

ONCOLOGY

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

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RESUME

Understanding the molecular mechanisms underlying the pathogenesis of lung cancer is critical for developing effective therapeutic strategies. It is important to study the role of key DNA repair genes, such as BRCA1, CHEK1, etc., in the context of the presence of aberrations in them and their influence on long-term treatment outcomes.

*Thus, **the aim** of the work was to assess the presence of chromosomal aberrations and expression in the main genes of the homologous recombination system in lung tumor.*

Materials and methods. *The study included 111 patients with non-small cell lung cancer (NSCLC) stage IIA-IIIB. RNA and DNA were isolated from the surgical material of normal and tumor tissue. The expression level of HR genes was assessed using RT-PCR. The presence of chromosomal aberrations and loss of heterozygosity sites was determined using microarray analysis.*

Results. *Evaluation of the expression level showed that the presence of hyperexpression (more than 1) of the RAD51D gene is associated with 100 % metastasis-free survival (MFS) compared to the group with low expression (67.5 %), log-rank test $p = 0.05$. The effect of amplification in the ATM, BRIP1, BRCA1 genes on the increase in their expression was established (at $p < 0.05$). For the BRCA1 gene, an association was established between the presence of a deletion and gene hypoexpression ($p = 0.00006$). According to Kaplan – Meier curves, in the presence of amplifications in the PPP2R2A gene in the tumor, the 5-year survival rate is only 50 %, compared to normal copy number with 85 % survival ($p = 0.02$). The opposite picture is observed for the BRCA2 gene: with a deletion, the survival rate is 54 % versus 88 % in the norm ($p = 0.001$).*

Conclusion. *It remains important to further research to better understand the interaction between aberrations in the genes of the HR system and the assessment of the expression profile of these genes and therapeutic responses, which may ultimately lead to more effective and individualized treatment strategies for NSCLC.*

Keywords: *non-small cell lung cancer, homologous recombination genes, DNA copy number aberrations, prognosis, survival, personalized treatment*

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ВЛИЯНИЕ НАЛИЧИЯ АБЕРРАЦИЙ ЧИСЛА КОПИЙ ДНК И ИЗМЕНЕНИЙ В УРОВНЕ ЭКСПРЕССИИ ГЕНОВ ГОМОЛОГИЧНОЙ РЕКОМБИНАЦИИ НА ВЫЖИВАЕМОСТЬ БОЛЬНЫХ ПЕРВИЧНО-ОПЕРАБЕЛЬНЫМ НЕМЕЛКОКЛЕТОЧНЫМ РАКОМ ЛЕГКОГО

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РЕЗЮМЕ

Понимание молекулярных механизмов, лежащих в основе патогенеза рака легкого, является критически важным для разработки эффективных терапевтических стратегий. Важным является изучение роли ключевых генов репарации ДНК, таких как BRCA1, CHEK1 и др., в контексте наличия нарушений в них и их влияния на отдаленные результаты лечения.

Цель. Оценка наличия хромосомных aberrаций и экспрессии в основных генах системы гомологичной рекомбинации (ГР) в опухоли легкого.

Материалы и методы. В исследование было включено 111 больных немелкоклеточным раком легкого (НМРЛ) IIA–IIIB стадии. РНК и ДНК выделяли из операционного материала нормальной и опухолевой ткани. Уровень экспрессии генов ГР оценивали при помощи ОТ-ПЦР. Наличие хромосомных aberrаций и участков потери гетерозиготности проводили при помощи микроматричного анализа.

Результаты. Оценка уровня экспрессии показала, что наличие гиперэкспрессии (более 1) гена RAD51D сопряжена со 100 % безметастатической выживаемостью (БМВ) по сравнению с группой с низкой экспрессией (67,5 %), log-rank test $p = 0,05$. Установлено влияние наличия амплификации в генах ATM, BRIP1, BRCA1 на рост их экспрессии (при $p < 0,05$). Для гена BRCA1 установлена связь наличия делеции с гипоекспрессией гена ($p = 0,00006$). Согласно кривым Каплана – Майера, при наличии амплификаций в гене PPP2R2A в опухоли показатель 5-летней безметастатической выживаемости (БМВ) составляет всего 50 %, по сравнению с нормальной копийностью при 85 % выживаемости ($p = 0,02$). Для гена BRCA2 наблюдается противоположная картина: при делеции показатель БМВ составляет 54 % против 88 % в норме ($p = 0,001$).

Заключение. Важным остается необходимость дальнейших исследований для более глубокого понимания взаимодействия между нарушениями в генах системы ГР и оценкой экспрессионного профиля данных генов, и терапевтическими ответами, что в конечном итоге может привести к более эффективным и индивидуализированным стратегиям лечения НМРЛ.

Ключевые слова: немелкоклеточный рак легкого, гены гомологичной рекомбинации, aberrации числа копий ДНК, прогноз, выживаемость, персонализированное лечение

