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Different patterns of allelic imbalance in sporadic tumors and tumors associated with long-term exposure to gamma-radiation

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ABSTRACT

The study aimed to reveal cancer related mutations in DNA repair and cell cycle genes associated with chronic occupational exposure to gamma-radiation in personnel of the Siberian Group of Chemical Enterprises (SGCE). Mutations were analyzed by comparing genotypes of malignant tumors and matched normal tissues of 255 cancer patients including 98 exposed to external gamma-radiation (mean dose 128.1 ± 150.5 mSv). Also a genetic association analysis was carried out in a sample of 149 cancer patients and 908 healthy controls occupationally exposed to gamma-radiation (153.2 ± 204.6 mSv and 150.5 ± 211.2 mSv, respectively). Eight SNPs of genes of DNA excision repair were genotyped (rs13181, rs1052133, rs1042522, rs2305427, rs4244285, rs1045642, rs1805419 and rs1801133). The mutation profiles in heterozygous loci for selected SNP were different between sporadic tumors and tumors in patients exposed to radiation. In sporadic tumors, heterozygous genotype Arg/Pro of the rs1042522 SNP mutated into Arg/0 in 15 cases (9.6%) and 0/Pro in 14 cases (8.9%). The genotype Lys/Gln of the rs13181 SNP mutated into Lys/0 and 0/Gln in 9 and 4 cases, respectively. In tumors of patients exposed to low-level radiation, the rs1042522 Arg/0 mutated genotype was found in 12 cases (12.1%), while in 2 cases (2%) *0/Pro* mutation was observed. Finally, the rs13181 *0/Gln* mutated genotype was observed in 15 cases (16,5%) . Thus, our study showed the difference in patterns of allelic imbalance in tumors appeared under low-level radiation exposure and spontaneous tumors for selected SNPs. This suggests different mechanisms of inactivation of heterozygous genotypes in sporadic and radiation-induced tumors.

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1. Introduction

Cancer diseases have been the subject of genetic and genomic studies for a long period of time. However, high proportion of genetically attributable risk of sporadic cancer remains unexplained. Recent genome-wide association studies failed to identify variants that could be clear indicators of the genetic risk of cancer [1,2,3].

An alternative approach for identifying genes with low penetrance associated with cancer is the analysis of allelic imbalance in heterozygous loci that are likely to be differentially expressed in tissues carrying alternative alleles [4,5,6]. In sporadic tumors, the phenomenon of loss of heterozygosity (LOH) can be observed as the result of allele deletions. Also, gene duplication, as well as amplification of one of the alleles or cis-regulation, can result in only one allele of a gene being expressed while the other allele is silenced [7,8]. The differential expression of one allele is a common feature of the human genome (up to 60% of genes) and has a genetic basis [9,10,11].

The phenomenon of allelic imbalance in tumor cells was described by A. Knudson in 1971. According to his two-stage model of carcinogenesis, the inactivation of tumor suppressor genes requires two successive mutational events: the loss of the first allele due to LOH and somatic mutation (or methylation) of

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the other allele [12]. The allelic imbalance and LOH cases have been shown for many genes in sporadic tumors of various organs [8].

The phenomenon of allelic imbalance in radiation-induced carcinogenesis is not well studied. We set out to compare radiation-induced and spontaneous carcinogenesis and to identify genetic markers specific for radiation carcinogenesis. To do so, we compared the prevalence of genotypes of polymorphic genetic loci in tumors and normal tissues of cancer patients who have been exposed to radiation during their professional activities at the Siberian Group of Chemical Enterprises (SGCE) and cancer patients never exposed to radiation, including residents of the nearby city of Seversk and the SGCE employees. Additionally, we estimated associations between genetic markers and cancer development on the background of low-level exposure to radiation. Two main questions have been addressed: (1) is there any difference between the radiation-induced and spontaneous carcinogenesis on genetic level? and (2) is it possible to identify genetic marker(s) of radiation-induced tumors?

For the purpose of the current study we chose eight single nucleotide polymorphisms (SNP) of genes known to be involved in carcinogenesis (Table 1).

ERCC2 (*XPD1*) is a component of the nucleotide excision repair pathway which is involved in the effective maintenance of genome integrity [13]. Defects in *ERCC2* gene can result in cancer-prone syndrome xeroderma pigmentosum complementation group D, trichothiodystrophy, and Cockayne syndrome. The Lys751Gln polymorphism of the gene chosen for the current study, is associated with the increased risk of lung cancer and other tobacco-related cancers [14,15].

hOGG1 gene encodes the enzyme responsible for the excision repair of 8-oxoguanine, a mutagenic base byproduct which occurs as a result of exposure to reactive oxygen species. Loss of heterozygosity at the *hOGG1* locus was frequently detected in lung cancer cells [16,17]. The Ser326Cys polymorphism of the gene is associated with the reduced capacity to repair 8-oxoguanine-related mutations [16].

TP53 gene is the most common target for genetic alteration in human tumors. This gene encodes a tumor suppressor protein which responds to diverse cellular stresses to regulate expression of target genes, apoptosis, senescence, DNA repair, or changes in metabolism. Mutations in this gene are associated with a variety of human cancers. The alleles of the polymorphism in codon 72, chosen for the current study, encode an arginine amino acid (CGC; Arg72) with a positive-charged basic side chain and a proline residue (CCC; Pro72) with a nonpolar-aliphatic side chain. Significant association between the codon 72 polymorphism and risk of most solid cancers [18].

