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Peer-Reviewed Scientific and Practical Journal

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Рецензируемый научно-практический журнал

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South Russian Journal of Cancer

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The "South Russian Journal of Cancer" is a professional medical publication that reflects the results of current research on the subject of publications: diagnosis and treatment of oncological diseases, issues of carcinogenesis and molecular oncology, new medicines and technologies. It was founded in 2019.

The purpose of the Journal:

- To promote the development of oncological medicine in the South of Russia and the implementation of its achievements in practice.
- High-quality published content that includes the latest and trustworthy scientific papers, research or work on oncology issues.

Tasks of the Journal:

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- Facilitating the exchange of experience and transfer of advanced knowledge between specialists;
- Informing readers about the results of major medical forums;
- Giving scientists the opportunity to publish the results of their research;
- Achieving an international level in scientific publications;
- Promotion of the magazine on the international and Russian markets;
- Drawing attention to relevant, promising and interesting areas of scientific research related to the journal's subject matter;
- Involvement of reputable national and international high-level authors;
- Expansion of the editorial board and reviewers by attracting well-known experts from Russia and other countries;
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- Increasing the impact factor of the journal.

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the results of original research, literature reviews, and descriptions of clinical cases.

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Журнал входит в рекомендованный ВАК РФ перечень рецензируемых научных журналов и изданий для опубликования основных научных результатов диссертаций на соискание учёной степени кандидата и доктора наук.

«Южно-Российский онкологический журнал» – профессиональное медицинское издание, в котором отражаются результаты актуальных исследований по тематике публикаций: диагностика и лечение онкологических заболеваний, вопросы канцерогенеза и молекулярной онкологии, новые лекарственные средства и технологии. Основан в 2019 г.

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- Продвижение журнала на международном и российском рынках;
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- Расширение состава редакционной коллегии и рецензентов путем привлечения известных экспертов из России и других стран;
- Обеспечение полнотекстового доступа к научным статьям и повышение доступности и открытости журнала в России и за рубежом;
- Повышение импакт-фактора журнала.

Журнал принимает к публикации: результаты оригинальных исследований, обзоры литературы, описание клинических случаев.

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ORIGINAL ARTICLE

The content of steroid hormones in the mitochondria of unchanged and tumor tissue of the uterine body

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ABSTRACT

Mitochondria regulate a wide range of processes, including stress responses, metabolism, immunity, differentiation, redox homeostasis, and steroidogenesis, and also serve as the principal intracellular source of reactive oxygen species (ROS). Mitochondrial dysfunction has been linked to the development of various pathological conditions, including the growth of both benign and malignant tumors.

Purpose of the study. Determination of the level of steroid hormones in the mitochondria of various tissues of the uterine body.

Materials and methods. The study included 65 patients with benign and malignant diseases of the uterus: 25 patients with endometrioid adenocarcinoma of the uterus (EAC) of low differentiation (G3) stage II–III; 15 patients with leiomyosarcoma of the uterus stage I–III; and 25 patients with uterine myoma. Mitochondria from native samples of uterine tumors were isolated by differential centrifugation in a high-speed refrigerated centrifuge Avanti J-E, Becman Coulter. For the comparison group, mitochondria were isolated from intact uterine tissue. The levels of estradiol (E2), testosterone (T), progesterone (P4), and cortisol were determined using standard ELISA kits (Monobind, USA) in mitochondria isolated from the indicated tissues. A statistical analysis of the results was conducted using the Statistica 10.0 software package.

Results. Irrespective of the nature of the tumor process (benign or malignant), a decrease in the P4 level by 2.7 to 9.1 times, but an increase in the content of cortisol by 1.3 to 3.7 times and T by 2.1 to 3.7 times were detected in the mitochondria of uterine tumors. Conversely, the concentration of E2 in the mitochondria of uterine fibroids exhibited an increase of 2.2 times compared to the indicators in the mitochondria of the intact uterus. No significant differences were observed in the mitochondria of EAC, while a decrease of 1.4 times was noted in the mitochondria of uterine sarcoma.