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INTRODUCTION

Currently, non-small cell lung cancer (NSCLC) is diagnosed at a resectable stage in a significant number of patients [1]. Approximately 20 % of patients are diagnosed with the disease at stages I and II, and 30 % are diagnosed at stage III. For stages II–III, complete resection is the preferred treatment method [2]. However, the 5-year recurrence rate for these patients can reach 62 % for stage II and 76 % for stage III. It is important to note that approximately 48–57 % of patients with resectable NSCLC who are diagnosed in these stages receive adjuvant chemotherapy [1]. Non-platinum cytotoxic chemotherapy, such as docetaxel or pemetrexed, is used as monotherapy after first-line treatment. Patients who receive this type of treatment in the adjuvant setting, however, have a poor prognosis, with a response rate below 10 % and median progression-free survival no more than three months [3]. According to current guidelines, platinum-based chemotherapy is the standard treatment for NSCLC in both the preoperative and postoperative periods [4, 5]. A meta-analysis evaluating the efficacy of adjuvant cisplatin therapy has shown that platinum-based chemotherapy increases the 5-year overall survival (OS) rate by 5.4 % in patients with resectable NSCLC [6]. Another meta-analysis of 10 studies on patients with NSCLC who received platinum rechallenge found that the objective response rate to treatment regimens containing platinum as a second-line therapy was 27.5 %. The median progression-free survival was 3.9 months, and the median overall survival was 8.7 months [7]. These results indicate that the use of platinum doublets as subsequent chemotherapy may be an effective treatment option. Nevertheless, one of the reasons for chemotherapy inefficiency may be the DNA repair process, which occurs in response to platinum drugs and other DNA-damaging chemotherapeutics. The process of homologous recombination is defined as the exchange of nucleotide sequence involving specific enzymes encoded by genes involved in homologous recombination, one of which is the *BRCA1* gene [8]. While *BRCA1* mutations and epigenetic alterations are present in both hereditary and sporadic forms of breast and ovarian cancer, deficiency of *BRCA1* due to epigenetic changes or other mechanisms is rare in lung tumors [9]. Additionally, *BRCA1* is a molecular marker for a number of cytotoxic agents, including platinum-based drugs and those that disrupt the mitotic spindle, which can be utilized as first-line treatment for lung tumor patients [10]. Therefore, studying the various aberrant forms of the *BRCA1* gene and other genes involved in homologous recombination may serve as an additional indicator of chemotherapy efficacy. For example, in a pioneering study, Taron et al. demonstrated the potential role of *BRCA1* mRNA expression in determining the sensitivity of NSCLC to chemotherapy [11]. In patients treated with gemcitabine and cisplatin in the neoadjuvant setting, there was

a correlation between the treatment effect and the level of *BRCA1* expression. Patients with a level below 0.61 showed a better outcome, whereas those with a level above 2.45 showed a worse outcome. Analysis of mutations in the *BRCA1* and *BRCA2* genes in 6,220 NSCLC patients revealed 64 pathogenic mutations and 699 variants of uncertain significance [12]. The overall frequency of identified mutations was 1.03 % (64/6,220 cases), with a predominance of mutations in the *BRCA2* gene (49/64, 76.5 %). A positive association was found between the presence of these mutations and the risk of NSCLC development.

With regard to other genes of the homologous recombination (HR) system, it has been established that genes, such as serine/threonine protein kinase (*ATM*), *BRCA1*-associated RING domain 1 (*BARD1*), *BRCA1*-interacting protein C-terminal helicase 1 (*BRIP1*), and poly(ADP-ribose) polymerase 1 (*PARP1*), among others, also have high potential for predicting and prognostication [13]. Drug therapy targeting homologous recombination deficiency (HRD), such as PARP inhibitors, is commonly used in the treatment of breast cancer, ovarian cancer, and prostate cancer [14]. However, due to the presence of genomic instability and sensitivity to DNA-damaging agents in lung tumors, PARP inhibitors represent a promising option for targeted therapy research in this field. Nevertheless, this necessitates an understanding of the mutational profiles of these genes in patients with lung cancer.

AIM

To assess the presence of chromosomal aberrations and expression levels of the main genes involved in the homologous recombination (HR) system in lung tumors.

MATERIALS AND METHODS

The study included 111 patients with non-small cell lung cancer (NSCLC) stages IIA–IIIB, of central and peripheral localization, and a morphologically confirmed diagnosis. The study was conducted in accordance with the Helsinki Declaration of 1964 (amended in 2013), and with the approval of the local ethics committee of the institute (Protocol No. 1 dated January 15, 2016). All patients signed informed consent for their involvement in the study. The patients were treated at the clinic of the Cancer Research Institute, Tomsk National Research Medical Center, where they underwent pneumonectomy or lobectomy. Following surgery, patients received three courses of adjuvant chemotherapy (ACT) with “platinum doublet” regimens, including vinorelbine 25 mg/m² (days 1 and 8)/carboplatin AUC 6 (day 2); doxorubicin 50 mg/m²/carboplatin AUC 6 (day 2); gemcitabine 1,250 mg/m² (days 1 and 8)/

carboplatin AUC 6 (day 2); carboplatin AUC 6 (day 2)/paclitaxel 175 mg/m². The interval between chemotherapy courses was 3 weeks. Chemotherapy was administered when the patient's general condition and laboratory values within satisfactory and without deviations from the norm. The main clinical and pathological characteristics of the patients studied are presented in Table 1.

Tissue samples. The study material consisted of surgical material from normal and tumor lung tissues (~60–70 mm²). Tissue samples were immersed in RNAlater solution (Ambion, USA) and incubated for 24 hours at +4°C. Following this, the tumor samples were stored at -80°C for subsequent RNA isolation.

RNA isolation. RNA was isolated from postoperative tissue samples (both normal and tumor lung tissues) from 46 patients using the RNeasy Plus Mini Kit (Qiagen, Germany #74134), following the manufacturer's instructions. The RNA concentration was measured using a Qubit 4.0 fluorometer (Thermo Fisher Scientific, USA) with an appropriate RNA quantification kit. The concentration ranged from 20 to 500 ng/μL. RNA integrity was assessed using capillary electrophoresis on a TapeStation system (Agilent Technologies, USA) and R6K Screen Tape kit. The RIN value ranged from 5.6 to 9.0. The isolated RNA was then stored at -80°C in a low-temperature freezer (Sanyo, Japan) and used for gene expression analysis via quantitative reverse transcription PCR (RT-qPCR).