CYP2C19 gene encodes a member of the cytochrome P450 superfamily of enzymes, which is an important phase I enzyme expressed abundantly in endothelial and smooth muscle cells [19]. It is a key enzyme responsible for the metabolism of numerous therapeutic drugs and is also suspected to play a major role in the detoxification or inactivation of potential carcinogens and bioactivation of certain environmental pro-carcinogens to produce toxic DNA binding metabolites [20,21]. The 681G > A polymorphism of the gene, analyzed in the current study, is associated with reduced enzyme activity [22].

ABCB1(MDR1) gene encodes a membrane-associated protein, a member of the superfamily of ATP-binding cassette (ABC) transporters. This protein belongs to the MDR/TAP subfamily of the ABC-transporters involved in multidrug resistance and is an ATP-dependent drug efflux pump for xenobiotic compounds with broad substrate specificity. It is responsible for decreased drug accumulation in multidrug-resistant cells and often mediates the development of resistance to anticancer drugs. It was shown that the minor allele of the Ile1145Ile polymorphism of the *ABCB1* gene, chosen for the current study, was associated with a better response to chemotherapy in advanced NSCLC patients [23] and with a survival in III to IV lung cancer patient [24].

MTHFR gene encodes protein which catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for homocysteine remethylation to methionine. Genetic variations in this gene are associated with various diseases, including colon cancer and acute leukemia. A common non-synonymous SNP, C677T (A222 V), which we chose for the current study, has been extensively studied for associations with colorectal cancer [25]. One of the recent studies showed that the risk of death from cancer significantly increased by the rare allele counts [26]. Also a significant association between the C677T genotypes and the risk of breast cancer was established [27].

BAX gene encodes BCL2-associated X protein belonging to the BCL2 protein family exhibiting anti- or pro-apoptotic effects and involved in a wide variety of cellular activities. The expression of this gene is regulated by the tumor suppressor p53 and has been shown to be involved in p53-mediated apoptosis. Low expression of Bax protein is associated with tumors in rectum, while its positive expression correlates with the presence of cancer cell infiltration to lymph and blood vessels [28]. To the best of our knowledge, the rs1805419 polymorphism chosen for the study, has not been analyzed regarding its associations with cancer; however, it was found to be associated with longevity [29].

The protein that encodes by the *RB1CC1* gene interacts with signaling pathways to coordinately regulate cell growth, proliferation and apoptosis. *RB1CC1* is a tumor suppressor gene which amplifies retinoblastoma 1 gene expression in cancer cells. To the best of our knowledge, the polymorphism rs2305427, chosen for analysis, was not found to be associated with cancer risk; however, it is listed in SNPCancer500 database, so is of interest.

2. Material and methods

2.1. The description of the settings

For the purpose of the study we used the data obtained during the long-term observations of the health status of the SGCE employees. The SGCE was established in 1949 with the purpose of nuclear weapon components production. As a result of later conversion, the Enterprise became the leading producer of nuclear fuel for the purposes of nuclear energy. The SGCE integrates five main production facilities and a number of auxiliary sub-divisions. The personnel of auxiliary facilities have not been involved into professional contact with the radioactive materials and sources of external irradiation. The city of Seversk was initiated to provide the settlement for the SGCE personnel and their families.

Importantly, during the whole period of the SGCE activity there were no accidents which could cause the overexposure of the SGCE employees. Also, the technologies and procedures excluded the possibility of getting the high doses of radiation in compliance with safety regulations by staff. To reduce the risk of negative consequences of irradiation, personal protective equipment and technological processes were improved to increase radiation safety. The average annual dose in the initial period was about 10 mSv; at present it does not exceed 1 mSv.

Individual dosimetric control of the employees has been carrying out since the moment of starting the main technological processes in 1953. The data regarding the individual doses of external γ-radiation measured with the film badge dosimeters and thermoluminescence dosimeters have been routinely kept at the Department of Radiation Safety of the SGCE. The database of the SGCE personnel contains the data about the individual radiation doses of SGCE personnel for every year since the beginning of

Table 1
Characteristics of the studied single nucleotide polymorphisms

Gene	Protein	rs	Alleles	Contig position	Function	Protein position	Residue change
OGG1	*8-Oxoguanine DNA glycosylase*	rs1052133	C/G	9738773	missense	326	S [Ser] ⇒ C [Cys]
ERCC2(XPD1)	*Excision repair cross-complementing rodent repair deficiency, complementation group 2*	rs13181	A/C	18123137	Missense	751	K [Lys] ⇒ Q [Gln]
TP53	*Tumor protein p53*	rs1042522	C/G	7182846	Missense	72	P [Pro] ⇒ R [Arg]
RB1CC1	*RB1-inducible coiled-coil 1*	rs2305427	T/C	5419470	Synonim	1393	R [Arg] ⇒ R [Arg]
CYP2C19	*cytochrome P450, family 2, subfamily C, polypeptide 19*	rs4244285	G/A	47346080	Synonim	227	P [Pro] ⇒ P [Pro]
ABCB1	*ATP-binding cassette, sub-family B (MDR/TAP), member 1*	rs1045642	T/C	25171488	Synonim	1145	I [Ile] ⇒ I [Ile]
BAX	*BCL2-associated X protein*	rs1805419	G/A	21727322	Intronic	NA	NA
MTHFR	*Methylenetetrahydrofolate reductase (NAD(P)H)*	rs1801133	T/C	7861110	Missense	222	A [Ala] ⇒ D [Asp]

employment as well as the information about the cumulative dose of radiation exposure which a person has received during the entire career.

