ABSTRACT

of the dissertation for the degree Doctor of Philosophy (Ph.D.)
«6D070100- Biotechnology» **Botbayev Dauren Muslimovich**
«Development of a method for assessing genetic stability under chronic low-dose radiation exposure».

General Characteristics of the Work

Kazakhstan is one of the world's leading countries in uranium reserves, and workers involved in its extraction, as well as local residents, may be exposed to chronic low-dose radiation. This dissertation is devoted to the study of genetic polymorphisms associated with resistance to chronic exposure to low doses of ionizing radiation, as well as the analysis of radiation-induced changes in the genetic material of uranium industry workers, residents of nearby areas, and medical personnel exposed to chronic radiation in the workplace.

The chronic effects of low doses of ionizing radiation on the human body, particularly on its genetic material, remain a poorly studied area, especially in the context of genetic resistance. The investigation of genetic polymorphisms associated with radiosensitivity or resistance to low-dose radiation is of great importance, since such polymorphisms can be used as markers. These markers may allow for the assessment of individual radiosensitivity among people chronically exposed to low-dose radiation, thereby enabling optimization of professional selection and disease prevention in at-risk groups.

The limited data on genetic factors of resistance to chronic low-dose radiation exposure highlights the significance of this research.

Relevance of the Research Topic

The effects of high doses of radiation on human genetic material are well studied and are associated with severe DNA damage, mutations, and an increased risk of oncological diseases. However, the impact of low-dose radiation under conditions of prolonged exposure remains an insufficiently investigated aspect. This is particularly relevant for individuals exposed to occupational or environmental radiation, where low doses accumulate in the body over an extended period.

Kazakhstan, ranking second in the world in terms of proven uranium reserves and producing a significant share of the global uranium supply (according to the *World Nuclear Association*), faces particular challenges associated with chronic radiation exposure. Long-term exposure to low doses of radiation may lead to the accumulation of mutations in genetic material, thereby increasing the risk of developing diseases, primarily oncological, as well as the potential emergence of hereditary pathologies. Thus, uranium mining and processing create potential health risks for both workers and local residents, which highlights the importance of studying the long-term effects of low-dose radiation.

DNA repair systems play a crucial role in protecting genetic material by restoring damage caused by radiation. Individual differences in the efficiency of these protective mechanisms can significantly affect radiosensitivity and resistance to chronic low-dose radiation exposure. Understanding the mechanisms of DNA repair systems and their variability will make it possible to develop methods for assessing individual radiosensitivity, optimize occupational selection, and introduce effective preventive measures for health protection. Therefore, research in this field has high scientific and practical significance, contributing to risk reduction and improved safety under radiation exposure, which is particularly important for countries with a developed uranium industry such as Kazakhstan.

Chronic exposure to low doses of radiation may also affect the immune system, potentially weakening it and reducing the body's ability to resist infections, although the available data remain contradictory. Furthermore, particular interest is drawn to the body's

response through epigenetic changes (DNA methylation, histone modifications, and non-coding RNA expression) under conditions of prolonged low-dose radiation exposure. These effects are complex and clearly depend on many factors, including individual characteristics. Thus, studies in this area are essential for understanding the consequences of long-term low-dose radiation exposure on human health, for developing preventive measures, and for ensuring the safety of risk groups such as uranium industry workers and populations living in regions with elevated radiation background.

Research Goal

To identify molecular markers for assessing genetic stability and individual sensitivity to radiation exposure by investigating polymorphisms and mutations in functionally important genes in individuals exposed to low doses of radiation.

Research Objectives

1. To establish a collection of blood samples and DNA samples from uranium industry workers, residents of nearby areas, medical personnel exposed to low doses of radiation in the workplace, as well as residents of regions with a normal radiation background (control group).
2. To study the associations of key polymorphisms in genes regulating the cell cycle, apoptosis, and angiogenesis, as well as genes involved in the DNA repair system, with resistance to chronic low-dose radiation exposure.
3. To investigate the level of mutational changes in immune system genes under chronic low-dose radiation exposure.
4. To develop a method for assessing genetic stability under chronic low-dose radiation exposure.