TABLE 1
CLINICAL AND PATHOLOGICAL PARAMETERS OF PATIENTS WITH LC

Clinical and pathological parameter		Number of patients (n = 111), n (%)
Sex	Male	82 (73.9)
	Female	29 (26.1)
Age (years)	Mean age	60.2 ± 0.76 years
	≤ 50 years	20 (18.0)
	> 50 years	91 (82.0)
Tumor size	T ₁	19 (17.1)
	T ₂	41 (37.0)
	T ₃	42 (37.8)
	T ₄	6 (5.4)
	No data	3 (2.7)
Lymphogenous metastasis	N ₀	46 (41.5)
	N ₁	24 (21.6)
	N ₂	34 (30.6)
	N ₃	3 (2.7)
	No data	4 (3.6)
Clinical and anatomical form of lung cancer	Central	30 (27.0)
	Peripheral	81 (73.0)
Histological tumor type	Squamous cell carcinoma	52 (46.8)
	Adenocarcinoma	59 (53.2)
Type of surgery	Pneumonectomy	26 (23.4)
	Pneumonectomy	85 (76.6)
Chemotherapy regimen	Vinorelbine/carboplatin	36 (32.1)
	Doxorubicin/carboplatin	21 (18.9)
	Gemcitabine/carboplatin	41 (37.7)
	Carboplatin/paclitaxel	13 (11.3)

DNA isolation. DNA was isolated from lung tumor samples ($n = 111$) using the QIAamp DNA Mini Kit (Qia-gen, Germany #51304), following the manufacturer's instructions. DNA concentration and purity were assessed using a NanoDrop-2000 spectrophotometer (Thermo Scientific, USA). Concentration ranged from 50 to 300 ng/ μ L, with $A_{260}/A_{280} = 2.10$ – 2.35 , and $A_{260}/A_{230} = 2.15$ – 2.40 . DNA integrity was assessed using capillary electrophoresis on a TapeStation system (Agilent Technologies, USA) with the Agilent Genomic DNA ScreenTape System Quick Guide (Agilent Technologies, USA #5067–5365). The isolated DNA was stored at -80°C in a low-temperature freezer (Sanyo, Japan) and used for subsequent microarray studies.

Quantitative Real-time PCR. The expression level of homologous recombination genes was assessed in 46 patients. The expression of *BRCA1*, *BRCA2*, *ATM*, *BARD1*, *BRIP1*, *CDK12*, *CHEK1*, *CHEK2*, *FANCL*, *PALB2*, *PPP2R2A*, *RAD51B*, *RAD51C*, *RAD51D*, *RAD54L*, and *PARP1* was assessed using reverse transcription quantitative real-time PCR (RT-qPCR) with TaqMan technology on a Rotor-Gene-6000 amplifier (Corbett Research, Australia). PCR was performed in triplicates in a volume of 15 μ L containing 250 μ M dNTPs (Sibenzyme, Russia), 300 nM of forward and reverse primers, 200 nM probes, 2.5 mM MgCl_2 , 1 $\times$ SE buffer (67 mM Tris-HCl pH 8.8 at 25°C , 16.6 mM $(\text{NH}_4)_2\text{SO}_4$, 0.01 % Tween-20), 2.5 U HotStart Taq polymerase (Sibenzyme, Russia), and 50 ng cDNA. The amplification program consisted of a two-step process: initial denaturation at 94°C for 10 min., followed by 40 cycles of 94°C for 10 sec., 60°C for 20 sec. Two reference genes were used as housekeeping genes: *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase) and *ACTB* (actin beta). The gene expression levels were measured in arbitrary units and normalized to the levels of these genes in the normal tissue. The relative expression of each gene was assessed using the Pfaffl method [15], and the following formula (1) was applied to calculate the expression ratio of the sample relative to the calibrator:

$$\text{Ratio} = \frac{(E_{\text{target}})^{\Delta\text{Ct, target(calibrator-test)}}}{(E_{\text{ref}})^{\Delta\text{Ct, ref(calibrator-test)}}} \quad (1)$$

where E is the reaction efficiency, Ct is the threshold cycle of target genes and reference genes. $\Delta\text{Ct, target(calibrator-test)} = \text{Ct of the target gene in the calibrator} - \text{Ct of the target gene in the test sample}$; $\Delta\text{Ct, ref(calibrator-test)} = \text{Ct of the reference gene in the calibrator} - \text{Ct of the reference gene in the test sample}$. The RNA isolated from normal tissue was used as the calibrator.

Microarray analysis was conducted to assess the presence of copy number variations in the DNA. High-density CytoScan™ HD Array microarrays (Affymetrix, USA), containing 1.9 million non-polymorphic

markers for copy number aberration analysis, were used. Sample preparation, hybridization, and scanning were performed according to the manufacturer's protocol on an Affymetrix GeneChip® Scanner 3000 7G system (Affymetrix, USA). Chromosome Analysis Suite 4.1 software (Affymetrix, USA) was used to analyze the results obtained from the CytoScan™ HD Arrays. The software identified unbalanced chromosomal aberrations – deletions and amplifications (loss and gain) – as well as loss of heterozygosity (LOH) sites. In the event of “loss”, the software detected a loss of gene copy number; in the event of “gain”, it identified the presence of three or four copies of a gene.