For the current study, the main source of medical information was the archive of medical documentation of the Seversk Biophysical Research Centre. The archive contains circa 135242 storage units including more than 24,000 outpatient records of former employees of the SGCE, protocols of autopsies from 1949 to 2010, journals of histological studies from 1950 to 2010. Also the archive contains more than 65,000 medical records of the SGCE employees and residents of the city of Seversk examined and treated in the Clinical Hospital #81 for the following diseases: malignant neoplasms (solid tumors, regardless of location); hemoblastoses; thyroid gland diseases; congenital malformations; acute myocardial infarction; hemorrhagic and ischemic stroke; diabetes mellitus. Current medical documentation of the Clinical Hospital #81 and medical records from the health care institutions of the regional centre (Tomsk) were also used as the source of information.

2.2. Samples overview

Two cohorts were analyzed to establish allelic imbalance in tumor tissues associated with radiation exposure (Table 2). The first group comprised 98 cancer patients with tumors of the digestive system who have been exposed to gamma-irradiation during their service at the SGCE. This group was denoted as Radiation Tumor (RT) group. The median dose of external gamma-radiation in this group was 50.4 mSv and interquartile range of 8.2–197.0 mSv. The second cohort comprised 157 cancer patients with tumors of the digestive system, including SGCE workers and other residents of the city of Seversk who have never been in contact with industrial sources of gamma-radiation. This group was denoted as Sporadic Tumor (ST) group. The two groups were analyzed to reveal allelic imbalance by comparing genotypes in tumors and adjucent normal tissues.

For the association study, two additional groups were recruited. They comprised 149 cancer patients and 908 healthy individuals (control) who all have been exposed to long-term external γ-radiation during their service at the SGCE. These groups were matched by sex, dose of external γ-radiation and tumor localizations (Table 2).

Informed consent was obtained for all study participants and the Ethical Committee of the Seversk Biophysical Research Centre and Cancer Research Institute approved the study protocol.

2.3. DNA isolation

For the analysis of allelic imbalance, DNA was isolated from tumors and adjacent normal tissues from formalin-fixed paraffin-embedded (FFPE) sections. Ten slices from FFPE sections were placed into xylene ("Panreac", Spain) to remove paraffin followed by washing with 100% ethanol, cell lysis with proteinase K ("Sibenzyme", Russia) and column-based DNA extraction ("Biosilica", Russia).

For the association study, genomic DNA was extracted from peripheral white blood cells using standard phenol-chloroform extraction/ethanol precipitation method [30]. The blood cell samples were obtained from the Bank of Biological Material of the Seversk Biophysical Research Centre.

2.4. Genotyping

Genotyping was performed by real-time PCR using "ICycler IQ5" or "CFX96" instrument ("Bio-Rad Laboratories", USA). Primers and probes (Table 3) were designed using "Oligo 7.5" program and the following databases: http://www.ncbi.nlm.nih.gov/pubmed/ and http://snp500cancer.nci.nih.gov/.

The reaction mix (total volume 15 μl) contained 1 μl DNA, 1.5 μl 10x Taq-polymerase Buffer (160 mM $(NH_4)_2SO_4$, 670 mM Tris–HCl, 15 mM $MgCl_2$, 0.1% Tween-20, 1.5 μl of 25 mM $MgCl_2$), 1.5 μl of 2.5 mM dNTP (Deoxyribonucleotide triphosphate) ("Sibenzyme", Russia), 0.2 μl Taq DNA polymerase ("Sibenzyme", Russia), 0.7 μl of each primer ("Syntol", Russia) at the concentration of 10 pmol/μl and 0.7 μl of probes for wild-type (ROX-tagged) and mutant (FAM-tagged) alleles at the concentration of 10 pmol/μl and up to 15 μl miliQ water. PCR were performed for each using the following amplification program: initial denaturation at 94 °C for 5 min, followed by 42 cycles of 94 °C for 10 s and for 25 s at an annealing temperature specific for primers (Table 3).

The allelic imbalance was observed in case of the loss of an allele in heterozygous genotype in tumor tissue as compared with matched normal tissue from the same patient (for example, A/B → A/0 or A/B → 0/B).

2.5. Statistical analysis

Fisher's exact test was used to compare genotypes prevalence in tumors and normal tissues as well as in sporadic tumors and radiation-induced tumors. For the association study, codominant and multiplicative models were used to determine the significance of risk for genotypes and alleles, respectively (http://gen-expert.ru/calculator_or.php). Odds ratio (OR) and 95% confidence intervals (CI) were calculated as a measure of the risk size.

3. Results

This study was designed to consider the effects of low-dose gamma-radiation on the risk of the development of cancer under

Table 2
Characteristics of the examined groups of SGCE workers.

