ABSTRACT

of the dissertation for the degree Doctor of Philosophy (Ph.D.)
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«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 terms of uranium reserves, and workers employed in uranium mining, as well as residents of nearby areas, may be chronically exposed to low doses of ionizing radiation. This dissertation is devoted to identifying associations between polymorphisms of genes involved in key molecular and cellular processes underlying radioresistance and long-term occupational exposure to low-dose ionizing radiation, as well as to the analysis of radiation-induced genetic alterations in uranium miners, residents of adjacent areas, and medical workers chronically exposed to ionizing radiation.

The chronic effects of low-dose ionizing radiation on the human body, particularly on the genetic material, remain insufficiently understood. The investigation of genetic variants associated with radiosensitivity or radioresistance is of considerable scientific and practical importance, as these variants may serve as potential genetic markers. In the future, such markers may be used to assess individual genetic susceptibility to radiosensitivity among people chronically exposed to low doses of ionizing radiation, improve occupational selection strategies, and develop preventive measures for high-risk groups.

The limited knowledge regarding genetic factors underlying individual variability in the response to chronic exposure to low-dose ionizing radiation highlights the need for further research in this field.

Relevance of the Research Topic

The effects of high doses of ionizing radiation on the human genome have been extensively studied and are known to be associated with severe DNA damage, mutations, and an increased risk of cancer. In contrast, the biological effects of chronic exposure to low doses of ionizing radiation remain insufficiently understood. This issue is particularly relevant for individuals who are occupationally or environmentally exposed to ionizing radiation, where low radiation doses accumulate over extended periods.

Kazakhstan ranks second in the world in terms of identified natural uranium reserves and is one of the world's leading uranium producers (according to the World Nuclear Association). Consequently, the country faces specific challenges associated with chronic radiation exposure. Long-term exposure to low doses of ionizing radiation may lead to the accumulation of genetic alterations, thereby increasing the risk of various diseases, particularly cancer, and potentially contributing to the occurrence of hereditary disorders. Therefore, uranium mining and processing may pose health risks to both workers and residents of nearby communities, highlighting the importance of investigating the long-term biological effects of chronic low-dose radiation exposure.

DNA repair systems play a critical role in maintaining genome integrity by repairing radiation-induced DNA damage. Genetic variability in DNA repair genes may contribute to interindividual differences in the efficiency of DNA damage repair and, consequently, in individual radiosensitivity. A better understanding of DNA repair mechanisms and the impact of genetic variability on their function provides a foundation for developing approaches to assess individual radiosensitivity, improving occupational selection strategies, and implementing effective preventive measures. Therefore, research in this field is of

considerable scientific and practical importance, contributing to risk reduction and improved radiation safety, particularly in countries with a well-developed uranium industry, such as Kazakhstan.

In addition to DNA repair pathways, the potential contribution of the immune response to individual susceptibility to chronic low-dose ionizing radiation exposure is also of considerable interest. It has been suggested that prolonged radiation exposure may affect immune system function; however, the available evidence remains limited and, in many cases, inconsistent. Further investigation of genetic factors involved in immune responses may provide a more comprehensive understanding of the molecular mechanisms underlying individual radiosensitivity.

Research Goal

Identification of genetic variants associated with radioresistance through the investigation of polymorphisms and mutations in functionally important genes in individuals chronically exposed to low-dose ionizing radiation.

Research Objectives

1. To establish a collection of blood and DNA samples from uranium industry workers, residents of adjacent areas, medical workers occupationally exposed to low-dose ionizing radiation, and residents of regions with natural background radiation (control group).
2. To investigate the association between polymorphisms of genes involved in the regulation of the cell cycle, apoptosis, angiogenesis, and DNA repair pathways and resistance to low-dose ionizing radiation.
3. To investigate the level of mutational changes in immune system genes under chronic exposure to low-dose ionizing radiation.
4. To develop a methodological approach for assessing genetic predisposition to chronic exposure to low-dose ionizing radiation.

Objects of the Study

The object of this study was DNA samples obtained from workers of uranium mining and processing enterprises and residents of nearby areas in Kazakhstan, as well as medical workers in Italy who had been exposed to low-dose ionizing radiation. These groups represent a unique source of data for investigating the consequences of long-term radiation exposure. DNA samples from healthy donors residing in regions with natural background radiation were used as the control group.

Subject of the Study

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

Research Methods

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

Scientific Novelty

This study is the first to evaluate the effects of low-dose ionizing radiation on genomic alterations in workers of uranium mining enterprises in Kazakhstan and to report associations between allelic variants of selected genes involved in the regulation of the cell cycle, apoptosis, angiogenesis, and DNA repair pathways and resistance to chronic low-dose

ionizing radiation exposure. Significant differences in genotype and allele frequencies of TP53 and CDKN1A polymorphisms, which are involved in the regulation of the cell cycle and apoptosis, were identified between occupationally exposed groups and the control group. These findings support the potential use of these genes as biomarkers of genetic susceptibility to radiation exposure. In contrast, the investigated polymorphisms in the VEGF, RAD51, XPD, XRCC1, and IFNα-2b genes showed no association with resistance to chronic low-dose ionizing radiation exposure. This study represents one of the first fundamental investigations in Kazakhstan addressing the genetic aspects of occupational radiation risk and provides a scientific foundation for future research in radiation epidemiology, genetic screening, and preventive medicine.