Statistical analysis was performed using Statistica 8.0 software (StatSoft Inc., USA). For each sample, the arithmetic mean and standard error of the mean were calculated. The Wilcoxon – Mann – Whitney test was used to test for significant differences between the groups. Statistical significance was determined at $p < 0.05$. For the analysis of metastasis-free survival (MFS), Kaplan – Meier survival curves were used [16]. The log-rank test was used to compare the statistical significance of differences between the groups.

RESULTS

At the initial stage of the study, the expression levels of the major homologous recombination genes in lung tumor tissues were assessed in 46 patients (Fig. 1A). The lowest mean expression was characteristic of the *BRCA2* gene: 0.3 ± 0.12 . The serine/threonine protein phosphatase 2A gene (*PPP2R2A*) also had a relatively low mean expression of 0.53 ± 0.08 . In contrast, three genes showed significantly higher expression levels: *CHEK2* (1.20 ± 0.17), *CHEK1* (1.74 ± 0.21), and *RAD51L* (1.93 ± 0.54).

Next, using the Kaplan – Meier method, the association between MFS and the expression of HR genes was assessed in a group of 46 patients. Distant metastases developed in 11 patients (23.9 %) within 5–52 months after diagnosis. The one-year MFS was 87.8 %, the 2-year MFS was 85.0 %, and the 5-year MFS was 68.6 %. Considering that the assessment of gene expression levels was performed relative to normal tissue, according to the statistical characteristics of our sample, patients can be divided into two analysis groups: patients with hyperexpression of the gene (expression >1) and patients with hypoexpression (expression <1). Only for one gene were statistically significant differences shown at the borderline level (Fig. 1B). For patients with *RAD51D* hyperexpression, a 100 % metastasis-free survival was observed compared to patients with low expression (67.5 %). The log-rank test showed a p -value of 0.05.

Given that, there are various regulatory mechanisms of gene expression, microarray analysis was used to investigate large-scale chromosomal rearrangements that lead to either loss or gain in gene copy number,

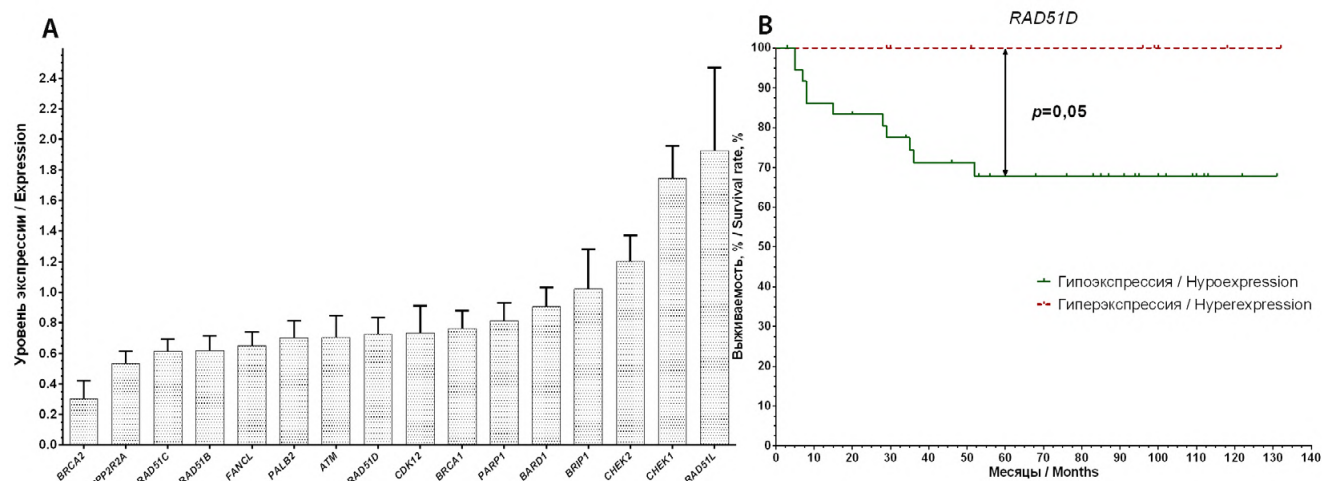


FIG. 1.
The expression level of the main genes of homologous recombination in lung tumor tissue (**A**) and the curves of metastasis-free survival depending on the expression level of the RAD51D gene (**B**)

