CHANGES IN EXPRESSION OF HOMOLOGOUS RECOMBINATION GENES IN CHEMOTHERAPY-INDUCED TUMORS *IN VIVO*

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Studying molecular mechanisms of carcinogenesis, including abnormalities of the homologous recombination (HR) system, is an important objective when studying malignization. Dysfunction of HR genes, such as *BRCA1/2*, contributes to genomic instability and the development of more aggressive tumor clones. The use of chemical carcinogens, such as dimethylbenz(a)anthracene (DMBA), allows one to simulate tumorigenesis processes and assess changes in expression of repair genes. It is important to study such changes to understand the mechanisms underlying adaptation of tumor cells to genotoxic stress and develop personalized approaches to cancer treatment. The study aimed to assess the expression of major HR genes in chemotherapy-induced carcinogenesis in mice. The study involved female outbred ICR laboratory mice (CD-1; $n = 20$). Two groups of animals were formed: the control group and the treatment group that was administered DMBA. Histological analysis of autopsy specimens was conducted to identify tumors. Gene expression levels were assessed using RT-PCR, and testing for chromosomal aberrations was performed using digital PCR. Tumors were found in four animals. Zero expression of the genes *Brca1, Brca2, Cdk12, Chek2, Palb2, Bard1, Brip1* and *Rad* paralogues was observed in three tumor samples. One sample showed high expression of the genes *Cdk12* (14.3), *Chek1* (27.6), *Rad51d* (38.5). Predominance of deletions in the test genes was reported in the majority of cases. Thus, tumorigenesis is associated with the decrease in expression of major repair genes, chromosomal aberration formation, which can contribute to the emergence of more aggressive clones and increase sensitivity to chemotherapy drugs.

Keywords: chemotherapy-induced carcinogenesis; homologous recombination genes, *Brca1*; expression; dimethylbenzanthracene

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ИЗМЕНЕНИЯ В ЭКСПРЕССИИ ГЕНОВ ГОМОЛОГИЧНОЙ РЕКОМБИНАЦИИ В ХИМИЧЕСКИ ИНДУЦИРОВАННЫХ ОПУХОЛЯХ *IN VIVO*

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Изучение молекулярных механизмов канцерогенеза, включая нарушение в системе гомологичной рекомбинации (ГР), является важной задачей при изучении процесса малигнизации. Дисфункция генов ГР, таких как *BRCA1/2*, способствует геномной нестабильности и развитию более агрессивных опухолевых клонов. Использование химических канцерогенов, таких как диметилбенз(а)антрацен (ДМБА), позволяет моделировать процессы опухолеобразования и анализировать изменения экспрессии генов репарации. Изучение этих изменений важно для понимания механизмов адаптации опухолевых клеток к генотоксическому стрессу и разработки персонализированных подходов к терапии рака. Целью работы было оценить экспрессию основных генов ГР при химиоиндуцированном канцерогенезе у мышей. Исследование проводили на самках аутбредных лабораторных мышей ICR (CD-1; $n = 20$). Сформировано две группы животных: контрольная и группа исследования с введением ДМБА. Для идентификации опухолей аутопсийный материал подвергали гистологическому анализу. Уровень экспрессии генов оценивали при помощи ОТ-ПЦР, наличие хромосомных аберраций — посредством цифровой ПЦР. Наличие опухолей установлено у четырех животных. В трех образцах опухоли наблюдали нулевую экспрессию *Brca1, Brca2, Cdk12, Chek2, Palb2, Bard1, Brip1* и паралогов *Rad*. У одного образца зафиксированы высокие уровни генов *Cdk12* (14,3), *Chek1* (27,6), *Rad51d* (38,5). В большинстве случаев зафиксировано преобладание делеций в исследуемых генах. Таким образом, при опухолеобразовании происходит снижение экспрессии основных генов репарации, формирование хромосомных аберраций, что может способствовать появлению более агрессивных клонов, а также увеличивать чувствительность к химиопрепаратам.

Ключевые слова: химиоиндуцированный канцерогенез, гены гомологичной рекомбинации, *Brca1*, экспрессия, диметилбензантрацен

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Studying the mechanisms underlying the emergence and progression of solid tumors remains one of the most urgent tasks of modern biomedicine [1]. Alteration of homologous recombination system genes involved in double-stranded DNA breakrepair, cell cycle regulation, and other cellular processes can be one of the key events underlying carcinogenesis [2]. Such alterations can manifest itself as dysfunction (accumulation of mutations, major chromosomal rearrangements, changes in expression, etc.) of the key homologous recombination genes, specifically *BRCA1* and *BRCA2*. Dysfunction leads to deficiency of the repair of double-stranded DNA breaks, or homologous recombination deficiency (HRD) [3]. At the same time, the expression levels of *BRCA1/2* and other HR system genes represent the major ultimate factors determining not only the genetic and functional dysfunction extent, but also the tumor cell sensitivity to various chemotherapeutic agents.

According to the working hypothesis of the present study, the mechanisms underlying the development of homologous recombination deficiency are enhanced, increasing genetic instability during the tumor transformation, growth, and progression. From an evolutionary perspective, it is "beneficial" for the tumor to form a mutator phenotype, primarily through the homologous recombination dysregulation. Moreover, the mechanisms underlying the development of HRD in tumor cells have to be enhanced through the increase of the rate and expansion of the range of abnormalities. Baseline mutations in the HR genes can be complemented by deletions, methylation, gene repression, etc., which should lead to generation of more aggressive clones. However, this process remains practically unexplored. The study of the process will help predict the risk of malignant transformation for precancerous disorders and the aggressiveness of early-stage tumors.