Theoretical Significance of the Study

The theoretical significance of this study lies in expanding current knowledge of the molecular genetic mechanisms underlying individual differences in the response to chronic exposure to low-dose ionizing radiation. The study identified associations between specific allelic variants of genes involved in DNA repair, cell cycle regulation, and apoptosis and long-term chronic exposure to low-dose ionizing radiation. The obtained findings suggest that the molecular mechanisms of radioresistance may operate at multiple levels of biological regulation. Furthermore, the study broadens the understanding of the multilevel nature of radioresistance by suggesting interactions among different biological systems. The findings provide a scientific foundation for future research in this field.

Practical Value

The practical significance of this study lies in generating data that may serve as a foundation for the development of genetic markers for assessing individual susceptibility to chronic low-dose ionizing radiation exposure among uranium miners, medical workers in radiological specialties, and residents of radiation-prone areas. In the future, such markers may be used to identify individuals with increased radiosensitivity or radioresistance, facilitate evidence-based occupational selection, and enable the timely implementation of preventive measures. The obtained results may find application in radiation hygiene, occupational medicine, and occupational genetics, as well as in the development of regulatory and methodological guidelines and the improvement of genetic monitoring programs.

Key Provisions Submitted for Defense

1. The intron 6 polymorphism of the *TP53* gene is associated with resistance to chronic low-dose ionizing radiation exposure: the A allele is associated with higher radioresistance, whereas the G allele is associated with lower radioresistance. The intron 3 insertion polymorphism of the TP53 gene is also associated with resistance to chronic low-dose ionizing radiation exposure: the presence of the insertion is associated with higher radioresistance, whereas the deletion is associated with lower radioresistance.
2. The Arg72Pro (rs1042522) polymorphism located in exon 4 of the *TP53* gene is associated with resistance to chronic low-dose ionizing radiation exposure: the C (Pro) allele is associated with higher radioresistance, whereas the G (Arg) allele is associated with lower radioresistance.
3. The codon 31 polymorphism of the *CDKN1A* gene is associated with resistance to chronic low-dose ionizing radiation exposure: the C allele is associated with higher radioresistance, whereas the A allele is associated with lower radioresistance.
4. A methodology for assessing genetic predisposition to chronic low-dose ionizing radiation exposure has been proposed. It may be applied in the selection of personnel for work under conditions of elevated radiation background.

Main Results and Conclusions of the Study

1. A collection of blood and DNA samples was established from uranium industry workers (511 samples), residents of nearby areas (100 samples), medical workers occupationally exposed to low-dose ionizing radiation (21 samples), and residents of regions with natural background radiation serving as the control group (300 samples).

2. Significant associations were identified between four polymorphic loci of the *TP53* and *CDKN1A* genes and resistance to chronic low-dose ionizing radiation exposure. Statistically significant differences in genotype and allele distributions were observed between workers of the Stepnogorsk Mining and Chemical Combine (SMCC) and the control group, as well as between miners of the Balkhashinskoye uranium deposit and the control group, for polymorphisms located in intron 3, intron 6, and exon 4 of the *TP53* gene and codon 31 of the *CDKN1A* gene. Although some differences in allele and genotype distributions were observed between ethnic groups, they did not substantially affect the overall pattern of the identified associations. The observed discrepancies are likely attributable to the limited sample size, particularly among participants of Kazakh ethnicity. These findings indicate the potential utility of *TP53* and *CDKN1A* polymorphisms as molecular genetic markers of susceptibility to radiosensitivity/radioresistance that may be applied for occupational risk assessment and evidence-based medical genetic screening of individuals working under ionizing radiation exposure. Although V*EGF, RAD51, XPD, XRCC1,* and *APC* play important roles in the mechanisms of radiation response, no significant differences in allele or genotype distributions were detected for the investigated polymorphisms of these genes.

3. Analysis of the *IFNα-2b* gene revealed no mutational changes in uranium industry workers or medical workers chronically exposed to low-dose ionizing radiation in the workplace. Although no evidence of an increased level of mutational changes associated with chronic low-dose ionizing radiation exposure was observed in this study, the inconsistent findings reported in the literature and the relatively limited sequencing coverage warrant further investigations to confirm or refute these results.

4. Based on the identified molecular genetic variants, a potential approach for assessing genetic predisposition to the individual response to chronic low-dose ionizing radiation exposure was proposed. Following further validation, this approach may be considered for use in occupational selection for professions involving elevated radiation exposure.

Relation of the Work to State Programs and Plans

The study was carried out within the framework of the following Scientific and Technical Programs (STPs): STP O.0457, "Preservation and Expansion of Collections of Plants, Animals, and Microorganisms, and Conservation of Unique Genetic Banks to Ensure the Preservation of Kazakhstan's Biodiversity" (2011–2015). Task 1: "Establishment of a DNA bank of uranium miners chronically exposed to low-dose ionizing radiation and their molecular genetic characterization. Development of an electronic database of genotypes of individuals chronically exposed to low-dose ionizing radiation."STP O.0592, "Development of National Genomic, Proteomic, and Cell Technologies for Medicine, Agriculture, and Environmental Protection in Kazakhstan" (2012–2014). Task 2: "Molecular genetic monitoring of the effects of low-dose ionizing radiation exposure in personnel of uranium mining and processing enterprises." The experimental part of the research was conducted 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 comprises 148 pages and includes the following sections: definitions, abbreviations and symbols, introduction, literature review, materials and methods, results and discussion, conclusion, references, and appendices.The reference list includes 282 sources. The dissertation contains 26 tables and 19 figures.