Conclusion. There is a change in the content of steroid hormones in In the mitochondria of uterine tumors, consisting in an increase in the concentrations of cortisol and testosterone and progesterone deficiency regardless of the type of pathology, but a relative or absolute deficiency of estrogens only in the mitochondria of malignant tumors. Changes in the steroid background of tumor mitochondria, compared with the mitochondria of the intact uterus, probably have a significant effect on both the energy balance of cells and the production of ROS, as well as on proliferative processes.

Keywords: mitochondria, estradiol, progesterone, testosterone, cortisol, uterine adenocarcinoma, uterine myoma, uterine leiomyosarcoma

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Compliance with ethical standards: the study followed the ethical principles set forth by the World Medical Association Declaration of Helsinki, 1964, ed. 2013. Written informed consent was obtained from all patients for the collection and transfer of biological material for scientific research and state-funded projects conducted for public and socially beneficial purposes. The protocol of the Ethics Committee of the National Medical Research Center for Oncology, Ministry of Health of the Russian Federation (Protocol No. 22), was approved on September 5, 2023.

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ОРИГИНАЛЬНАЯ СТАТЬЯ

Содержание стероидных гормонов в митохондриях неизмененной и опухолевой ткани тела матки

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РЕЗЮМЕ

Митохондрии регулируют множество процессов, включая стресс, метаболизм, иммунитет, дифференцировку, окислительно-восстановительный баланс и синтез стероидов, а также являются основным внутриклеточным источником активных форм кислорода (АФК). Нарушение митохондриальной функции связано с развитием различных патологических состояний, включая рост доброкачественных и злокачественных опухолей.

Цель исследования. Определение уровня стероидных гормонов в митохондриях различных тканей тела матки.

Материалы и методы. В исследование включены 65 больных с доброкачественными и злокачественными заболеваниями матки: 25 больных с эндометриоидной аденокарциномой матки (ЭАК) низкой степени дифференцировки (G3) II–III стадии; 15 больных с лейомиосаркомой матки I–III стадии и 25 больных с миомой матки. Митохондрии из нативных образцов опухолей матки выделяли методом дифференциального центрифугирования на высокоскоростной рефрижераторной центрифуге Avanti J-E, Becman Cjulter. Для группы сравнения митохондрии выделяли из интакной ткани матки. В митохондриях, выделенных из указанных тканей, с использованием стандартных ИФА наборов Monobind (США) определяли уровни эстрадиола (Е2), тестостерона (Т), прогестерона (Р4) и кортизола. Статистический анализ результатов проводили с помощью пакета программ Statistica 10.0.

Результаты. Независимо от характера опухолевого процесса (доброкачественного или злокачественного), в митохондриях опухолей матки выявлено снижение уровня Р4 в 2,7–9,1 раза, но повышение содержания кортизола в 1,3–3,7 раза и Т в 2,1–3,7 раза. Концентрация Е2 в митохондриях миомы матки была повышена в 2,2 раза по сравнению с показателями в митохондриях интактной матки, не имела значимых отличий в митохондриях ЭАК, и снижалась в 1,4 раза в митохондриях саркомы матки.

Заключение. В митохондриях опухолей матки происходит изменение содержания стероидных гормонов, заключающееся в повышении концентраций кортизола и тестостерона и прогестероновом дефиците вне зависимости от типа патологии, но относительном или абсолютном дефиците эстрогенов только в митохондриях злокачественных опухолей. Изменение стероидного фона митохондрий опухолей, по сравнению с митохондриями интактной матки, вероятно оказывает существенное влияние как на энергетический баланс клеток и выработку активных форм кислорода (АФК), так и на пролиферативные процессы.

Ключевые слова: митохондрии, эстрадиол, прогестерон, тестостерон, кортизол, аденокарцинома матки, миома матки, лейомиосаркома матки

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Соблюдение этических стандартов: в работе соблюдались этические принципы, предъявляемые Хельсинкской декларацией Всемирной медицинской ассоциации (World Medical Association Declaration of Helsinki, 1964, ред. 2013). От всех пациентов получено подписанное информированное согласие на взятие и передачу биологического материала для проведения научных исследований, государственных заданий в общественно и социально-полезных целях. Протокол этического комитета ФГБУ «НМИЦ онкологии» Минздрава России № 22 утвержден 05.09.2023.