Index	N[a]	Gender(M/F)	Age(M ± SD),years	Length of service(M ± SD),years	External γ-irradiation dose, mSv			
					Med[b]	M ± SD	Min–Max[c]	Interquartile range
Cancer cases	149	115/34	64.2 ± 8.1	33.9 ± 9.9	63.1	153.2 ± 204.6	1.1–1 555.7	17.6–264.4
Healthy control	908	734/174	55.1 ± 11.5	28.3 ± 11.7	72.3	150.5 ± 211.2	1.1–1 631.1	15.8–195.3
p-value	–	0.35[*]	9.9×10^{-20e}	2.5×10^{-6e}	–	0.88[**]	–	–
RT-group	98	73/25	62.3 ± 9.6	31.9 ± 12.3	50.4	128.1 ± 150.5	1.2–609.6	8.2-197.0
ST-group	157	112/45	64.1 ± 12.3	–	–	–	–	–
p-value	–	0.55[†]	0.12[†]	0.23[‡]	–	0.39[‡]	–	–

[a] Number of examinees in the groups.
[b] Median.
[c] Minimum-maximum.
[*] *p*-Value for differences between groups (Cancer cases and Healthy control) by Pearson χ with Yates'
[**] *p*-Value for differences between groups (Cancer cases and Healthy control) by Student's *t*-test.
[†] *p*-Value for differences between groups (RT-group and ST-group) by Pearson χ with Yates'
[‡] *p*-Value for differences between groups (RT-group and Healthy control) by Student's *t*-test.

Table 3
The primers and probes for genotyping of polymorphisms.

SNP	Primer/Probe	Sequence	Ta[a]
hOGG1	Forward	5′-acacagactccaccctcc-3′	61 °C
977C > G	Reverse	5′-cgctttgctggtggct-3′	
Ser326Cys	Probe 1	ROX 5′-cgccaatcccgccatg-3′ BHQ2 allele C	
rs1052133	Probe 2	FAM 5′-cgccaatgccgccat-3′ BHQ1 allele G	
XPD1 2251A > C	Forward	5′-gtgccccctctccct-3′	61 °C
Lys751Gln	Reverse	5′-acccgccccactcaga-3′	
rs13181	Probe 1	ROX 5′-agaggagacgctg**a**agaggatagag-3′ BHQ2 allele A	
	Probe 2	FAM 5′-agaggagacgctg**c**agaggatag-3′ BHQ1 allele C	
TP53+119C > G	Forward	5′-atgaagctcccagaatgc-3′	64 °C
Arg72Pro	Reverse	5′-gccggtgtaggagct-3′	
rs1042522	Probe 1	ROX 5′-ctgctcccc**c**cgtggccc-3′ BHQ2 allele C	
	Probe 2	FAM 5′-ctgctcccc**g**cgtggccc-3′ BHQ1 allele G	
RB1CC1	Forward	5′-tttgggtgtccttcagatttg-3′	61 °C
Ex18 + 83T > C	Reverse	5′-gctacatatggtgaaggaacaaa-3′	
Arg1393Arg	Probe 1	ROX 5′-aagaagaagtcagtaagttgcg**t**agtagc-3′ BHQ2 allele T	
rs2305427	Probe 2	FAM 5′-agaagaagtcagtaagttgcg**c**agta-3′ BHQ1 allele C	
CYP2C19	Forward	5′tgttttctcttagatatgcaata-3′	61 °C
681G > A	Reverse	5′ggtgttcttttactttctccaa-3′	
Pro227Pro	Probe 1	ROX 5′-actatcattgattatttccc**g**ggg-3′ BHQ2 allele G	
rs4244285	Probe 2	FAM 5′-actatcattgattatttccc**a**ggaac-3′ BHQ1 allele A	
ABCB1 Ex27–55T > C	Forward	5′-agcccatcctgtttgactg-3′	62 °C
Ile1145Ile rs1045642	Reverse	5′-gcatgtatgttggcctcct-3′	
	Probe 1	ROX 5′-caggaagagatt**g**tgagggcagc-3′ BHQ2 allele T	
	Probe 2	FAM 5′-acaggaagagat**c**gtgagggca-3′ BHQ1 allele C	
BAX IVS3 + 14	Forward	5′-acgaactggacagtaacatgg-3′	61 °C
G > A rs1805419	Reverse	5′-ctgagagtcctgtgtcctgaag-3′	
	Probe 1	ROX 5′-ctggacttctgggtcc**c**cag-3′ BHQ2 allele G	
	Probe 2	FAM 5′-tggacttctgggtcc**t**cagg-3′ BHQ1 allele A	
MTHFR Ex5 + 79	Forward	5′-gctgacctgaagcacttgaa-3′	63 °C
T > C A222V	Reverse	5′-gtgtcagcctcaaagaaaagc-3′	
rs1801133	Probe 1	ROX 5′-aaggtgtctgcgggag**t**cgattt-3′ BHQ2 allele T	
	Probe 2	FAM 5′-tgtctgcgggag**c**cgatttc-3′ BHQ1 allele C	

[a] Annealing temperature.

long-term exposure and to reveal genetic markers of this risk. Accordingly, two approaches were used. First approach included the analysis of the allelic imbalance phenomenon in tumors from patients who have or have not been exposed to gamma-radiation during the service at a nuclear enterprise. The second approach was based on the comparison of the prevalence of alleles and genotypes in cancer patients and healthy people exposed to low-dose gamma-radiation.