Chemicals that cause mutations and other genotoxic changes are particularly valuable for carcinogenesis modeling, since these are direct triggers of tumor development. The 7,12-dimethylbenz[a]anthracene (DMBA) having the pronounced carcinogenic properties and widely used in pre-clinical trials for simulation of tumorigenesis processes *in vivo* and *in vitro* is one of the most common polycyclic aromatic hydrocarbons in our environment [4, 5]. Currently, the data on expression profiles of the tumor genes induced by specific chemical carcinogens remain scarce. The available data suggest the association between the effects of chemicals and the homologous recombination abnormalities. For example, it has been shown that the exposure to the PAH doses appropriate to the environmental conditions results in the dose-dependent *BRCA1* expression decrease in breast cancer cells [6]. The earlier research showed that PAH also suppressed *BRCA1 in vitro* and *in vivo* [7]. In this context, the use of mouse models has become a powerful instrument for *in vivo* assessment of cancer etiology and progression. However, studying the homologous recombination suppression effects *in vivo* is still a challenging task, especially in the context of chemotherapy-induced carcinogenesis. For example, it has been shown that the *Rad51* mutations strongly predispose mice to lymphomas, while the *Brca1* mutations contribute to the development of tumors of other types [8]. Mouse models with the partial loss of *Brca2* function also show increased carcinogenesis levels with predispodition to lymphomas [9]. In addition to the *BRCA1* and *BRCA2* key HR genes, other components of the pathways play an important role in both DNA repair and carcinogenesis. The mouse model studies have shown that the decrease in activity of the same *Rad51* gene in vivo does not contribute to tumor development, but rather ensures protection against the tumor. These data suggest that the *Rad51*-mediated repair can contribute to tumor progression rather than function as a tumor suppressor [10, 11]. Other HR genes with low penetrance, including *ATM*, *CHEK2*, *BRIP1*, and *BARD1*, have been extensively studied in the context of human breast carcinogenesis [12]. However, their function and contribution to tumorigenesis in mouse models are still poorly understood. Today, the data on the regulation of these genes in mouse models are limited, which hinders full understanding of their role in DNA repair and carcinogenesis [12]. That is why the analysis of the HR gene expression changes during carcinogenesis can make it possible to determine, how the cells respond to genotoxic stress and how these processes can be disrupted in tumor tissues [13]. Understanding of these dynamic changes can determine the tumor cell sensitivity to DNA-damaging agents, which is important for personalized cancer treatment. Thus, the present study aimed to assess the expression of the key homologous recombination system genes in chemotherapy-induced carcinogenesis in mice.

METHODS

Animals

Chemotherapy-induced carcinogenesis was assessed in 20 female outbred ICR laboratory mice (CD-1). The animals were handled in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS N 123). The animals were kept in standard conditions at a temperature of 22 ± 2 °C, relative humidity of 50–60%, with the 12-h light/dark cycle (8:00 to 20:00). Food and water were provided freely. All the procedures involving animals were conducted in the morning (9:00 to 11:00 local time) in accordance with the rules and guidelines on humane treatment of animals used for experimental and other scientific purposes. The health and behavioral aspects were monitored daily, and any signs of discomfort or illness were immediately eliminated by specialists.

Experimental design

The design was developed in accordance with the 3R principles, reducing the number of animals to the necessary minimum and minimizing discomfort. Two groups of animals were formed for the experiment. Weighing and randomization (based on the mean body weight, mean ± 10%) were used to divide the animals into the control (n = 10) and treatment (n = 10) groups. The mean body weight was 27.2 ± 0.62 g in the control group and 26.4 ± 0.56 g in the treatment group. Dimethylbenz(a)anthracene (DMBA) (100 mg, 1,3-dimethylbutylamine, 98%, Sigma-Aldrich #108-09-8) was used as a chemical agent possessing direct or indirect genotoxicity to induce carcinogenesis.

Dose selection

Since DMBA is not soluble in water, but is well soluble in organic solvents, when preparing the working solution, 100 mg of the substance was dissolved in 10 mL of toluene until completely dissolved, thereby obtaining a matrix solution with the substance concentration of 1 mg per 0.1 mL (100 μL) of the solvent. A total of 0.07 mg (70 μg) of the substance was taken for the course. Recalculated on the solution: 70 μL of the substance dissolved in toluene were adjusted to 30 ml of vegetable oil. With this dilution, the substance dose was 23 μg/kg, provided that 0.25 mL of the solution per 25 g of live weight would be administered. The resulting solution was

intragastrically administered weekly throughout 3 months. It should be noted that according to published data, toluene can have a toxic effect on behavioral characteristics of laboratory animals and some body's molecular parameters [14]. However, with minimal toluene doses cytochrome P4502A13 can effectively metabolize this substance in the body [15] without any negative effects.

Euthanasia

Humane euthanasia of animals was accomplished using the CO_2 camera with the gradually increasing gas concentration. Autopsy of the animals aimed at identifying the tumor was performed in accordance with the method for laboratory animal autopsy and organ harvesting [16]. After harvesting the tissue was placed in the RNAlater solution (Thermo Fisher Scientific, USA). After the 24-h incubation at +4 °C the tissue samples were stored at a temperature of –80 °C for further DNA and RNA extraction.

Morphological examination

To assess the pattern of morphological changes and confirm the presence of tumor tissue in the samples, the tissue fragments removed from the suspected tumor site, as well as metastases, were examined. The tissue fragments sized 5 mm^3 were placed in the 10% pH-neutral formalin (6.5–7.5). The duration of fixation was 18–24 h. Then the material was processed in accordance with the standard method and paraffin embedded. The 4–5 μm thick serial sections were prepared from paraffin blocks [17]. Slides were stained using the hematoxylin and eosin solutions prepared in accordance with the generally accepted protocols. Morphological examination was performed using the Axio Scope.A1 light microscope (Karl Zeiss, Germany). Microscopic evaluation was carried out according to the generally accepted criteria [18].

DNA and RNA extraction

RNA and DNA were extracted from the tumor tissue using the RNeasy Plus mini Kit and QIAamp DNA mini Kit (Qiagen, Germany), respectively, in accordance with the manufacturer's instructions.