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BACKGROUND

Mitochondrial intracellular and extracellular communication networks regulate a vast array of processes, including stress response, metabolism, immunity, differentiation, redox balance, and steroid biosynthesis, and are responsible for the generation of reactive oxygen species (ROS) [1]. In addition, mitochondria play a key role in maintaining chromatin integrity and in the execution of acrosomal reactions [2]. Mitochondria contain their own circular DNA (mtDNA), which, due to the absence of DNA-binding proteins such as histones, is approximately 100 times more susceptible to ROS-induced damage and mutations than nuclear DNA (nDNA), which is protected by histones. Moreover, mtDNA repair processes are less efficient than those of nDNA, and the mutation rate of mtDNA is 10–17 times higher [3]. Disruption of mitochondrial activity is closely associated with a number of pathological conditions, including neurological and metabolic disorders, as well as tumor development [1].

Uterine tumors are among the most common pathologies of the female reproductive system. In malignant transformation, one of the earliest metabolic alterations is the reprogramming of cellular energy metabolism [4]. Mitochondria play a direct role in this metabolic reprogramming, supporting tumor cell survival and proliferation. Mitochondrial dysfunction manifests through disturbances in Ca^{2+} homeostasis, elevated ROS levels, and alterations in the steroid balance that contribute to genetic instability [5].

Mitochondria in uterine tissues contain all the essential enzymes involved in steroid hormone biosynthesis. Within mitochondria, the cytochrome P450 side-chain cleavage enzyme plays a key role in the degradation of the aliphatic tail of the cholesterol molecule, initiating the steroidogenic pathway that produces pregnenolone [1].

Steroid hormones such as estrogens, progesterone, androgens, and glucocorticoids influence mitochondrial function through their receptors localized within mitochondria. These hormones regulate the expression of genes involved in energy metabolism, apoptosis, and redox homeostasis [6, 7].

Benign and malignant uterine tumors exhibit distinct metabolic characteristics that affect their progression and response to therapy. Steroid hormones modulate mitochondrial activity, including ATP production and intracellular ROS generation, which regulate cell maintenance, viability, and overall physiological integrity [8]. It has been shown that tissues of affected endometrium display increased oxidative damage and mtDNA deletions, which correlate with altered levels of sex hormones and their receptors [9].

There is evidence that mitochondria in uterine fibroids exhibit enhanced activity, as indicated by increased mitochondrial mass and membrane potential, associated with high sensitivity to progesterone. Through the mitochondrial receptor RP4-M, progesterone enhances oxidative phosphorylation [10]. In contrast, malignant tumors often shift toward glycolysis (the Warburg effect), resulting in reduced mitochondrial activity and altered steroid hormone regulation [11, 12].

Purpose of the study: to determine the levels of steroid hormones in mitochondria isolated from uterine tissue unaffected by tumor processes (intact mitochondria) and in various uterine tumor formations.

MATERIALS AND METHODS

The study included 65 patients with benign and malignant uterine diseases who underwent surgery at the Gynecology Department of the National Medical Research Center for Oncology in 2023–2024: 25 patients with low-grade (G3) endometrioid adenocarcinoma of the uterus (EAC) stage II–III; 15 patients with uterine leiomyosarcoma stage I–III; and 25 patients with uterine myoma. All patients had morphologically verified diagnoses confirmed by postoperative histological examination. The age of the patients in all groups ranged from 52 to 84 years.

The study was conducted on native intact and pathological tissues obtained during hysterectomy from 65 patients. For EAC and uterine leiomyosarcoma, tumor tissues were used, while for uterine myoma, samples were taken from the myomatous node and a fragment of visually and morphologically unchanged uterine tissue (intact uterine tissue).

Mitochondria were isolated from all tissues obtained during surgery using differential centrifugation

on a high-speed refrigerated centrifuge Avanti J-E, Beckman Coulter, USA, according to the methods of M. V. Egorova, S. A. Afanasyev (2011) [13] and A. P. Gureeva et al. (2015) [14]. To disrupt intercellular connections, cell walls, and plasma membranes, tissues were mechanically processed by mincing with scissors and homogenizing in a glass homogenizer with a Teflon pestle (Potter–Elvehjem homogenizer). For each gram of tissue, 10 ml of isolation medium was added (0.22 M mannitol, 0.3 M sucrose, 1 mM EDTA, 2 mM TRIS-HCl, 10 mM HEPES, pH 7.4).