TABLE 2
FREQUENCY OF CHROMOSOMAL ABERRATIONS IN HOMOLOGOUS RECOMBINATION GENES IN LUNG TUMORS

Gene	Localization	Chromosomal aberrations, n (%)			Loss of heterozygosity sites	
		Gain	n	Loss	LOH	n
BRCA2	13q13.1	2 (1.8)	96 (86.5)	13 (11.7)	18 (16.2)	93 (83.8)
PPP2R2A	8p21.2	6 (5.4)	87 (78.4)	18 (16.2)	17 (15.3)	94 (84.7)
RAD51C	17q22	18 (16.2)	91 (82)	2 (1.8)	44 (39.6)	67 (60.4)
RAD51B	14q24.1	6 (5.4)	99 (89.2)	6 (5.4)	70 (63.1)	41 (36.9)
FANCL	2p16.1	13 (11.7)	97 (87.4)	1 (0.9)	23 (20.7)	88 (79.3)
PALB2	16p12.2	8 (7.2)	99 (89.2)	4 (3.6)	33 (29.7)	78 (70.3)
ATM	11q22.3	4 (3.6)	98 (88.3)	9 (8.1)	32 (28.8)	79 (71.2)
RAD51D	17q12	13 (11.7)	95 (85.6)	3 (2.7)	21 (18.9)	90 (81.1)
CDK12	17q12	13 (11.7)	95 (85.6)	3 (2.7)	21 (18.9)	90 (81.1)
BRCA1	17q21.31	21 (18.9)	85 (76.6)	5 (4.5)	57 (51.4)	54 (48.6)
PARP1	1q42.12	32 (28.8)	77 (69.4)	2 (1.8)	21 (18.9)	90 (81.1)
BARD1	2q35	2 (1.8)	104 (93.7)	5 (4.5)	18 (16.2)	93 (83.8)
BRIP1	17q23.2	11 (9.9)	98 (88.3)	2 (1.8)	79 (71.2)	32 (28.8)
CHEK2	22q12.1	13 (11.7)	91 (82)	7 (6.3)	28 (25.2)	83 (74.8)
CHEK1	11q24.2	8 (7.2)	92 (82.9)	11 (9.9)	15 (13.5)	96 (86.5)
RAD54L	1p34.1	8 (7.2)	98 (88.3)	5 (4.5)	39 (35.1)	72 (64.9)

Note. Gain – increase in copy number; Loss – deletion; n – normal gene copy number (no changes); LOH – loss of heterozygosity.

as well as the presence of loss of heterozygosity (LOH) sites in the genes under study. It was found that these aberrations can significantly affect the expression profile of genes, and consequently, the chemosensitivity and reparative capacity of tumor cells.

In order to assess the frequency of chromosomal abnormalities and LOH sites in HR genes within lung tumor tissue, 111 patient samples were analyzed (Table 2).

It was shown that the highest frequency of deletions occurred in the *BRCA2* (11.7 %, 13/111 cases), *PPP2R2A* (16.2 %, 18/111 cases), and *CHEK1* (9.9 %, 11/111 cases) genes. At the same time, the highest frequency of copy number increase was established for *PARP1* (28.8 %, 32/111 cases), *RAD51C* (16.2 %, 18/111 cases), and *BRCA1* (18.9 %, 21/111 cases) genes. With regard to loss of heterozygosity sites, maximum values were reached in the *BRIP1* gene (71.2 %, 79/111 cases). Additionally, 63.1 % and 51.4 % of patients had LOH in *RAD51B* and *BRCA1* genes, respectively. The lowest frequency of LOH was characteristic of two genes: *CHEK1* and *PPP2R2A*, at 13.5 % and 15.3 % respectively (15/111 and 17/111 cases).

Then, using the expression values for the studied genes (for 46 patients), we assessed the influence of DNA copy number aberrations and loss of heterozygosity on the expression of these genes (Fig. 2). As a result of the analysis, a direct relationship was found between increased copy number in the *ATM* gene and high expression levels (Fig. 2A). The mean expression value with a normal gene state was 0.5 ± 0.07 compared to 3.39 ± 0.72 , $p = 0.001$. A similar result was found for the *BRIP1* gene: an increase in copy number was associated with hyperexpression: 0.88 ± 0.29 versus 2.27 ± 0.67 , respectively ($p = 0.004$) (Fig. 2C). For the *BRCA1* gene, the presence of a deletion was statistically significantly associated with a low expression level: 0.02 ± 0.01 ($p = 0.00006$), and an increase in copy number with a high level: 1.64 ± 0.36 ($p = 0.05$), compared to the normal copy number of this gene (0.87 ± 0.12) (Fig. 2B).

Survival analysis established that the aberrant states only in the *PPP2R2A* and *BRCA1* genes affect this parameter (Fig. 2D and 2E). In particular, in the presence of an increased copy number in the *PPP2R2A* gene in lung tumor surgical material, the 5-year