Real-time qPCR

Expression levels of the homologous recombination genes *Brca1, Brca2, Atm, Bard1, Brip1, Cdk12, Chek1, Chek2, Fancl, Palb2, Ppp2r2a, Rad51b, Rad51c, Rad51d, Rad54l, Parp1* were assessed using the reverse transcription quantitative PCR in the real-time mode (RT-qPCR) based on the TaqMan technology in the Rotor-Gene-6000 thermal cycler (Qiagen, Germany), as previously reported [19]. Two genes were used as reference ones: *Gapdh* (glyceraldehyde 3-phosphate dehydrogenase) and *Actb* (beta-actin); expression levels of these genes were normalized with respect to normal expression values of the genes and measured in arbitrary units. The gene relative expression was assessed by the Pfaffl method [5]. RNA extracted from the normal tissue was used for calibration.

Digital PCR

Digital PCR in the QIAcuity Digital PCR System (Qiagen, Germany) was used as a method to analyze the *Brca1, Brca2, Cdk12, Chek1, Parp1*, and *Rad51c* gene copy number. The copy number variation analysis involved determination of the number of targets and reference loci by duplex amplification. The *Ap3b1* (adaptor related protein complex 3 subunit beta 1) gene recommended by the manufacturer was selected as a reference gene.

Statistical processing of the results

Statistical data processing was performed using the Statistica 8.0 software package (StatSoft Inc., USA).

RESULTS

In the first phase of the study, we assessed the dynamic changes in body weight in the studied groups of animals between 30 December 2023 and 16 November 2025 (Fig. 1). Animals of the control group showed no changes in body weight during the follow-up period. DMBA administration caused significant changes in the animals' body weight within six weeks (Fig. 1). In particular, the mean body weight of animals of the control group in this period was 30.5 ± 0.84 compared to that of the DMBA group (28.3 ± 0.54; $p = 0.05$). In three weeks, the differences became more pronounced ($p = 0.02$), with the body weight of the DMBA group (28.7 ± 0.69) and that of the control group (31.4 ± 1.07), respectively. Later the upward trend of the animals' body weight was observed in both control and experimental groups. However, no significant differences were reported throughout the experiment.

Upon palpating (and visually examining), tumors were found in four animals on weeks 46, 55, 56, and 61 of the experiment (Fig. 1).

After euthanasia, autopsy of the animals was performed, as well as testing for tumors and metastasis to distant organs (if any). A total of four mice out of 10 developed tumors. No tumors were found in the control group. Analysis of specimen obtained from the laboratory animal No. 7, which was located subcutaneously in the cervical region, revealed a morphological pattern of a pleomorphic cellular tumor with the invasive structure forming solid areas, variously sized sockets and cords, as well as trabecular and glandular structures, composed of moderately polymorphic cells of medium size with the moderately pronounced eosinophilic cytoplasm and rounded, hyperchromatic nuclei. Multiple tumor necrosis foci were found in the central areas of glandular structures. The stroma was moderately pronounced. It was represented by various-sized strands of mature fibrous connective tissue with hyalinosis and uneven lymphoplasmacytic infiltration. There was an extremely small fragment of skin with the subcutaneous fat along the edge of the fragment (Fig. 2A). Tumors with similar morphological structure, but localized in the stomach area on the peritoneum, were found in another two laboratory mice (specimens No. 8 and No. 9). Subtotal tissue fragments were represented by necrosis with signs of active inflammation. Structures of the invasive tumor described above were found along the periphery of a tissue fragment in some fields of view (specimen No. 7). The signs of uneven moderate inflammatory infiltration with the presence of neutrophils and corpuscular purulenta were seen throughout the fragment. There were sporadic small calcifications in the thickness of necrotic areas (Fig. 2B and C).

In one more laboratory animal (No. 6), the tumor was localized in the lung tissue (Fig. 2D). Morphological examination revealed lung tissue fragments subtotally substituted with the tumor of polymorphic structure within the slide. A large part of the tumor was represented by merged solid

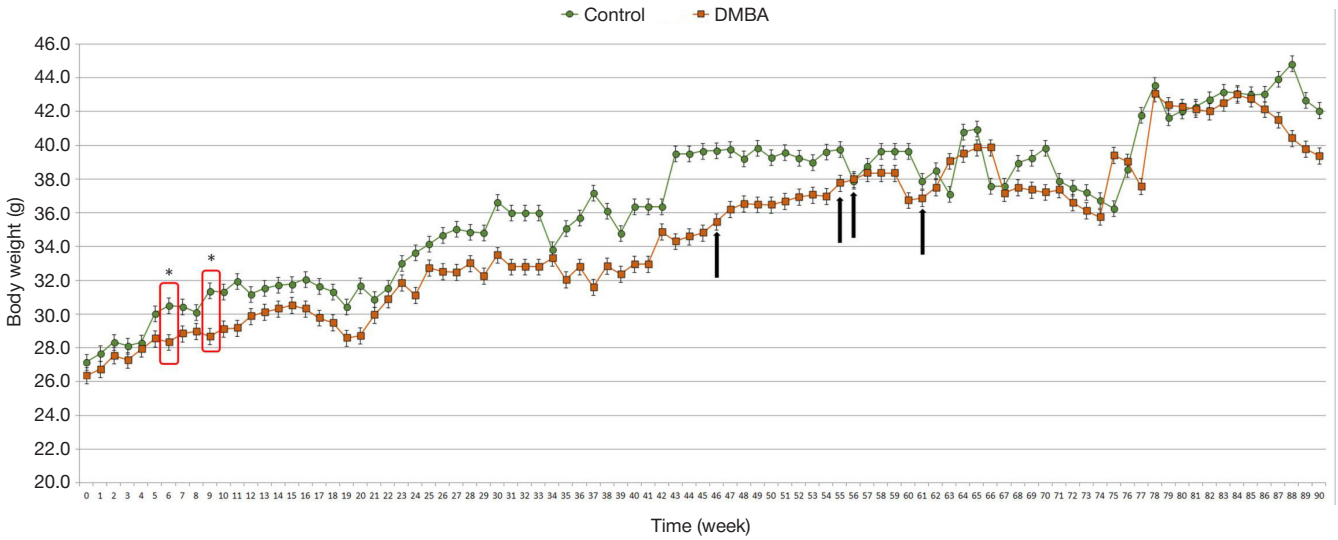

Fig. 1. Dynamic changes in body weight in the studied groups of animals during the follow-up period. DMBA — group of animals administered dimethylbenz[a] anthracene; * — significant differences; the time of tumor emergence in the studied group of animals is marked with *arrows*

