus, Borghini et al. showed that the level of circulating mtDNA in interventional cardiologists exposed to X-rays was two times higher than in the control group not exposed to occupational exposure [20].

Our previous study also showed a correlation between the level of circulating mitochondrial DNA and radon levels in residents of radon-prone regions in Kazakhstan, who were exposed to alpha radiation [21].

In the present study, a similar trend of increased levels of cf mtDNA in the blood plasma was observed in rats exposed to a total dose of 1 Gy. On average, the number of copies of cf mtDNA in the blood plasma of the irradiated rats was 2.7 times higher than in the samples from the control group. Presumably, the difference in the level of circulating mitochondrial DNA observed between the experimental and control groups of the study is associated with the destructive effects of ionizing radiation on cells, leading to apoptotic cell death and the release of circulating mitochondrial DNA into the bloodstream. Moreover, radiation has the ability to enhance mitochondrial biogenesis, expression of mitochondrial genes, and subsequently increase the number of mitochondrial copies in order to generate more ATP [12, 19].

The use of dosimeters allows monitoring individual radiation exposure and establishing appropriate regulatory limits to ensure safety. This allows for an assessment of the proximity to the regulatory limits of radiation exposure and enables the implementation of necessary precautionary measures.

However, it is important to note that the assessment of radiation dose and the effects of radiation exposure is a complex process that should be based on a combination of information from dosimeters,

epidemiological data, biological effects, and other factors. Dosimeters provide quantitative data on radiation exposure, and a comprehensive approach is required for a full assessment of the effects of radiation exposure. The search for easily accessible biomarkers to track the biological effects of radiation on the human body is an important task.

It is important to note that the number of articles providing information about the role of cf mtDNA as a marker of radiation damage is currently limited. Our previous research [21] and the results of the current study convincingly demonstrate that the level of cf mtDNA is highly responsive to radiation exposure, regardless of the type of radiation.

The detection and analysis of cf mtDNA as a biomarker of radiation exposure hold promise for various applications, including biodosimetry, early detection of radiation-related health effects, and monitoring the effectiveness of radiation therapy. However, further research is needed to validate and refine these biomarkers, develop standardized protocols for their measurement, and understand their full potential in clinical and environmental settings.

Conclusions

This study demonstrated a significant increase in the levels of circulating cell-free mitochondrial DNA (cf mtDNA) in the blood plasma of rats exposed to ionizing X-ray radiation, with values 2.7 times higher than those observed in the control group. These findings support the hypothesis that ionizing radiation induces cellular damage, including apoptosis, which contributes to the release of mitochondrial DNA into the bloodstream. Furthermore, radiation may stimulate mitochondrial biogenesis and gene expression as a compensatory mechanism to maintain cellular energy metabolism.

Given the growing interest in identifying reliable and minimally invasive biomarkers of radiation exposure, cf mtDNA emerges as a promising candidate. Its sensitivity to radiation, as demonstrated both in this and previous studies, highlights its potential utility in biodosimetry, occupational health monitoring, and assessment of environmental radiation exposure.

However, the current literature on cf mtDNA as a biomarker of radiation damage remains limited. Further research is required to validate these findings, standardize quantification methods, and determine the clinical and environmental applicability of cf mtDNA measurement in radiation risk assessment.

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Жануарларда рентген сәулеленуіне ұшырауды көрсететін ықтимал биомаркер ретінде қан плазмасындағы митохондриялық ДНҚ көшірме саны

А.А. Кусаинова, О. В. Булгакова*

Еуразия ұлттық университетінің Жасуша биологиясы және биотехнологиясы институтының жалпы биология және геномика кафедрасы. Л.Н. Гумилев, Астана, 010008, Қазақстан

Аңдатпа.

Иондаушы сәулеленудің тірі ағзаларға зиянды әсері – Қазақстанды қоса алғанда, көптеген елдер үшін өзекті мәселе болып табылады. Зерттеудің маңыздылығы Қазақстанның кейбір аймақтарында, әсіресе уран кен орындарына бай өңірлер мен бұрынғы Семей ядролық сынақ полигонының аумағында ұзақ уақыт бойы ыдырамайтын радиоактивті изотоптардың болуына байланысты, радиациялық фон деңгейінің жоғары болуымен түсіндіріледі. Сонымен қатар, медицина саласындағы кәсіби сәулелену адам денсаулығына елеулі әсер етуі мүмкін. Физикалық дозиметрлер жеке зақымдану деңгейін дәл бағалауда шектеулі мүмкіндіктерге ие болғандықтан, радиобиологиядағы негізгі міндеттердің бірі – жеке радиорезистенттіліктің биомаркерлерін іздеу болып табылады. Бұл зерттеудің мақсаты – иондаушы сәулеленудің (рентген) айналымдағы митохондриялық ДНҚ деңгейіне әсерін зерттеу. Рентген сәулесіне ұшыраған егеуқұйрықтардан қан плазмасы үлгілері алынды. Митохондриялық ДНҚ көшірмелерінің санын анықтау үшін нақты уақыт режиміндегі сандық полимеразды тізбекті реакция (qPCR) әдісі қолданылды. Нәтижесінде, сәулеленген егеуқұйрықтардың қан плазмасындағы айналымдағы митохондриялық ДНҚ көшірме саны (4.51×10^7 copies/mL) бақылау тобымен (1.67×10^7 copies/mL) салыстырғанда 2.7 есе жоғары екені анықталды. Алынған нәтижелер айналымдағы митохондриялық ДНҚ-ның рентген сәулеленуін бағалауға арналған биомаркер ретінде әлеуеті бар екенін көрсетеді.

Түйінді сөздер: иондаушы сәулелену, рентген, айналымдағы митохондриялық ДНҚ (cf mtDNA), биомаркер.

Количество копий митохондриальной ДНК в крови как потенциальный индикатор воздействия рентгеновского излучения у животных

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Аннотация.

Опасные эффекты ионизирующего излучения на живые организмы являются ключевой проблемой для многих стран, включая Казахстан. Актуальность исследования обусловлена тем, что в некоторых регионах Казахстана наблюдается повышенный уровень радиационного фона, что связано преимущественно с богатым содержанием урановых месторождений, а также с присутствием долгоживущих радиоактивных изотопов на территории бывшего Семипалатинского ядерного полигона. Кроме того, профессиональное воздействие медицинского излучения может привести к серьезным последствиям для здоровья человека. Поскольку физические дозиметры имеют ограничения в точной оценке степени индивидуального повреждения, одной из основных задач радиобиологии является поиск биомаркеров индивидуальной радиочувствительности. Целью данного исследования было изучение влияния ионизирующего излучения (рентгена) на уровень свободно циркулирующей митохондриальной ДНК. Образцы плазмы крови были собраны у облучённых крыс, подвергшихся рентгеновскому воздействию. Для определения количества копий митохондриальной ДНК использовалась количественная ПЦР в режиме реального времени. В среднем количество копий свободно циркулирующей митохондриальной ДНК в плазме крови облучённых крыс (4.51×10^7 copies/mL) было в 2,7 раза выше по сравнению с контрольной группой (1.67×10^7 copies/mL). Полученные результаты указывают на потенциальную роль свободно циркулирующей митохондриальной ДНК как биомаркера для оценки воздействия рентгеновского излучения.

Ключевые слова: ионизирующее излучение, рентген, свободно циркулирующая митохондриальная ДНК (cf mtDNA), биомаркер.