The tissues were homogenized and centrifuged for the first time for 10 min at 3000 g, at a temperature of 0–2 °C. The second and third centrifugations were performed at 20,000 g for 20 min at 0–2 °C. Between centrifugations, the mitochondrial pellet was resuspended in the isolation medium. Mitochondria were further purified from lysosomes, peroxisomes, melanosomes, etc., by centrifugation in a 23 % Percoll gradient. The suspension of subcellular structures was layered on the Percoll gradient and centrifuged for 15 min at 21,000 g, resulting in separation into three phases; the lower mitochondrial layer was collected and resuspended in the isolation medium. The subsequent washing of mitochondria was performed by centrifugation for 10 min at 15,000 g at 0–2 °C.

Mitochondrial samples (protein concentration 4–6 g/L) were stored at –80 °C in the isolation medium until analysis. Before ELISA analysis, mitochondrial samples were subjected to freeze–thaw cycles to disrupt mitochondrial membranes and release intramitochondrial contents. The purity of mitochondrial fractions isolated by the described method was confirmed by electron microscopy, which revealed no nuclear or cytoplasmic components, and by flow cytometry analysis.

In mitochondria isolated from the above tissues, the levels of estradiol (E2), testosterone (T), progesterone (P4), and cortisol were determined using standard ELISA kits (Monobind, USA) and an immunoassay analyzer Infinite F50 (Austria).

Statistical Analysis

Statistical analysis of the results was performed using the Statistica 10.0 software package. The data were tested for normality using the Shapiro–Wilk test (for small samples). Comparison of quantitative data between groups was carried out using Student's t-test and the Mann–Whitney test. Data in the tables are presented as $M \pm m$, where M is the arithmetic mean and m is the standard error of the mean. A value of $p < 0.05$ was considered statistically significant. The results were processed in accordance with general recommendations for medical research.

STUDY RESULTS

The content of steroid hormones in mitochondria of intact uterine tissue and in various tumor processes is presented in Table 1. It was found that the level of E2 in mitochondria of uterine myoma was 2.2 times higher, while in mitochondria of uterine leiomyosarcoma it was 1.4 times lower ($p < 0.05$) compared with the indicators in mitochondria of intact uterine tissue. No significant differences were found in E2 content in mitochondria of EAC (G3).

The content of P4 was found to be reduced to varying degrees in mitochondria of uterine tumors compared with mitochondria of intact uterine tissue: in myoma by 4.9-fold, in low-grade EAC by 9.1-fold, and in sarcoma by 2.7-fold. At the same time, mitochondria of uterine tumors were oversaturated with T. Its level was higher than in mitochondria of intact uterus: in myoma by 2.1-fold, in EAC by 3.7-fold, and in sarcoma by 2.1-fold. The cortisol content in mitochondria of uterine myoma, EAC, and sarcoma was higher than in mitochondria of intact uterine tissue by 2.6-, 3.7-, and 1.3-fold ($p < 0.05$), respectively.

When comparing the levels of steroid hormones in mitochondrial samples from malignant and benign uterine tumors, significant differences were found in E2 levels – they were lower in EAC and leiomyosarcoma compared with uterine myoma by 2-fold and 3.1-fold, respectively. The concentration of P4 in mitochondria of uterine myoma was 1.8-fold lower than in mitochondria of leiomyosarcoma, but 1.8-fold higher than in mitochondria of EAC. The content of T in mitochondria of uterine myoma was 1.7-fold lower than in EAC and showed no significant differences compared with leiomyosarcoma. The level of cortisol in mitochondria of uterine myoma was 1.5-fold

lower compared with EAC mitochondria but 2-fold higher compared with leiomyosarcoma mitochondrial samples.

Considering the metabolic precursors and products involved in the synthesis of steroid hormones, the ratios P4/T, P4/cortisol, E2/T, and E2/P4 were calculated (Table 2).

A significant decrease in the P4/cortisol ratio was found in mitochondria of uterine myoma by 12-fold, in EAC (G3) by 32.7-fold, and in sarcoma by 3.5-fold, indicating a predominance of glucocorticoid synthesis. The P4/T ratio also showed a marked decrease compared with that in mitochondria of intact uterus: in mitochondria of uterine myoma by 10.6-fold, in EAC (G3) by 33-fold, and in sarcoma by 5.5-fold.