areas of moderately polymorphic, moderately sized cells with moderately pronounced eosinophilic cytoplasm and rounded, hyperchromatic nuclei. In some fields of view, the tumor consisted of the acinar structures composed of medium and small sized relatively monomorphic cells. In some foci, the tumor formed pseudovascular fissures; a few small foci of necrosis were found. The moderately pronounced tumor stroma was represented by strands of the mature fibrous connective tissue with mild lymphoid infiltration.

In the next phase of the study, we assessed the expression of homologous recombination genes in the tumor tissue specimens collected (Fig. 3).

In particular, high expression of the genes Brca1 (2.06 AU); *Atm* (6.81 AU), *Bard1* (5.62 AU), *Cdk12* (14.36 AU), *Chek1* (27.68 AU), *Fancl* (5.82 AU), *Rad51d* (38.57 AU) was found for the tumor tissue specimen collected from the laboratory mouse No. 6 (Fig. 3A), which suggests that the homologous recombination system and its potential DNA damage repair activity were preserved in this tumor. It should be also noted that testing for chromosomal aberrations using digital PCR revealed the *Brca1* gene amplification (Table). Low expression of the test genes was observed in other tumor tissue specimens. In particular, in the laboratory mouse No. 7, normal expression was found typical only for *Parp1* (1.001 AU), and hyperexpression was reported only for *Rad51c* (2.9 AU). All other genes, including *Brca1/2*, showed zero or very low expression (Fig. 3B). Similar results were typical for another two samples. In the tumor specimen from the mouse No. 8, zero expression was typical for 8 test genes out of 16 (*Brca1, Brca2, Cdk12, Chek2, Palb2, Rad51b, Rad51c, Rad51d*). In the tumor of mouse No. 9, zero values were reported for 12/16 genes (*Brca1, Brca2, Atm, Bard1, Brip1, Chek2, Fancl, Palb2, Ppp2r2a, Rad51b, Rad51d*,

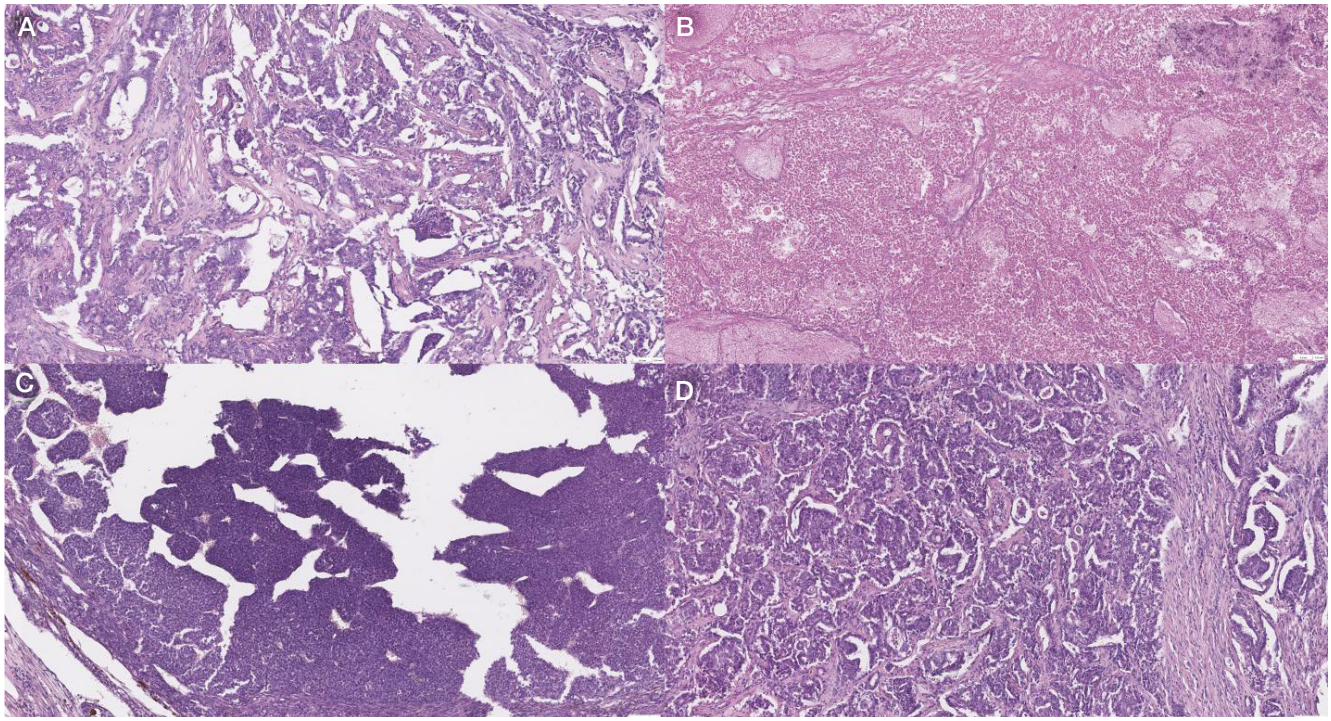

Fig. 2. Microphotographs of the sections of tumor specimens obtained from laboratory animals No. 7 (**A**), No. 8 (**B**), No. 9 (**C**), and No. 6 (**D**), 10× magnification. Hematoxylin and eosin stain