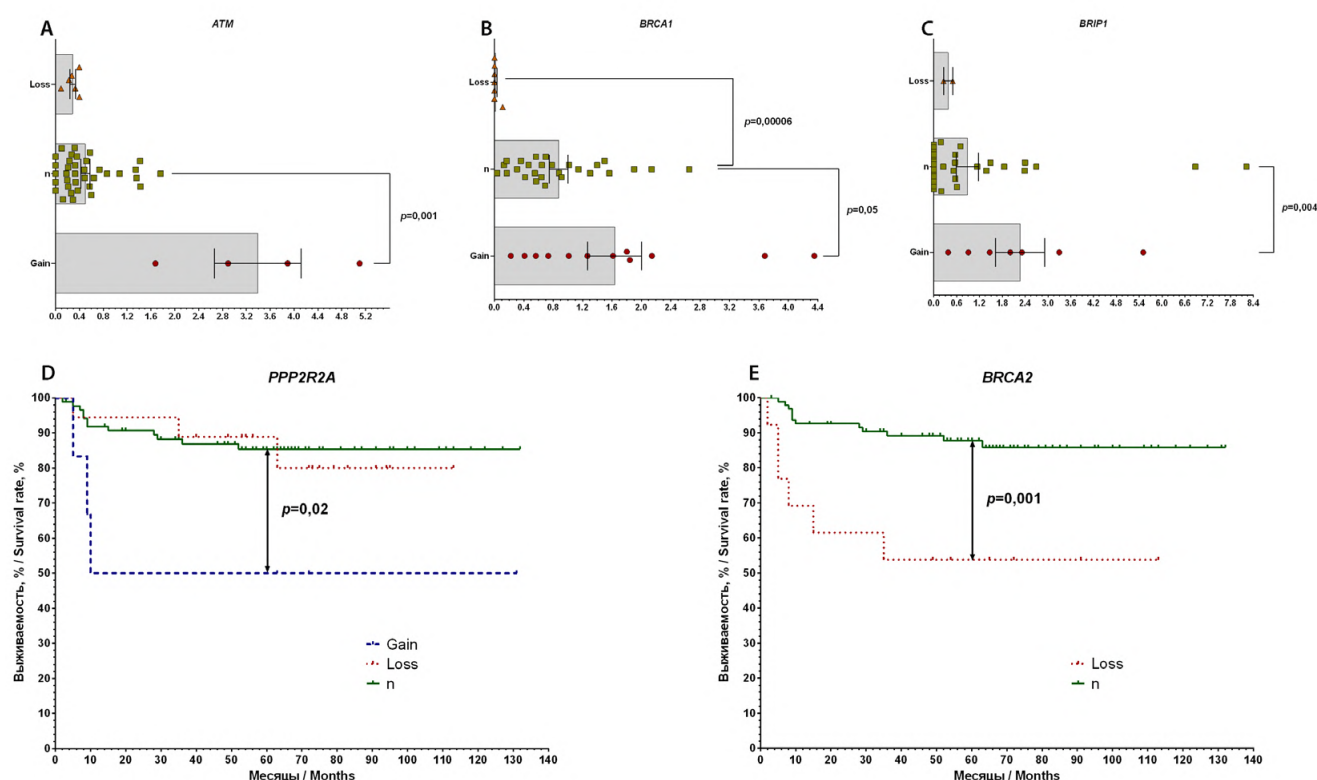


FIG. 2.

The influence of the presence of chromosomal aberrations and the level of expression of the genes *ATM* (A), *BRCA1* (B) and *BRIP1* (C), curves of metastasis-free survival depending on the presence of aberrations in the number of DNA copies of the gene *PPP2R2A* (D) and *BRCA1* (E), $n = 46$

metastasis-free survival rate is only 50 %, compared to a normal copy number with 85 % survival (log-rank test $p = 0.02$). Interesting data were shown for the *BRCA2* gene. According to previously obtained data [17] and literature data, the presence of a deletion, as well as other mechanisms of gene expression suppression, should affect the formation of homologous recombination deficiency and, as a consequence, higher treatment efficacy. In this case, the opposite picture is observed for the *BRCA2* gene: with a deletion, the MFS rate is 54 % versus 88 % in the norm (log-rank test $p = 0.001$). In addition, for some genes, a correlation was shown between increased copy number and MFS rates (data not shown). In particular, for the *RAD51C* gene, it was shown that with increased copy number, the survival rate was 70 %, which is lower compared to the norm (86 %), $p = 0.1$. A similar trend was observed for the *CHEK2* gene, with a survival rate of 67 % compared to 85 %, $p = 0.06$. Although the presence of loss of heterozygosity sites in the studied gene group was not statistically significantly associated with survival, a strong trend was shown for two genes, *ATM* and *BRIP1* ($p = 0.06$ and $p = 0.1$). Moreover, the presence of loss of heterozygosity favorably influenced long-term treatment results: 94 % and 87 % 5-year MFS rates for *ATM* and *BRIP1*, respectively, compared to 80 % and 77 % under normal conditions.

DISCUSSION

DNA copy number variations are a type of genetic variation that can affect cell physiology and the development of various diseases by disrupting gene function or altering their expression. In tumor cells, this is quite a common phenomenon, and the presence of this type of aberration may be associated with the effectiveness of treatment with certain chemotherapeutic drugs and affect long-term treatment outcomes [18]. It has been established that *CHEK1* amplification causes overexpression of this gene and is associated with a poor prognosis in lung cancer patients. Moreover, gene expression varies within a single tumor, and cells with high expression levels are more prone to metastasis [19].

The *RAD51* gene family plays a crucial role in the homologous recombination process and DNA repair, as well as genomic stability. It may also be involved in carcinogenesis and cancer treatment [20]. Our study found that high *RAD51D* expression is associated with 100 % MFS. However, other studies have found that the expression of a related gene, *RAD51C*, is associated with resistance to platinum-based chemotherapy in 56 NSCLC patients. In these cases, high *RAD51C* expression in 67 % of cases (23/34 patients) was found to be associated with lack of response to chemotherapy (stabilization and progression), compared to 36 % (8/24 patients) with low expression, $p = 0.021$ [20]. In addition, high *RAD51C*

expression closely correlated with lower overall survival ($p = 0.036$) and disease-free survival ($p = 0.02$) [20]. Interestingly, high expression levels of *RAD51* paralogous genes in breast cancer was also associated with improved progression-free survival ($p < 0.01$) [21]. The opposite result was shown in lung cancer: a high level of *RAD51* expression was observed in 29.4 % (100 out of 340) of cases. Patients with tumors that demonstrated high levels of *RAD51* expression had a significantly shorter median overall survival (19 vs. 68 months in the low expression group), $p < 0.0001$ [22]. It has been established that *RAD51D* is overexpressed in many tumors, and this phenomenon is especially observed in cells with BRCA deficiency [23]. A possible explanation for this may be compensation for genetic instability during malignant progression caused by the presence of homologous recombination deficiency [24]. Supporting this hypothesis, studies have found that a decrease in *BRCA1* expression often correlates with an increase in *RAD51* expression, suggesting that cells are attempting to compensate for HRD. However, such compensation is not always effective and may, on the contrary, lead to increased genomic instability and, as a result, affect treatment efficacy [25].