The E2/T ratio in mitochondria of uterine myoma did not differ significantly from that in mitochondria of intact uterus, whereas in EAC (G3) and sarcoma it decreased by 3.3-fold and 2.9-fold, respectively, indicating a shift in sex steroid balance toward hyperandrogenism. In contrast, the E2/P4 ratio was increased in all mitochondrial samples: by 10.8-foldin myoma, 10.1-fold in EAC (G3), and 2-fold in sarcoma.

Compared with mitochondria of uterine myoma, in EAC mitochondria the following ratios were decreased: P4/T by 3.2-fold, E2/T by 3.4-fold, and P4/ cortisol by 2.7-fold. In mitochondria of leiomyosarcoma, compared with uterine myoma, the ratios P4/T and P4/cortisol were increased 1.9-fold and 3.5-fold, respectively, while E2/P4 and E2/T ratios were decreased 5.8-fold and 3.1-fold, respectively.

DISCUSSION

Mitochondria are multifunctional centers regulating the synthetic and energetic components of ho-

Table 1. Levels of steroid hormones in mitochondria of uterine body tissue

Mitochondrial samples	E2, nmol/g protein	P4, nmol/g protein	T, nmol/g protein	Cortisol, nmol/g protein
Intact uterine tissue (*n* = 25)	0,10 ± 0,007	0,59 ± 0,05	0,18 ± 0,01	3,2 ± 0,23
Uterine myoma (*n* = 25)	0,22 ± 0,02 p^1 = 0,0000	0,12 ± 0,009 p^1 = 0,0000	0,38 ± 0,03 p^1 = 0,0000	8,2 ± 0,71 p^1 = 0,0000
EAC (G3) (*n* = 25)	0,11 ± 0,007 p^2 = 0,0000	0,065 ± 0,005 p^1 = 0,0000 p^2 = 0,0000	0,66 ± 0,04 p^1 = 0,0000 p^2 = 0,0000	11,9 ± 0,91 p^1 = 0,0000 p^2 = 0,0023
Uterine leiomyosarcoma (*n* = 15)	0,07 ± 0,007 p^1 = 0,0000 p^2 = 0,0000	0,22 ± 0,02 p^1 = 0,0000 p^2 = 0,0000	0,37 ± 0,037 p^1 = 0,0000	4,16 ± 0,35 p^1 = 0,0230 p^2 = 0,0001

Note: p^1 – statistically significant compared with the value in intact tissue; p^2 – statistically significant compared with the value in myoma.

Table 2. Ratios of steroid hormone levels in mitochondria of uterine body tissues (arbitrary units)

Tissue samples	E2, nmol/g protein	P4, nmol/g protein	T, nmol/g protein	Cortisol, nmol/g protein
Intact tissue (*n* = 25)	3,3 ± 0,09	0,17 ± 0,005	0,56 ± 0,005	0,18 ± 0,003
Uterine myoma (n = 25)	0,32 ± 0,008 p^1 = 0,0000	1,84 ± 0,05 p^1 = 0,0000	0,58 ± 0,006	0,015 ± 0,0004 p^1 = 0,0000
EAC (G3) (*n* = 25)	0,10 ± 0,003 p^1 = 0,0000 p^2 = 0,0000	1,72 ± 0,03 p^1 = 0,0000	0,17 ± 0,004 p^1 = 0,0000 p^2 = 0,0000	0,0055 ± 0,00007 p^1 = 0,0000 p^2 = 0,0000
Uterine leiomyosarcoma (*n* = 15)	0,6 ± 0,008 p^1 = 0,0000 p^2 = 0,0000	0,32 ± 0,005 p^1 = 0,0000 p^2 = 0,0000	0,19 ± 0,005 p^1 = 0,0000 p^2 = 0,0000	0,052 ± 0,002 p^1 = 0,0000 p^2 = 0,0000

Note: p^1 – statistically significant compared with the value in intact tissue; p^2 – statistically significant compared with the value in myoma.