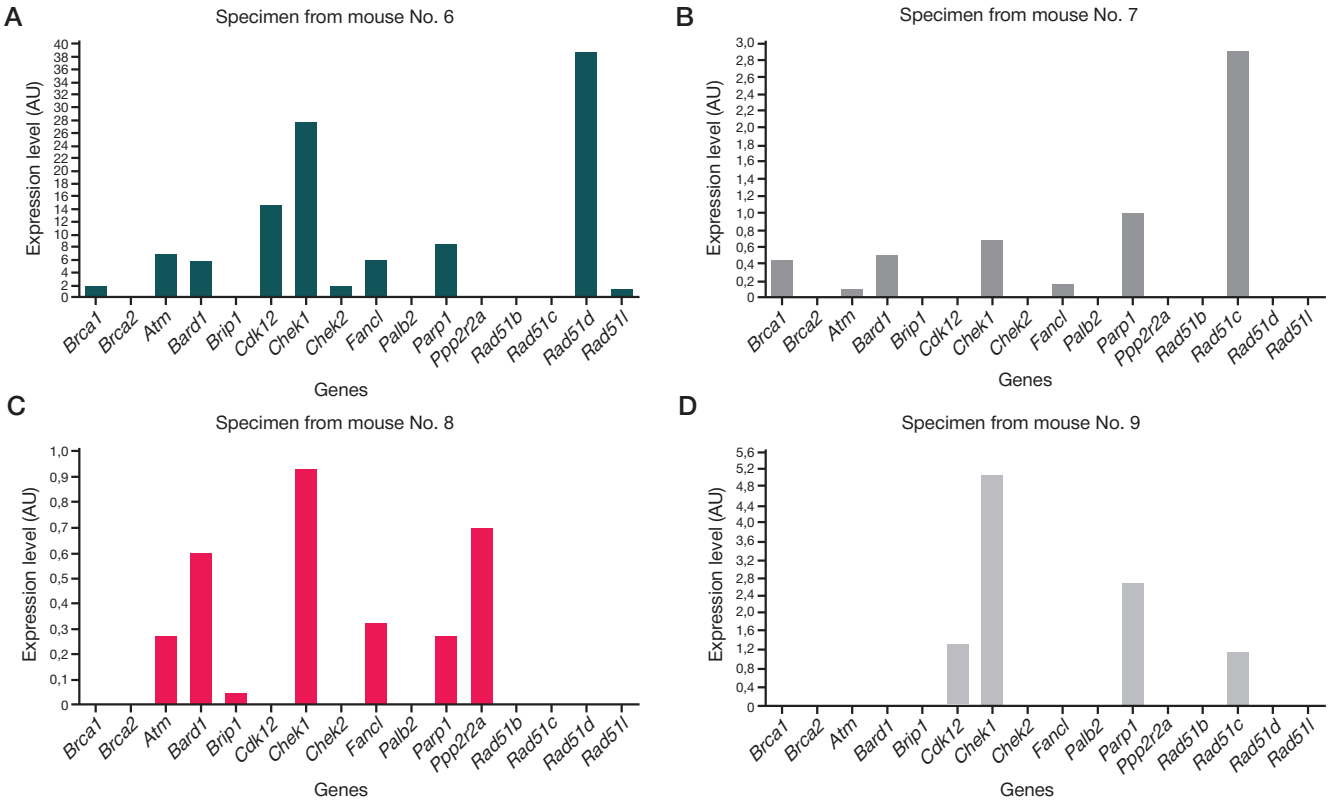

Fig. 3. Homologous recombination gene expression levels in the group of mice administered carcinogen (DMBA)

Rad51l) (Fig. 3C and D). The deletion in the *Brca1* was reported for all specimens. Furthermore, Table 1 provides the data of the analysis of chromosomal rearrangements in some test genes in tumor specimens. The analysis results demonstrate high genomic rearrangement heterogeneity. In particular, in the specimen No. 6, amplification is reported for the genes *Cdk12*, *Chek1*, *Parp1*, along with *Brca1*, and only two deletions are reported for the genes *Brca2* and *Rad51c*. Predominance of deletions in the test genes or normal gene copy number was observed in other specimens. In general, this suggests that the emergence of major chromosomal rearrangements and the DNA repair gene activity decrease can represent one of the carcinogenesis primary events. Furthermore, the fact of identifying amplification and/or normal gene copy number is correlated with high expression of the gene, which is in line with the literature data [20].

Thus, considering the experimental data, it has been found that disturbances of the homologous recombination mechanisms lead to the accumulation of genomic abnormalities and decreased reparative activity, thereby increasing the risk of tumors. However, if the tumor already exists, such disturbances make it more susceptible to DNA-damaging agents. This has been shown in clinical material, where the presence of the deletion and low *BRCA1* expression affected the efficacy of chemotherapy with platinum-based drugs in patients with breast cancer [21], as well as metastasis-free survival of patients with non-small cell lung cancer [22]. The studies of animal models show that such alterations (in HR genes) can contribute to both the increase in the number of tumor cases and the emergence of various tumor clones with different molecular genetic characteristics.