3.1. Association study

A comparison of prevalence of alleles and genotypes between cancer patients and healthy controls revealed no significant differences for CYP2C19 681G > A, ABCB1 Ex27-55T > C, BAX IVS3 + 14 G > A, and MTHFR Ex5 + 79C > T polymorphisms.

However, the *Arg* allele and *Arg/Arg* genotype of the *TP53* gene statistically significantly increased the risk of cancer development on the background of gamma-radiation (OR = 1.69, 95% CI = 1.27–2.26, $p = 0.0003$; OR = 1.57, 95% CI = 1.11–2.23, $p = 0.0005$ for the allele and genotype, respectively); conversely, the *Pro/Pro* genotype showed a protective anti-tumor effect (Table 4).

The *Gln/Gln* genotype of the *XPD1* gene was found to be associated with an increased risk of the development of cancer with a background of gamma-radiation (OR = 1.73, 95% CI = 1.15–2.60, $p = 0.03$) (Table 4).

Finally, the *hOGG1* wild-type genotype (*Ser/Ser*) and *Ser* allele were found to be associated with the development of cancer on the background of radiation (OR = 1.65, 95% CI = 1.12–2.42, $p = 0.01$; OR = 1.52, 95% CI = 1.08–2.14, $p = 0.01$ for genotype and allele, respectively) (Table 4).

Table 4
The prevalence of genotypes and alleles of SNP in SGCE workers exposed to low-dose gamma-radiation (149 cancer cases and 908 healthy controls).

TP53 + 119C > G Arg72Pro: rs1042522					
Groups	Genotypes			Alleles	
	Arg/Arg	Arg/Pro	Pro/Pro	Arg	Pro
Cancer cases	85 (57.0%)	61 (40.9%)	3 (2.1%)	231 (0.775)	67 (0.225)
Healthy control	416 (45.8%)	386 (42.5%)	106 (11.7%)	1218 (0.671)	598 (0.329)
OR (95% CI)	1.57	0.94	0.16	1.69	
	1.11–2.23	0.66–1.33	0.05–0.50	1.27–2.26	
p-Value	0.0005			0.0003	
RB1CC1 Ex18+83T>C Arg1393Arg: rs2305427					
Groups	Genotypes			Alleles	
	T/T	T/C	C/C	T	C
Cancer cases	99 (66.4%)	42 (28.2%)	8 (5.4%)	240 (0.805)	58 (0.195)
Healthy control	590 (65.0%)	278 (30.6%)	40 (4.4%)	1458 (0.803)	358 (0.197)
OR(95% CI)	1.07	0.89	1.23	1.02	
	0.74–1.54	0.61–1.31	0.56–2.68	0.75–1.38	
p-Value		0.76		0.92	
XPD1 2251A > C Lys751Gln: rs13181					
Groups	Genotypes			Alleles	
	Lys/Lys	Lys/Gln	Gln/Gln	Lys	Gln
Cancer cases	53 (35.6%)	58 (38.9%)	38 (25.5%)	164 (0.550)	134 (0.450)
Healthy control	344 (37.9%)	414 (45.6%)	150 (16.5%)	1102 (0.607)	714 (0.393)
OR (95% CI)	0.91	0.76	1.73	0.79	
	0.63–1.30	0.53–1.08	1.15–2.60	0.61–1.02	
p-Value		0.03		0.07	
hOGG1 977C>G Ser326Cys: rs1052133					
Groups	Genotypes			Alleles	
	Ser/Ser	Ser/Cys	Cys/Cys	Ser	Cys
Cancer cases	109 (73.1%)	36 (24.2%)	4 (2.7%)	254 (0.852)	44 (0.148)
Healthy control	566 (62.3%)	305 (33.6%)	37 (4.1%)	1437 (0.791)	379 (0.209)
OR (95% CI)	1.65	0.63	0.65	1.52	
	1.12–2.42	0.42–0.94	0.23–1.85	1.08–2.14	
p-Value		0.01		0.01	

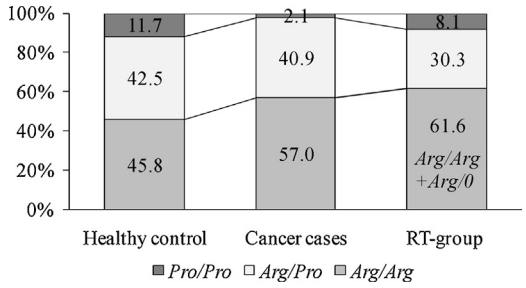

Fig. 1. The prevalence of *TP53 + 119C > G (Arg72Pro)* genotypes in SGCS workers exposed to long-term gamma-radiation and in tumor tissues
Healthy control — healthy SGCS workers; Cancer cases — SGCS workers affected by cancer; RT-group — tumors of SGCS workers exposed to gamma-irradiation.