meostasis, as in addition to energy production they serve as sites for the synthesis of various hormones, neurotransmitters, and biogenic amines [1, 15]. It is known that mitochondria can dynamically and reversibly adapt to energetic, environmental, and other endogenous or exogenous stress factors. The basis of this adaptation lies in temporary molecular and functional changes rather than necessarily dysfunctional processes. Mitochondria act as systemic signaling hubs, transmitting information both within and between cells [16]. Alterations in mitochondrial function are believed to be involved in the pathogenesis of many diseases, such as cancer, cardiovascular, and neurodegenerative disorders, and understanding mitochondrial mechanisms and implementing adaptive strategies may offer an integrated approach to treating chronic diseases and restoring health [17]. Since the enzyme P450scc, responsible for the initiation of steroid hormone synthesis, is localized on the matrix side of the inner mitochondrial membrane, mitochondria occupy a central role in steroidogenesis [18].

This study examined changes in the content of steroid hormones in endometrial mitochondria depending on the underlying uterine pathology – whether it was benign tumor growth (uterine myoma) or malignant (low-differentiated endometrioid adenocarcinoma, EAC G3, or uterine leiomyosarcoma). It was found that mitochondria of all uterine tumors demonstrated unidirectional changes in the levels of progesterone, testosterone, and cortisol compared with mitochondria from intact uterus, while E2 levels varied depending on the tumor type. These alterations in mitochondrial steroid profiles may be related to the diverse functions of the studied hormones. Regardless of benign or malignant nature, all tumor mitochondria showed decreased progesterone levels but elevated testosterone and cortisol levels, differing only in magnitude. The most pronounced changes were observed in mitochondria from EAC (G3), showing minimal progesterone concentrations and maximal testosterone and cortisol levels compared with mitochondria from intact uterus.

It is known that mitochondrial steroid hormones, including glucocorticoids, androgens, and estrogens, exert both physiological and pathological effects, contributing to aging and the development of various diseases [19].

Glucocorticoid hormones penetrate mitochondria and directly interact with mtDNA, which may enhance oxidative stress and release of cytosolic mtDNA [20]. Elevated cortisol concentrations in mitochondria of uterine tumors may promote the accumulation of reactive oxygen species (ROS). Mitochondria are both the main target of ROS-induced epithelial cell damage and the primary ROS producer during oxidative phosphorylation [21]. Studies have shown that significantly higher levels of MDA and 8-OHdG (a modified nucleoside reflecting DNA damage) are found in endometrioid lesions compared with normal endometrium. mtDNA mutations are associated with elevated MDA and 8-OHdG levels, whereas E2 or an ERβ-selective agonist stimulates increased activity and expression of MnSOD [22].

P4 is believed to protect epithelial cells from oxidative damage and mitochondrial dysfunction through the c-MYC/SIRT1/PGC-1α signaling pathway [23]. It has been reported that P4, the second major endogenous female steroid hormone after estradiol, inhibits chronic inflammation and oxidative stress in mouse models [24]. Moreover, P4 possesses well-documented anti-inflammatory and antioxidant properties across various conditions [25] and has been shown to protect different cell types from oxidative damage [26].

The progesterone deficiency identified in this study in mitochondria of uterine tumors may therefore indicate reduced cellular protection against oxidative injury in all investigated formations.

Testosterone affects mitochondrial function in multiple ways, including altering the structure of these organelles. Androgens stimulate mitochondrial biogenesis via the AR/PGC-1α/TFAM pathway, increasing mitochondrial content through induction, transcription, and replication of mtDNA, which encodes 13 essential components of the respiratory chain [27]. However, mtDNA mutations or copy number alterations are known risk factors for mitochondrial dysfunction, leading to excessive ROS production and ATP deficiency, frequently observed in hereditary metabolic diseases [28]. Evidence also

suggests the presence of androgen receptors in mitochondria, whose overexpression, particularly in prostate cancer cell lines, reduces the activity of respiratory chain complexes I, II, and III [29]. However, sex-specific differences exist in testosterone's impact on mitochondria: in men, testosterone promotes energy expenditure and prevents metabolic disorders such as obesity and type 2 diabetes, whereas in women, elevated androgen levels increase the risk of type 2 diabetes and are commonly observed in patients with polycystic ovary syndrome [30].

It can be assumed that on one hand, mitochondrial hyperandrogenization in uterine tumors contributes to mitochondrial biogenesis and maintenance of cellular energy balance, while on the other hand, it may promote excessive ROS production and mitochondrial dysfunction.