DISCUSSION

Homologous recombination responsible for the repair of double-strand DNA breaks plays an important role in maintaining genomic stability and preventing of carcinogenesis [8]. However, when exposed to carcinogens, such as DMBA, the HR system can show reduced activity, resulting in accumulation of mutations and, therefore, to tumorigenesis [23]. Our study demonstrates hyperexpression of the genes *Cdk12, Brca1, Atm, Bard1, Fancl, Chek1*, and *Parp1*. Furthermore, according to the literature data, disturbances in *Cdk12* functional activity lead to DNA repair abnormalities, causing

Table. Presence of the homologous recombination gene DNA copy number aberrations in tumor specimens

Genes/samples	Tumor specimen from animal No. 6	Tumor specimen from animal No. 7	Tumor specimen from animal No. 8	Tumor specimen from animal No. 9
Brca1	Gain	Loss	Loss	Loss
Brca2	Loss	Loss	Loss	Loss
Cdk12	Gain	Loss	Loss	Loss
Chek1	Gain	n	n	n
Parp1	Gain	n	n	Gain
Rad51c	Loss	Gain	Loss	n

Note: the table provides the data on the presence of major chromosomal rearrangemens for some homologous recombination genes, where gain — amplification; *n* — normal gene copy number; loss — deletion

genomic instability and the decrease in expression of some homologous recombination genes, such as *Brca1*, *Fanci*, and *Fancd2* [21, 22]. Moreover, hyperexpression of the test genes was observed in one specimen, which is not consistent with the hypothesis provided. Unfortunately, since the expression at the mRNA level is not always positively correlated with the quantity and activity of appropriate proteins, the actual functional activity of HR system in tumor cells may differ from the data obtained. This phenomenon can be explained by the influence of post-transcriptional regulatory mechanisms, such as mRNA degradation, alternative splicing, microRNA influence, post-translational modifications of proteins, etc., which can considerably modify the ultimate activity of genes in tumor cells [24]. This emphasizes the need for further research, including the study of HR system activity at the level of post-transcriptional factors.

Special attention was paid to the *Brca1* gene, the expression of which was significantly decreased in tumor tissues. Cases have been reported, when mutant mice with *Brca1* defects (*Brca1tr/tr*) developed tumors of various types, including breast cancer and lymphomas, without any additional genetic alterations, such as *Trp53* gene inactivation. Our data confirm these results and suggest the importance of *Brca1* dysfunction for the mechanism of carcinogenesis [10]. Moreover, assessment of the *Brca1* gene copy number using digital PCR revealed the *Brca1* gene deletion in three animals and amplification and the gene expression level of 2.06 in one animal. Low expression of this gene in tumor tissues was observed in the presence of the *Brca1* gene deletion. We also revealed hyperexpression of the genes *Rad51d* and *Rad51c* in three tumor specimens (Fig. 3). It can be assumed that upregulation of those in tumors can represent a compensatory mechanism in the context of *Brca1/2* dysfunction [25]. A number of studies confirm this hypothesis [10, 11]. As for *Bard1*, it has been shown that the *Bard1* inactivation induces basal-like carcinomas of the breast with the rate, latency, and histopathological characteristics indistinguishable from those observed in mice with the *Brca1* mutation or double *Bard1/Brca1* mutation [13]. Such results suggest that *Bard1* functions as the key tumor suppressor gene, along with *Brca1*, and that the *Brca1*-mediated tumor suppression is largely dependent on the *Bard1/Brca1* heterodimer. However, the question remains, why high *Bard1* expression is observed despite the decreased *Brca1* expression. In our previous *in vitro* study, it was found that when continuously exposed to cytostatic agents, the cell lines with *BRCA1* dysfunction acquired genetic alterations characterized by the HR gene amplification (including *BARD1*) and increased expression [14].

Among the genes tested, it is also important to highlight the *Rad51* gene paralogues that are involved in attracting *Rad51* to the sites of DNA damage [26] and contribute to formation and stabilization of the *Rad51* nucleoprotein filament. However, the exact role of each paralogue is not yet fully defined. Nevertheless, none of the *RAD51* mutations are associated with predisposition to cancer, which constitutes the "*RAD51* paradox" [25]. One potential explanation of the "*RAD51* paradox" is that the mutations affecting the mediator genes/accessory genes (such as *BRCA1* or *BRCA2*) in cancer result in the lack of *RAD51* in damaged DNA, leaving access to alternative, exclusively mutagenic repair processes [25]. Thus, it has been shown in the mouse model that decreasing the *Rad51* activity in vivo not only contributes to tumorigenesis, but also protects against tumors. These data suggest that the *Rad51*-controlled repair is not a tumor suppressor, but rather contributes to tumor progression [10, 11].

CONCLUSIONS

Thus, carcinogenesis is accompanied by developing the homologous recombination deficiency, and the related repair gene abnormalities are enhanced at early stages of transformation and tumor progression. We found significant changes in the copy number and expression profiles of the genes involved in biotransformation of xenobiotics, apoptosis, and cell proliferation. The findings emphasize the need for comprehensive analysis of the homologous recombination gene aberrant states aimed at understanding the carcinogenesis mechanisms and suggest potential directions for the development of novel diagnostic and therapeutic strategies in oncology. Understanding of abnormalities in the test genes, their homo- or heterogeneity can contribute to the development of the algorithms to determine the tumor chemosensitivity to DNA-damaging agents in the future. Such an approach will shed more light on the role of genetic instability in carcinogenesis and open new avenues for treatment methods.