3.2. Allelic imbalance study

The analysis of the *TP53* polymorphism in sporadic tumor tissues (ST group) revealed a loss of one of the alleles of heterozygous *Arg/Pro* genotype due to mutation into Arg/0 (9.6%) and 0/Pro (8.9%). In the ST group, the prevalence of genotypes for this SNP in tumors and normal tissues was statistically significantly different ($p = 0.0016$; Table 5). In tumor tissues with a background of low-level radiation (RT group), heterozygous *Arg/Pro TP53* genotype mainly mutated into *Arg/0* genotype (12.1%), while *0/Pro* mutation was observed only in 2 cases (2%) (Table 5). The prevalence of genotypes for this SNP differed significantly between tumor tissues in RT and ST groups ($p = 0.043$). This supports the hypothesis about the difference between radiation-induced and spontaneous carcinogenesis at the genetic level.

A gradual increase of the *Arg/Arg* genotype prevalence was observed in a comparison between healthy SGCE workers, cancer patients, and tumors from the RT group (Fig. 1). There was a significant difference between the genotype frequencies in the RT group tumors and healthy SGCE workers (OR = 1.90, 95% CI = 1.23–2.93, $p = 0.01$).

The allelic imbalance in tumor samples was also observed for the SNP in the *RB1CC1* gene both in the RT and the ST groups; the direction of mutagenesis was different in these groups. Namely, on the background of radiation (RT group), the heterozygous genotype mutated only into *T/0* genotype (10.3%), while in sporadic tumors (ST group) it mutated both into *0/C* and *T/0* genotypes (5.3% and 3.5%, respectively; Table 5). The differences in the prevalence of the genotypes in sporadic tumors and tumors on the background of radiation were not statistically significant. Similarly, the differences between the prevalence of the genotypes between tumor and matched normal tissues within the RT and ST groups were not statistically significant. This is consistent with the observation of no statistically significant association of the polymorphism with cancer in our association study (Table 4).

The heterozygous genotype of the *XPD1* gene was found to be mutating into *Lys/0* (7.8%) and *0/Gln* (3.5%) in sporadic tumors (ST group) (Table 5). In tumors from RT group a significant loss *Lys* allele was observed due to mutation of 16.5% of the *Lys/Gln* genotype into the *0/Gln* genotype. The difference in genotypes prevalence in tumors in the RT and ST groups was statistically significant ($p = 2.78 \times 10^{-11}$) (Table 5), and this suggests the specificity of the allelic imbalance for this gene for tumors with a background of gamma-radiation. This is also supported by the results of the association study (Table 4).

The loss of the *Lys* allele was apparent in a comparison between healthy SGCE workers, cancer patients, and tumors from RT-group (Fig. 2), and this trend was statistically significant (OR = 8.67, 95% CI 5.44–13.80, $p = 1 \times 10^{-8}$). This is consistent with the hypothesis that the risk significance of the mutant glycine genotype is more frequent under genotoxic conditions [31,32,33].

For the *hOGG1* gene the loss of the *Cys* allele by heterozygotes was observed in the RT group (5.4%), while in the ST group, the

Table 5
The prevalence of genotypes and alleles of genetic loci in tumor and normal tissues of cancer patients exposed (RT-group) and not exposed (ST-group) to low-dose gamma-radiation

SNP	Genotype/allele	RT-group		*ST-group*		$p_{between}$ *
		Tumor	Normal	Tumor	Normal	
TP53	*Arg/Arg*	49 (49.5%)[c]	49 (49.5%)	66 (42.0%)	66 (42.0%)	0.0433[g]
+119C > G	*Arg/0*	12 (12.1%)	–	15 (9.6%)	–	
Arg72Pro	*Arg/Pro*	30 (30.3%)	44 (44.4%)	46 (29.3%)	75 (47.8%)	
rs1042522	*0/Pro*	2 (2.0%)	–	14 (8.9%)	–	
	Pro/Pro	6 (6.1%)	6 (6.1%)	16 (10.2%)	16 (10.2%)	
	Arg	140 (0.761)	142 (0.717)	193 (0.677)	207 (0.659)	0.0317[al]
	Pro	44 (0.239)	56 (0.283)	92 (0.323)	107 (0.341)	
p_{within} **		0.0899[g]	0.1966[al]	0.0016[g]	0.3524[al]	
RB1CC1	*T/T*	44 (64.7%)	44 (64.7%)	67 (58.8%)	67 (58.8%)	0.2144[g]
Ex18 +83T > C	*T/0*	7 (10.3%)	–	4 (3.5%)	–	
R1393R	*T/C*	14 (20.6%)	21 (30.9%)	34 (29.8%)	44 (38.6%)	
rs2305427	*0/C*	–	–	6 (5.3%)	–	
	C/C	3 (4.3%)	3 (4.4%)	3 (2.6%)	3 (2.6%)	
	T	109 (0.845)	109 (0.801)	172 (0.754)	178 (0.781)	0.0289[al]
	C	20 (0.155)	27 (0.199)	56 (0.246)	50 (0.219)	
p_{within} **		0.4218[g]	0.2222[al]	0.1140[g]	0.2897[al]	
XPD1	*Lys/Lys*	22 (24.2%)	23 (25.3%)	59 (51.3%)	59 (51.3%)	2.78×10^{-11}[g]
2251A > C	*Lys/0*	–	–	9 (7.8%)	–	
Lys751Gln	*Lys/Gln*	12 (13.2%)	27 (29.7%)	26 (22.6%)	39 (33.9%)	
rs13181	*0/Gln*	15 (16.5%)	–	4 (3.5%)	–	
	Gln/Gln	42 (46.2%)	41 (45.1%)	17 (14.8%)	17 (14.8%)	
	Lys	56 (0.335)	73 (0.401)	153 (0.705)	157 (0.683)	3.62×10^{-13}[al]
	Gln	111 (0.665)	109 (0.599)	64 (0.295)	73 (0.317)	
p_{within} **		0.0156[g]	0.1228[al]	0.1657[g]	0.3403 [al]	
	Ser/Ser	60 (64.5%)	60 (64.5%)	89 (67.4%)	89 (67.4%)	0.0421[g]
hOGG1	*Ser/0*	5 (5.4%)	–	–	–	
977C>G	*Ser/Cys*	23 (24.7%)	28 (30.1%)	23 (17.4%)	32 (24.2%)	
Ser326Cys	*0/Cys*	–		9 (6.8%)		
rs1052133	*Cys/Cys*	5 (5.4%)	5 (5.4%)	11 (8.3%)	11 (8.3%)	
	Ser	148 (0.823)	148 (0.796)	201 (0.788)	210 (0.795)	0.2631[al]
	Cys	33 (0.177)	38 (0.204)	54 (0.212)	54 (0.205)	
p_{within} **		0.7164[g]	0.3445[al]	0.1373[g]	0.4623[al]	