The study revealed diverse changes in estradiol levels in tumor mitochondria depending on the pathological process. E2 concentration in mitochondria of uterine myoma was increased compared with intact uterine mitochondria, showed no significant difference in EAC (G3), and was decreased in sarcoma mitochondria. Normally, estrogens protect mitochondria from oxidative stress, enhance their biogenesis, and improve energy metabolism, whereas a decline in E2 levels – for example, during menopause – leads to mitochondrial dysfunction, which may contribute to various pathological conditions, including neurodegenerative disorders and tumor growth [31, 32].

Each mitochondrion contains about 1,200 different protein types, of which 13 are encoded by mitochondrial DNA and the rest by nuclear DNA [33]. Cross-talk between nuclear and mitochondrial genomes is essential for mitochondrial biogenesis and is regulated by a network of transcription factors that include estrogen-related receptors [34].

Estrogen and androgen receptors share localization and activity within both mitochondria and the nucleus, suggesting a synergistic relationship between estrogens and androgens in regulating mitochondrial function [35].

The calculated E2/T ratio showed a significant decrease compared with intact uterus only in malignant processes, whereas mitochondria of uterine myoma exhibited no significant differences.

There is evidence of altered levels of mitochondrial estrogen receptors in endometrial mitochondria under various gynecological pathologies, including adenomyosis. It is suggested that mitochondrial estrogen receptor β (MtERβ) continues the estrogen-induced signaling pathway within mitochondria, influencing mtDNA transcription, interacting with mitochondrial respiratory complex V, and enhancing activity of complex IV, thereby promoting ATP generation [10]. Moreover, estrogen deficiency has been shown to reduce the expression of genes involved in mitochondrial respiratory chain, oxidative phosphorylation, and glucose and lipid metabolism in ovariectomized rats [36].

CONCLUSION

The conducted study revealed significant alterations in the content of steroid hormones in mitochondria of uterine tumors. The obtained data demonstrate a general trend across all examined neoplasms – a decrease in progesterone levels accompanied by increased concentrations of testosterone and cortisol compared with intact tissue. The key difference between benign and malignant growth appears to be associated with the balance between estradiol and testosterone. It can be assumed that the identified hormone concentration profiles create distinct metabolic environments within mitochondria. The preservation of estradiol–testosterone balance in myoma may favor oxidative phosphorylation, whereas the pronounced shift toward androgens in malignant tumors potentially promotes oxidative stress and may be associated with a metabolic switch to glycolysis

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ORIGINAL ARTICLE

Antiproliferative properties of a new plant alkaloid against cellular colorectal cancer cultures

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ABSTRACT

Purpose of the study. To evaluate the antiproliferative properties of the novel alkaloid (P1) against CRC cell lines HT-29, Caco-2, and HCT116.
Materials and methods. CRC cell lines (HCT116, HT-29, Caco-2) were used in the experiments. The alkaloid (P1) was isolated from *Petasites hybridus* (L.) G. Gaertn., B. Mey. & Scherb and identified using high-performance liquid chromatography (HPLC) and nuclear magnetic resonance spectroscopy (NMR). Cells were incubated with various concentrations of the alkaloid, and cell viability was assessed. Berberine, a well-known anticancer alkaloid, served as the reference compound.
Results. The alkaloid (P1) demonstrated pronounced antiproliferative activity across all tested colorectal cancer cell lines – HCT116, HT-29, and Caco-2. The highest sensitivity was observed in HCT116 cells, with an IC_{50} value of 15.73 μmol/L after 72-hour incubation, indicating a substantial inhibitory effect on tumor cell proliferation. Comparative analysis showed that (P1) exhibited greater cytostatic efficacy than berberine in Caco-2 ($IC_{50}^{(P1)}$ = 54.489 ± 8.3 μmol/L vs $IC_{50}^{(berb)}$ = 193.154 ± 13.1 μmol/L) and HT-29 cultures ($IC_{50}^{(P1)}$ = 55.375 ± 7.1 μmol/L vs $IC_{50}^{(berb)}$ = 90.22 ± 8.2 μmol/L).
Conclusion. The findings indicate that the alkaloid (P1) possesses significant antiproliferative potential against colorectal cancer cell lines, underscoring its promise as a prospective anticancer agent. Notably, its superior efficacy compared with berberine highlights the relevance of further investigation. These results support continued development of (P1) as a basis for novel therapeutic agents. Future work should include detailed preclinical and clinical studies to elucidate its mechanism of action, evaluate safety and *in vivo* efficacy, and optimize pharmacological properties for potential clinical application.