Objects of the Study

The object of the study was the DNA of workers employed in uranium mining and processing enterprises and residents of nearby areas in Kazakhstan, as well as medical personnel in Italy who were exposed to low-dose radiation. These groups represent a unique source of data for examining the effects of long-term radiation exposure. DNA from healthy donors residing in regions with normal radiation backgrounds was used as a control.

Subject of the Study

DNA polymorphisms associated with resistance to chronic low-dose radiation exposure, as well as DNA alterations induced by such exposure.

Research Methods

Polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP), real-time PCR (RT-PCR), Sanger sequencing, and TaqMan Assay.

Scientific Novelty

This study was the first to assess the impact of low doses of ionizing radiation on genomic alterations in workers of uranium mining enterprises in Kazakhstan and the first to present data on associations between allelic variants of certain genes regulating the cell cycle, apoptosis, and angiogenesis, involved in the DNA repair system, and resistance to chronic low-dose radiation exposure. Significant genotypic and allelic differences were identified for the *TP53* and CDKN1A (p21) genes, which regulate cell cycle and apoptosis, between occupationally exposed groups and the control cohort, thereby supporting the potential use of these genes as biomarkers of sensitivity to radiation exposure. At the same time, the studied polymorphisms in the *VEGF, RAD51, XPD, XRCC1,* and *IFNα-2b* genes showed no association with resistance to low-dose radiation exposure. This research represents one of the first fundamental studies in Kazakhstan devoted to the genetic aspects of occupational radiation risk, forming a scientific and practical foundation for further developments in the fields of radiation epidemiology, genetic screening, and preventive medicine.

Theoretical Significance of the Study

The theoretical significance of the dissertation lies in the elucidation of potential molecular mechanisms of radiation resistance. The conducted research established that certain allelic variants of genes involved in DNA repair, cell cycle regulation, apoptosis, and immune response are associated with resistance to chronic low-dose radiation exposure. The obtained data indicate that the molecular mechanisms of radiation resistance may operate at different levels of biological regulation. The study expands the understanding of the multilayered nature of radiation resistance, suggesting interactions between various systems. The research provides a foundation and impetus for further investigations in this field.

Practical Value

The practical value of this work lies in the possibility of using genetic polymorphisms as markers for assessing and systematically monitoring individual resistance to chronic low-dose radiation exposure among uranium mine workers, medical personnel in radiological fields, and residents of radiation-affected areas. Such markers will make it possible to identify individuals with increased sensitivity or resistance to radiation exposure, enabling informed professional selection and the timely implementation of preventive measures. This approach will contribute to improving occupational safety and reducing the risk of radiation-induced diseases. The results of the study can be applied in medical practice, radiation hygiene, and occupational genetics, as well as used in the development of regulatory and methodological documents and in improving genetic monitoring systems for at-risk groups.

Key Provisions Submitted for Defense

1. The polymorphism in intron 6 of the *p53* gene is associated with resistance to chronic low-dose radiation exposure, with allele **A** associated with increased radioresistance and allele **G** with decreased radioresistance.
2. The insertion polymorphism in intron 3 of the *p53* gene is associated with resistance to chronic low-dose radiation exposure, with the insertion associated with increased radioresistance and the deletion with decreased radioresistance.
3. The Arg72Pro (rs1042522) polymorphism in exon 4 of the *p53* gene is associated with resistance to chronic low-dose radiation exposure, with allele C (Pro) associated with increased radioresistance and allele G (Arg) with decreased radioresistance.
4. The polymorphism in codon 31 of the *p21* (CDKN1A) gene is associated with resistance to chronic low-dose radiation exposure, with allele A associated with increased radioresistance and allele C with decreased radioresistance.
5. A method for assessing genetic resistance to chronic low-dose radiation exposure has been developed, which can be applied for the selection of personnel in occupational fields associated with elevated radiation backgrounds.