* *p* Value by Fisher's exact test for comparison of differences in the prevalence of genotypes[g] and alleles[al] between the RT-group tumors and ST-group tumors.
** *p* Value by Fisher's exact test for comparison of differences in the prevalence of genotypes[g] and alleles[al] between tumors and normal tissues within one group.

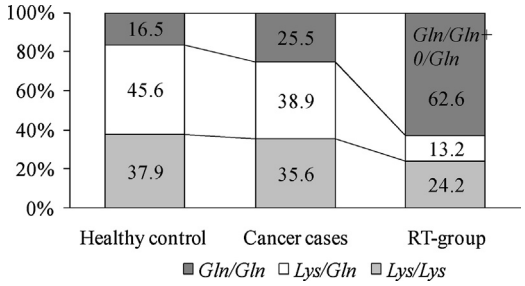

Fig. 2. The prevalence of *XPD1 2251A > C Lys751Gln* rs13181 genotypes in SGCE workers exposed to long-term gamma-radiation and in tumor tissues
Healthy control — healthy SGCS workers; Cancer cases — SGCS workers affected by cancer; RT-group — tumors of SGCS workers exposed to gamma-irradiation.

alternative allele loss was identified (6.8%). Genotypes prevalence in tumors of the RT group and in tumors of the ST group differed significantly ($p = 0.0421$) (Table 5). This result was consistent with the significant association between this polymorphism and cancer in our study (Table 4).

4. Discussion

The study was carried out to test a hypothesis of different genetic mechanisms of radiation-induced and spontaneous carcinogenesis. We did so by comparing the allelic imbalance in tumors developed under background of long-term radiation exposure and tumors developed with no contact with radiation exposure in personnel of a nuclear enterprise. We also employed a more established approach of genetic association analysis to compare genotype and allele prevalence in healthy people (controls) and cancer patients (cases) who have been subjected to a long-term occupational exposure to external gamma radiation.

Eight single nucleotide polymorphisms of DNA repair and cell cycle genes were chosen for the study (Table 1) and the analysis was carried out in samples of SGCE employee and the citisens of Seversk with well assessed radiation exposure and accurate medical records (Table 2).

We were able to identify different patterns of allelic imbalance in radiation-induced and spontaneous tumors as well as statistically significant associations with radiation-induced cancer for the *TP53*, *hOGG1*, *RB1CC1* and *XPD1* genes.

The p53 protein encoded by *TP53* gene is a transcription factor regulating multiple cellular functions critical for maintenance of genomic stability. The rs1042522 SNP is located in exon 4 of the *TP53* gene and causes *Arg-to-Pro* replacement at position 72 of the protein [34]. The *Arg* form was found to be associated with more efficient *P73*-mediated apoptosis as compared to the *Pro* form [35,36]. According to our earlier findings, *Pro/Pro* genotype is likely to be associated with more efficient DNA repair as it is liked to decreased levels of chromosomal aberrations in lymphocytes of patients with malignant tumors with a background of low-level radiation [37]. However, different studies showed the risk significance of both *Arg/Arg* and *Pro/Pro* genotypes for the development of cancer [38,39]. Differential allelic expression was found to be asso-

ciated with *Arg72Pro* polymorphism [40] further implicating this genetic variant into carcinogenesis.

In the present study, in spontaneous tumors, 18% of the *Arg/Pro* heterozygotes of *TP53* mutated into *Arg/0* and *0/Pro* (Table 5). The mutations in the heterozygote tumors on the background of radiation are clearly directed towards *Arg*-positive genotype and this corresponds to the risk significance of the *Arg* allele and *Arg/Arg* genotype for the development of tumors on the background of radiation as we identified in the association study (Table 4). This data suggest that *Arg* allele is a marker and a possible cause of malignant transformation associated with radiation exposure.