A similar result regarding expression was observed for the *PPP2R2A* gene. Kalev P., et al. showed that the expression level of *PPP2R2A* was lower in lung tumor tissue than in normal tissue [26]. Moreover, deletion or the presence of LOH sites in this gene are rare events [27] that correlate with decreased expression, which in turn is associated with high sensitivity of tumor cells to PARP inhibitors [26].

An interesting result was obtained for *BRCA2*. Our study showed that the expression level of this gene in tumor tissue is the lowest among all HR genes studied (Fig. 1) and there is a high frequency of deletions (Table 2). This correlates with a poor prognosis for the disease. However, according to literature data, the presence of HRD in tumor cells due to *BRCA2* mutation is associated with sensitivity to PARP inhibitors and DNA-damaging agents [28]. For example, the treatment results of a patient with metastatic lung adenocarcinoma showed that in the presence of a germline pathogenic mutation p.Phe2801LysfsTer11, a positive response to olaparib was observed after the disease had become resistant to chemotherapy, targeted therapy, and immunotherapy and progressed. The combination of olaparib and anlotinib achieved partial regression with increased survival [29], although studies in other localizations (gastric cancer, colorectal cancer, etc.) have shown that *BRCA2* hypoexpression leads to low overall survival rates (less than 20 %), $p < 0.001$ [30]. Regarding other genes of the homologous recombination system, it has been established that the *ATM*, *BARD1*, *BRIP1*, *PARP1*, and other genes have a high predictive and prognostic potential in various types of cancer [13]. Despite this, our study did not find a statistically significant association of the studied parameters with survival rates.

CONCLUSION

Thus, in this study, various genetic markers with prognostic potential were studied. Of these, the expression level of *RAD51D* was found to be particularly significant, with higher expression being associated with 100 % metastasis-free survival. This makes this marker a potentially valuable tool for prognosis. Analysis of chromosomal aberrations revealed that deletions in the *BRCA2* and *PPP2R2A* genes have a significant impact on survival and treatment efficacy. The presence of loss of heterozygosity sites in the *ATM* and *BRIP1* genes is associated with a more favorable prognosis, leading to increased 5-year survival rates, although this effect is only seen as a strong trend. The findings from this study are significant for understanding the biological mechanisms of tumor cells and for identifying genes and biomarkers that could serve as potential targets for predicting and monitoring the prognosis of patients with lung cancer during treatment.

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Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

1. Lim JU. Update on adjuvant treatment in resectable non-small cell lung cancer and potential biomarkers predicting postoperative relapse. *Tuberculosis and Respiratory Diseases*. 2022; 86(1): 14–22. doi: 10.4046/trd.2022.0081
2. Goldstraw P, Chansky K, Crowley J, Rami-Porta R, Asamura H, Eberhardt WE, et al. The IASLC lung cancer staging project: proposals for revision of the TNM stage groupings in the forthcoming (eighth) edition of the TNM classification for lung cancer. *Journal of Thoracic Oncology*. 2016; 11(1): 39–51. doi: 10.1097/jto.0b013e31812f3c1a
3. Hanna N, Shepherd FA, Fossella FV, Pereira JR, De Marinis F, Von Pawel J, et al. Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. *Journal of Clinical Oncology*. 2023; 41(15): 2682–90. doi: 10.1200/jco.22.02546
4. Miyawaki E, Kenmotsu H, Shintani Y, Sekine I, Shukuya T, Takayama K, et al. Efficacy of platinum agents for stage III non-small-cell lung cancer following platinum-based chemoradiotherapy: a retrospective study. *BMC Cancer*. 2022; 22(1): 342. doi: 10.1186/s12885-022-09441-3
5. Laktionov K, Artamonova E, Breder V, Gorbunova V, Demidova I, Dengina N, et al. Practical recommendations for drug treatment of non-small cell lung cancer. *Malignant neoplasms*. 2022; 12(3S2-1): 41–59. (In Russ.). [Лактионов К, Артамонова Е, Бредер В, Горбунова В, Демидова И, Деньгина Н, и др. Практические рекомендации по лекарственному лечению немелкоклеточного рака легкого. *Злокачественные опухоли*. 2022; 12(3S2-1): 41–59.].
6. Pignon J-P, Tribodet H, Scagliotti GV, Douillard J-Y, Shepherd FA, Stephens RJ, et al. Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group. *Journal of Clinical Oncology*. 2008; 26(21): 3552–9. doi: 10.1200/jco.2006.24.18_suppl.7008
7. Petrelli F, Coinu A, Cabiddu M, Ghilardi M, Ardine M, Barni S. Platinum rechallenge in patients with advanced NSCLC: a pooled analysis. *Lung Cancer*. 2013; 81(3): 337–42. doi: 10.1016/j.lungcan.2013.06.022
8. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, et al. A strong candidate for the breast and ovarian cancer susceptibility gene *BRCA1*. *Science*. 1994; 266(5182): 66–71. doi: 10.1126/science.7545954
9. Marsit CJ, Liu M, Nelson HH, Posner M, Suzuki M, Kelsey KT. Inactivation of the Fanconi anemia/*BRCA* pathway in lung and oral cancers: implications for treatment and survival. *Oncogene*. 2004; 23(4): 1000–4. doi: 10.1038/sj.onc.1207256
10. Tsyganov MM, Rodionov EO, Pevzner AM, Ibragimova MK, Miller SV, Cheremisina OV, et al. Prognostic significance of *ERCC1*, *RRM1*, *TOP1*, *TOP2A*, *TYMS*, *TUBB3*, *GSTP1* and *BRCA1* mRNA expressions in patients with non-small-cell lung cancer receiving a platinum-based chemotherapy. *Journal of BUON: official journal of the Balkan Union of Oncology*. 2020; 25(4): 1728–36.
11. Taron M, Rosell R, Felip E, Mendez P, Souglakos J, Ronco MS, et al. *BRCA1* mRNA expression levels as an indicator of chemoresistance in lung cancer. *Human molecular genetics*. 2004; 13(20): 2443–9. doi: 10.1093/hmg/ddh260
12. Hu X, Yang D, Li Y, Li L, Wang Y, Chen P, et al. Prevalence and clinical significance of pathogenic germline *BRCA1/2* mutations in Chinese non-small cell lung cancer patients. *Cancer biology and medicine*. 2019; 16(3): 556. doi: 10.20892/j.issn.2095-3941.2018.0506
13. Knijnenburg TA, Wang L, Zimmermann MT, Chambwe N, Gao GF, Cherniack AD, et al. Genomic and molecular landscape of DNA damage repair deficiency across the cancer genome atlas. *Cell reports*. 2018; 23(1): 239–54. e6. doi: 10.3410/f.732976037.793546302
14. González-Martín A, Pothuri B, Vergote I, De Pont Christensen R, Graybill W, Mirza MR, et al. Niraparib in patients with newly diagnosed advanced ovarian cancer. *New England Journal of Medicine*. 2019; 381(25): 2391–402. doi: 10.1056/nejmoa1910962
15. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic acids research*. 2001; 29(9): e45–e. doi: 10.1093/nar/29.9.e45
16. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *Journal of the American*