Main Results and Conclusions of the Study

1. A collection of blood samples and DNA samples was established, including workers of the uranium industry (511 samples), residents of nearby areas (100 samples), healthcare workers exposed to low-dose radiation in the workplace (21 samples), and residents of regions with normal radiation background (300 samples).
2. Significant associations of four polymorphic sites of the *p53* and *p21* genes with resistance to chronic low-dose radiation exposure were identified. Statistically significant differences in the distribution of genotypes and alleles were found between STCNC workers and controls, as well as between miners from the *Balkashinskoe* deposit and controls, specifically in intron 3, intron 6, exon 4 of the *p53* gene, and codon 31 of the *p21* gene. Reliable differences in allele and genotype distribution were also observed between ethnic groups for certain polymorphisms, along with discrepancies in the identified associations, indicating the importance of considering ethnic genetic background in such studies. The obtained data demonstrate the potential of *p53* and *p21* gene polymorphisms as molecular-genetic markers of individual radiosensitivity, which may be applied for occupational risk

assessment and justified medical-genetic selection of individuals working under ionizing radiation conditions. Despite the fact that the *VEGF, RAD51, XPD, XRCC1***,** and *APC* genes are key players in radiation resistance mechanisms, our study did not reveal significant differences in allele and genotype distributions across the investigated polymorphisms of these genes. Although no statistically significant differences were found for the *APC* gene, some identified trends require further investigation.

3. The level of mutational changes in the *IFNα-2b* gene among uranium industry workers and healthcare professionals exposed to low-dose radiation in the workplace did not differ significantly from that in the control group. Although our study did not provide evidence of an increased level of mutational changes under prolonged low-dose radiation exposure, given conflicting literature data and the relatively limited sequencing coverage in our work, additional studies are required to confirm or refute these findings.

4. Based on the identified molecular-genetic markers, a method for assessing genetic resistance to chronic low-dose radiation exposure has been developed, which can be applied for personnel selection in occupational fields associated with elevated radiation backgrounds.

Relation of the Work to State Programs and Plans

The dissertation was carried out in accordance with state programs and projects of the Ministry of Science and Higher Education and the Ministry of Health of the Republic of Kazakhstan. The research was conducted within the framework of the following initiatives: *"Development of the molecular-genetic characterization of DNA of uranium mine workers and creation of an electronic database of genotypes of individuals exposed to chronic low-dose radiation"* (2011–2015); *"Reduction of occupational risk of disease development among uranium industry workers of the Republic of Kazakhstan"* (2013–2015); as well as *"Research and development of methods to reduce radiation risk for the population living in the area affected by radioactive waste repositories"* (2018–2020). The practical part of the scientific work was carried out at the M.A. Aitkhozhin Institute of Molecular Biology and Biochemistry, as well as at the Department of Pharmacology and Biotechnology of the University of Bologna (Italy) in 2018.

Approbation of the Work

The dissertation materials were presented and discussed at the International Scientific Conference of Students and Young Scientists *"The World of Farabi"* (2014–2018, Almaty, Kazakhstan); the Conference *"Medical-Biological and Radioecological Problems of Uranium Mining Regions"* dedicated to the 10th anniversary of the Institute of Radiobiology and Radiation Protection (2015, Astana, Kazakhstan); the International Scientific Conference of Young Scientists dedicated to the 40th anniversary of the M.A. Aitkhozhin Institute of Molecular Biology and Biochemistry (2019, Almaty, Kazakhstan); the XVIII International Scientific and Practical Conference *"Russian Science in the Modern World"* (2018, Moscow, Russia); and the International Scientific and Practical Conference *"Satbayev Readings – 2021"* (2021, Almaty, Kazakhstan).

Structure of the Dissertation

The main results of the dissertation have been published in 14 printed works, including 2 articles in international journals indexed in the Scopus and Web of Knowledge databases with an impact factor, 7 articles in journals included in the list of the Committee for Quality Assurance in the Field of Science and Higher Education, 5 abstracts in the proceedings of international conferences, as well as 1 act of implementation, 1 patent, and 1 utility model patent.

Structure and Volume of the Dissertation:

The dissertation consists of 125 pages and includes the following sections: terminology, symbols and abbreviations, introduction, literature review, research materials

and methods, results and their discussion, and conclusion. The list of references comprises 256 sources. The work contains 18 tables and 14 figures.