References

1. Smith MT, Guyton KZ, Kleinstreuer N, Borrel A, Cardenas A, Chiu WA, et al. The key characteristics of carcinogens: relationship to the hallmarks of cancer, relevant biomarkers, and assays to measure them. Cancer Epidemiology, Biomarkers & Prevention. 2020; 29 (10): 1887–903.
2. Barnes JL, Zubair M, John K, Poirier MC, and Martin FL. Carcinogens and DNA damage. Biochemical Society Transactions. 2018; 46 (5): p. 1213–24.
3. Turner N, Reis-Filho J, Russell A, Springall R, Ryder K, Steele D, et al. BRCA1 dysfunction in sporadic basal-like breast cancer. Oncogene. 2007; 26 (14): p. 2126–32.
4. Morales-Herrero M and Ortega-Medina I. Experimental carcinogenesis with 7, 12-dimethylbenz (a) anthrazene (DMBA) and its inhibition with isothi-ocyanates. J oral res (Impresa). 2022: p. 1–13.
5. Allam AM, Abubakr HO, Yassin AM, Abdel-Razek AS, Khattab MS Gouda EM, et al. Potential chemopreventive effects of Broccoli extract supplementation against 7, 12 dimethyl Benz (a) anthracene (DMBA)-induced toxicity in female rats. Scientific reports. 2023; 13 (1): p. 1–19.
6. Davidson CJ, Svenson D, Hannigan JH, Perrine SA, and Bowen SE. A novel preclinical model of environment-like combined benzene, toluene, ethylbenzene, and xylenes (BTEX) exposure: Behavioral and neurochemical findings. Neurotoxicology and teratology. 2022; 91: 1–11.
7. Sahay D, Lloyd SE, Rivera JA, Jezioro J, Mcdonald JD, Pitiranggon M, et al. Prenatal polycyclic aromatic hydrocarbons, altered ERα pathway-related methylation and expression, and mammary epithelial cell proliferation in offspring and grandoffspring adult mice. Environmental research. 2021; 196: 1–15.
8. Xu X, Qiao W, Linke SP, Cao L, Li W-M, Furth PA, et al. Genetic interactions between tumor suppressors Brca1 and p53 in apoptosis, cell cycle and tumorigenesis. Nature genetics. 2001; 28 (3): 266–71.
9. Yossepowitch O, Olvera N, Satagopan JM, Huang H, Jhanwar S, Rapaport B, et al. BRCA1 and BRCA2 germline mutations in lymphoma patients. Leukemia & lymphoma. 2003; 44 (1): 127–31.
10. Matos-Rodrigues G, Barroca V, Muhammad AA, Dardillac E, Allouch A, Koundrioukoff S, et al. In vivo reduction of RAD51-mediated homologous recombination triggers aging but impairs oncogenesis. The EMBO journal. 2023; 42 (20): 1–21.

11. Kuznetsov SG, Haines DC, Martin BK, and Sharan SK. Loss of Rad51c leads to embryonic lethality and modulation of Trp53-dependent tumorigenesis in mice. Cancer research. 2009; 69 (3): 863–72.
12. Yamamoto H and Hirasawa A. Homologous recombination deficiencies and hereditary tumors. International journal of molecular sciences. 2021; 23 (1): 1–18.
13. Coates PJ, Lorimore SA, and Wright EG. Cell and tissue responses to genotoxic stress. The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland. 2005; 205 (2): 221–35.
14. Cruz SL, Rivera-García MT, and Woodward JJ. Review of toluene action: clinical evidence, animal studies and molecular targets. Journal of drug and alcohol research. 2014; 3: 1–15.
15. Fukami T, Katoh M, Yamazaki H, Yokoi T, and Nakajima M. Human cytochrome P450 2A13 efficiently metabolizes chemicals in air pollutants: naphthalene, styrene, and toluene. Chemical research in toxicology. 2008; 21 (3): 720–5.
16. Rathnamali K. Dissection of laboratory animal and sample collection for histology. International Journal of Scientific and Applied Research (IJSAR), eISSN: 2583–0279. 2022; 2 (3): 1–12.
17. Sadeghipour A and Babaheidarian P. Making formalin-fixed, paraffin embedded blocks. Biobanking: Methods and Protocols. 2018: 253–68.
18. Greenberg AK, Yee H, and Rom WN. Preneoplastic lesions of the lung. Respiratory research. 2002; 3: 1–10.
19. Tsyganov MM, Bulatova DZ, Fedorenko AA, Loos DM, Nikiforov PE, Tsydenova IA, et al. Assessment of Homologous Recombination System Gene Expression in Chemologically Induced Carcinogenesis In Vivo Models. Current Issues in Molecular Biology. 2026; 48 (3): 1–17.
20. Myhre S, Lingjærde O-C, Hennessy BT, Aure MR, Carey MS, Alsner J, et al. Influence of DNA copy number and mRNA levels on the expression of breast cancer related proteins. Molecular oncology. 2013; 7 (3): 704–18.
21. Tsyganov MM, Ibragimova MK, Garbukov EY, Bragina OD, Karchevskaya AA, Usynin EA, et al. Determination of BRCAness Phenotype in Breast Tumors for the Appointment of Neoadjuvant Chemotherapy Based on Platinum and Taxanes. International journal of molecular sciences. 2022; 24 (1): 1–13.
22. Tsyganov MM, Ibragimova MK, Tsydenova IA, Kravtsova EA, Bayanbaeva AA, Sharipkhanova Zh, Rodionov EO, Bragina OD, Mokh AA, Miller SV. Influence of DNA copy number aberrations and changes in the expression level of homologous recombination genes on the survival of primary operable non-small cell lung cancer patients. Acta Biomedica Scientifica.2026; 10 (6): 112–22.
23. Hollander MC, Kovalsky O, Salvador JM, Kim KE, Patterson AD, Haines DC, et al. Dimethylbenzanthracene carcinogenesis in Gadd45a-null mice is associated with decreased DNA repair and increased mutation frequency. Cancer research. 2001; 61 (6): 2487–91.
24. Verta J-P and Jacobs A. The evolutionary significance of post-transcriptional gene regulation. Heredity. 2024; 132 (3): 117–9.
25. Lopez BS. RAD51-mediated homologous recombination is a pro-tumour driver pathway. Oncogene. 2025; 44 (42): 4006–16.
26. Garcin EB, Gon S, Sullivan MR, Brunette GJ, Cian AD, Concordet J-P, et al. Differential requirements for the RAD51 paralogs in genome repair and maintenance in human cells. PLoS Genetics. 2019; 15 (10): 1–29.