The *RB1CC1* gene is located on chromosome 8q11. The RB1CC1 protein is a key regulator of the tumor suppressor gene *RB1*. The *RB1CC1* expression correlates with expression of *RB1* in many tumor cell lines, and inversely correlates with the expression of *MDR1* gene, associated with tumor drug resistance [41]. The *RB1CC1* is involved in the regulation of cell growth and proliferation [42,44,45], apoptosis regulation and autophagy [46–48]. The low level of *RB1CC1* expression is associated with poor prognosis for breast cancer [43]. In our study, allelic imbalance in tumor samples was observed for the rs2305427 SNP of the *RB1CC1* gene (Table 5). We did not find significant differences in the genotypes prevalence in sporadic tumors and tumors with a background of radiation as well as between matched normal tissues and tumors (Table 5). Also, no significant association between this polymorphism and cancer on the background of low-dose radiation was found (Table 4). These data suggest that the SNP cannot be considered as a marker of radiation-induced tumors.

The *XPD1* gene encodes a 5–3′ DNA helicase involved in nucleotide excision repair. A polymorphism has been described in exon 23 of the gene, which results in *Lys751Gln* amino acid substitution. The *XPD1 751Gln* allele was associated with risk of the development of lung cancer in Caucasians [49,50] and Asians [51,52]. Recent meta-analyses confirmed the relationship between the *751Gln/Gln* genotype and the risk of lung cancer in Caucasians [32,33]. Mutant genotype was also found to be associated with breast cancer in African-Americans, but not in white Americans and Europeans [31,53]. Also, mutant genotype was associated with gastric cancer in Asians, but not in Caucasians [54]. In general, the *Lys751Gln* polymorphism expresses mild-to-moderate association with cancer with OR range of 1.1–1.5.

According to our data, the *Gln/Gln* genotype of the *XPD1* gene is significantly associated with the development of malignant tumors with a background of gamma-radiation (Table 4). Also, about 56% of heterozygote tumors in patients exposed to radiation lost the *Lys* allele and mutated into the *0/Gln* genotype. No such the excess of the *0/Gln* genotype was observed in sporadic tumors (Table 5). Thus, the direction of mutagenesis at heterozygous locus in tumors with a background of radiation favors the Gln-positive genotype and it complies with the risk significance of the *Gln* allele observed in a comparison between cancer cases and healthy controls (Table 4).

The *hOGG1* gene encodes the 8-oxoguanine glycosylase I involved in repair of high mutagenic DNA adducts caused by the action of reactive oxygen metabolites. Consequently, the adducts are increased in peripheral blood lymphocyte and lung tissue of smokers as well as in lung tumors [55]. The rs1052133 polymorphism in the *hOGG1* gene results in *Ser326Cys* substitution and is associated with decreased enzyme activity [56]. The risk of lung cancer in carriers of the *326Cys/Cys* genotype is twice as high as in carriers of *326Ser/Ser* genotype [57]. The meta-analysis showed a significant association between the *326Cys/Cys* genotype and lung cancer (OR 1.22, 95% CI = 1.02–1.45) [58]. In contrast, a protective effect of the *hOGG1 326Cys* allele for the development of breast cancer was found in Europeans (OR = 0.71, 95% CI 0.51–0.98) [59].

We found that the wild-type *hOGG1 326Ser/Ser* genotype predisposes to the development of malignant tumors with a background of gamma-radiation (Table 4); moreover, in tumors of irradiated patients, we observed the loss of the *Cys* allele (from 20.4% to 17.7%) and mutation of the *Ser/Cys* genotype into *Ser/0* (Table 5), while in sporadic tumors no such mutation was identified, but a mutation of *Ser/Cys to 0/Cys* was observed. This data suggest that *Ser/0* mutation is a highly specific marker of radiation carcinogenesis.

5. Conclusion

Our study demonstrated the phenomenon of allelic imbalance in tumors developed under a background of low-level exposure to gamma-radiation and in spontaneous tumors for the selected SNPs. This indicates the universality of the mechanisms of inactivation of heterozygous genotypes. At the same time, the direction of mutagenesis in heterozygous loci is markedly different between sporadic tumors and tumors with a background of radiation. This indicates that there are genetic differences between spontaneous and radiation-induced carcinogenesis. These data are supported by the analysis of association between the studied polymorphisms and cancer in workers of a nuclear enterprise. This, in turn, suggests the significance of the studied genes for the development of malignant tumors and the functional role of the studied polymorphisms. Overall, the differences between patterns of mutagenesis in tumors and risk significance of the studied alleles can be taken into account for the development of panels of genetic markers of predisposition to radiation-induced carcinogenesis.

Competing interests

The author report no declarations of interest.

Author contributions

Nikolai V. Litviakov—study design, experiment, data analysis, writing the article; Maxim B. Freidin—data analysis, writing the article; Aleksey E. Sazonov—collection of clinical material, data analysis, overall guidance; Maria V. Khalyuzova—experiment, data analysis; Mikhail A. Buldakov—scientific editing; Mikhail S. Karbyshev—scientific editing; Elena N. Albakh—collection of clinical material; Daria S. Isubakova—collection of clinical material, experiment; Aleksey A. Gagarin—collection of clinical material; Gennadiy B. Nekrasov—collection of clinical material; Elena B. Mironova—collection of clinical material; Andrey S. Izosimov—collection of clinical material; Ravil M. Takhauov—study design, scientific editing, overall guidance; Andrei B. Karpov—scientific editing, overall guidance.

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