can statistical association. 1958; 53(282): 457–81. doi: 10.2307/2281868

17. 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): 207. doi: 10.3390/ijms24010207

18. The TRD, Fernandez KCJ, Cruz MKDM, Moreno PGG, Nacario RC, Completo GC, et al. Comparative Copy Number Variation Profiling of GL01, an Immortalized Non-small Cell Lung Cancer Cell Line Derived from a Filipino Patient, and A549 Lung Adenocarcinoma Cells. *Acta Medica Philippina*. 2024; 1(1): 1–15. doi: 10.47895/amp.vi0.10777

19. Tan Z, Chen M, Wang Y, Peng F, Zhu X, Li X, et al. CHEK1: a hub gene related to poor prognosis for lung adenocarcinoma. *Biomarkers in Medicine*. 2022; 16(2): 83–100. doi: 10.2217/bmm-2021-0919

20. Chen X, Qian D, Cheng J, Guan Y, Zhang B, Ding X, et al. High expression of Rad51c predicts poor prognostic outcome and induces cell resistance to cisplatin and radiation in non-small cell lung cancer. *Tumor Biology*. 2016; 37: 13489–98. doi: 10.1007/s13277-016-5192-x

21. Liu Y-C, Shen J. Meta-analysis of the association between overexpression of RAD51 family genes and prognosis and clinical features in breast cancer. *Scientific reports*. 2025; 15(1): 4229. doi: 10.1038/s41598-025-88763-1

22. Qiao G, Wu Y, Yang X, Zhong W, Xie D, Guan X, et al. High-level expression of Rad51 is an independent prognostic marker of survival in non-small-cell lung cancer patients. *British journal of cancer*. 2005; 93(1): 137–43. doi: 10.1016/s0169-5002(05)80265-x

23. Baker JL, Schwab RB, Wallace AM, Madlensky L. Breast cancer in a RAD51D mutation carrier: case report and review of the literature. *Clinical breast cancer*. 2015; 15(1): e71–e5. doi: 10.1016/j.clbc.2014.08.005

24. Martin RW, Orelli BJ, Yamazoe M, Minn AJ, Takeda S, Bishop DK. RAD51 up-regulation bypasses BRCA1 function and is a common feature of BRCA1-deficient breast tumors. *Cancer research*. 2007; 67(20): 9658–65. doi: 10.1158/0008-5472.can-07-0290

25. Scully R, Livingston DM. In search of the tumour-suppressor functions of BRCA1 and BRCA2. *Nature*. 2000; 408(6811): 429–32. doi: 10.1038/35044000

26. Kalev P, Simicek M, Vazquez I, Munck S, Chen L, Soin T, et al. Loss of PPP2R2A inhibits homologous recombination DNA repair and predicts tumor sensitivity to PARP inhibition. *Cancer research*. 2012; 72(24): 6414–24. doi: 10.1158/0008-5472.can-12-1667

27. Cheng Y, Liu W, Kim S-T, Sun J, Lu L, Sun J, et al. Evaluation of PPP2R2A as a prostate cancer susceptibility gene: a comprehensive germline and somatic study. *Cancer genetics*. 2011; 204(7): 375–81. doi: 10.1016/j.cancergen.2011.05.002

28. Walsh CS. Two decades beyond BRCA1/2: Homologous recombination, hereditary cancer risk and a target for ovarian cancer therapy. *Gynecologic oncology*. 2015; 137(2): 343–50. doi: 10.1016/j.ygygno.2015.02.017

29. Wu C, Fan M, Hu Y. Response to olaparib in metastatic lung adenocarcinoma with germline BRCA2 mutation: a case report. *Anti-cancer drugs*. 2022; 33(1): e734–e7. doi: 10.1097/cad.0000000000001160

30. Wang G-H, Zhao C-M, Huang Y, Wang W, Zhang S, Wang X. BRCA1 and BRCA2 expression patterns and prognostic significance in digestive system cancers. *Human pathology*. 2018; 71: 135–44. doi: 10.1016/j.humpath.2017.10.032

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