Литература

1. Smith MT, Guyton KZ, Kleinstreuer N, Borrel A, Cardenas A, Chiu WA, et al. The key characteristics of carcinogens: relationship to the hallmarks of cancer, relevant biomarkers, and assays to measure them. Cancer Epidemiology, Biomarkers & Prevention. 2020; 29 (10): 1887–903.
2. Barnes JL, Zubair M, John K, Poirier MC, and Martin FL. Carcinogens and DNA damage. Biochemical Society Transactions. 2018; 46 (5): p. 1213–24.
3. Turner N, Reis-Filho J, Russell A, Springall R, Ryder K, Steele D, et al. BRCA1 dysfunction in sporadic basal-like breast cancer. Oncogene. 2007; 26 (14): p. 2126–32.
4. Morales-Herrero M and Ortega-Medina I. Experimental carcinogenesis with 7, 12-dimethylbenz (a) anthrazene (DMBA) and its inhibition with isothi-ocyanates. J oral res (Impresa). 2022: p. 1–13.
5. Allam AM, Abubakr HO, Yassin AM, Abdel-Razek AS, Khattab MS Gouda EM, et al. Potential chemopreventive effects of Broccoli extract supplementation against 7, 12 dimethyl Benz (a) anthracene (DMBA)-induced toxicity in female rats. Scientific reports. 2023; 13 (1): p. 1–19.
6. Davidson CJ, Svenson D, Hannigan JH, Perrine SA, and Bowen SE. A novel preclinical model of environment-like combined benzene, toluene, ethylbenzene, and xylenes (BTEX) exposure: Behavioral and neurochemical findings. Neurotoxicology and teratology. 2022; 91: 1–11.
7. Sahay D, Lloyd SE, Rivera JA, Jezioro J, Mcdonald JD, Pitiranggon M, et al. Prenatal polycyclic aromatic hydrocarbons, altered ERα pathway-related methylation and expression, and mammary epithelial cell proliferation in offspring and grandoffspring adult mice. Environmental research. 2021; 196: 1–15.
8. Xu X, Qiao W, Linke SP, Cao L, Li W-M, Furth PA, et al. Genetic interactions between tumor suppressors Brca1 and p53 in apoptosis, cell cycle and tumorigenesis. Nature genetics. 2001; 28 (3): 266–71.
9. Yossepowitch O, Olvera N, Satagopan JM, Huang H, Jhanwar S, Rapaport B, et al. BRCA1 and BRCA2 germline mutations in lymphoma patients. Leukemia & lymphoma. 2003; 44 (1): 127–31.
10. Matos-Rodrigues G, Barroca V, Muhammad AA, Dardillac E, Allouch A, Koundrioukoff S, et al. In vivo reduction of RAD51-mediated homologous recombination triggers aging but impairs oncogenesis. The EMBO journal. 2023; 42 (20): 1–21.
11. Kuznetsov SG, Haines DC, Martin BK, and Sharan SK. Loss of Rad51c leads to embryonic lethality and modulation of Trp53-dependent tumorigenesis in mice. Cancer research. 2009; 69 (3): 863–72.
12. Yamamoto H and Hirasawa A. Homologous recombination deficiencies and hereditary tumors. International journal of molecular sciences. 2021; 23 (1): 1–18.
13. Coates PJ, Lorimore SA, and Wright EG. Cell and tissue responses to genotoxic stress. The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland. 2005; 205 (2): 221–35.
14. Cruz SL, Rivera-García MT, and Woodward JJ. Review of toluene action: clinical evidence, animal studies and molecular targets. Journal of drug and alcohol research. 2014; 3: 1–15.
15. Fukami T, Katoh M, Yamazaki H, Yokoi T, and Nakajima M. Human cytochrome P450 2A13 efficiently metabolizes chemicals in air pollutants: naphthalene, styrene, and toluene. Chemical research in toxicology. 2008; 21 (3): 720–5.
16. Rathnamali K. Dissection of laboratory animal and sample collection for histology. International Journal of Scientific and Applied Research (IJSAR), eISSN: 2583–0279. 2022; 2 (3): 1–12.
17. Sadeghipour A and Babaheidarian P. Making formalin-fixed, paraffin embedded blocks. Biobanking: Methods and Protocols. 2018: 253–68.
18. Greenberg AK, Yee H, and Rom WN. Preneoplastic lesions of the lung. Respiratory research. 2002; 3: 1–10.
19. Tsyganov MM, Bulatova DZ, Fedorenko AA, Loos DM, Nikiforov PE, Tsydenova IA, et al. Assessment of Homologous Recombination System Gene Expression in Chemologically Induced Carcinogenesis In Vivo Models. Current Issues in Molecular Biology. 2026; 48 (3): 1–17.
20. Myhre S, Lingjærde O-C, Hennessy BT, Aure MR, Carey MS, Alsner J, et al. Influence of DNA copy number and mRNA levels on the expression of breast cancer related proteins. Molecular oncology. 2013; 7 (3): 704–18.
21. Tsyganov MM, Ibragimova MK, Garbukov EY, Bragina OD, Karchevskaya AA, Usynin EA, et al. Determination of BRCAness Phenotype in Breast Tumors for the Appointment of Neoadjuvant

Chemotherapy Based on Platinum and Taxanes. International journal of molecular sciences. 2022; 24 (1): 1–13.
22. Цыганов М., Ибрагимова М., Цыденова И., Кравцова Е., Баянбаева А., Шарипханова Ж., и др. Влияние наличия аберраций числа копий ДНК и изменений в уровне экспрессии генов гомологичной рекомбинации на выживаемость больных первично-операбельным немелкоклеточным раком легкого. Acta Biomedica Scientifica. 2026; 10 (6): 112–22.
23. Hollander MC, Kovalsky O, Salvador JM, Kim KE, Patterson AD, Haines DC, et al. Dimethylbenzanthracene carcinogenesis in Gadd45a-null mice is associated with decreased DNA repair and increased mutation frequency. Cancer research. 2001; 61 (6): 2487–91.
24. Verta J-P and Jacobs A. The evolutionary significance of post-transcriptional gene regulation. Heredity. 2024; 132 (3): 117–9.
25. Lopez BS. RAD51-mediated homologous recombination is a pro-tumour driver pathway. Oncogene. 2025; 44 (42): 4006–16.
26. Garcin EB, Gon S, Sullivan MR, Brunette GJ, Cian AD, Concordet J-P, et al. Differential requirements for the RAD51 paralogs in genome repair and maintenance in human cells. PLoS Genetics. 2019; 15 (10): 1–29.