Keywords: colorectal cancer, plant alkaloid, berberine, cytostatic properties, cell cultures

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Compliance with ethical standards: the work was carried out in compliance with the ethical principles set forth in the World Medical Association Declaration of Helsinki (1964, revised 2013). The study was approved by the Ethics Committee National Medical Research Centre for Oncology (protocol No. 5/223 dated 06.09.2024). Informed consent was obtained from all participants of the study.

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Conflict of interest: Oleg I. Kit is the Editor-in-Chief of the Journal «South Russian Journal of Cancer» and one of the authors of the article. Elena A. Dzhenkova and Elena Yu. Zlatnik has been the Member of the Editorial Board of the Journal «South Russian Journal of Cancer» and one of the authors of the article. The article has passed the review procedure accepted in the Journal by independent experts. The authors did not declare any other conflicts of interest.

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ОРИГИНАЛЬНАЯ СТАТЬЯ

Антипролиферативные свойства нового растительного алкалоида в отношении клеточных культур колоректального рака

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РЕЗЮМЕ

Цель исследования. Оценить антипролиферативные свойства нового алкалоида (Р1) в отношении клеточных культур КРР HT-29, Caco-2 и HCT116.

Материалы и методы. В эксперименте использовались клеточные культуры КРР (HCT116, HT-29, Caco-2). Алкалоид (Р1) был выделен из *Petasites hybridus* (L.) G. Gaertn., B. Mey. & Scherb и идентифицирован с помощью методов ВЭЖХ и ядерного магнитного резонанса. Клетки инкубировали с различными концентрациями алкалоида и проводили анализ жизнеспособности клеток. Контрольным соединением являлся известный алкалоид берберин.

Результаты. В ходе эксперимента алкалоид (Р1) продемонстрировал выраженное антипрофелиферативное действие на все исследуемые клеточные линии колоректального рака – HCT116, HT-29 и Caco-2. Наиболее высокая чувствительность была выявлена у клеток линии HCT116, где значение IC_{50} составило 15,73 мкмоль/л при 72-часовой инкубации, что свидетельствует о значительной способности алкалоида подавлять пролиферацию этих опухолевых клеток. Кроме того, при сравнении активности алкалоида (Р1) с контрольным соединением – известным противоопухолевым алкалоидом берберином – было установлено, что (Р1) проявляет более высокую цитостатическую эффективность в культурах Caco-2 (IC_{50}^{P1} = 54,489 ± 8,3 мкмоль/л против IC_{50}^{berb} = 193,154 ± 13,1 мкмоль/л) и HT-29 (IC_{50}^{P1} = 55,375±7,1 мкмоль/л против IC_{50}^{berb} = 90,22 ± 8,2 мкмоль/л).

Заключение. Результаты проведенного исследования демонстрируют, что алкалоид (Р1) обладает выраженными антипролиферативными свойствами в отношении клеточных линий колоректального рака, что свидетельствует о его значительном потенциале в качестве противоопухолевого агента. Особенно важно отметить его высокую эффективность в сравнении с известным алкалоидом берберином, что подчеркивает перспективность дальнейших исследований данного соединения. Эти данные открывают новые возможности для разработки инновационных лекарственных препаратов на основе природных соединений. В дальнейшем необходимы углубленные доклинические и клинические исследования, направленные на изучение механизмов действия алкалоида (Р1), его безопасности и эффективности *in vivo*, а также оптимизацию его фармакологических свойств для возможного применения в клинической практике.

Ключевые слова: колоректальный рак, растительный алкалоид, берберин, цитостатические свойства, клеточные культуры

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Соблюдение этических стандартов: в работе соблюдались этические принципы, предъявляемые Хельсинкской декларацией Всемирной медицинской ассоциации (World Medical Association Declaration of Helsinki, 1964, ред. 2013). Исследование было одобрено Этическим комитетом ФГБУ «Национальный медицинский исследовательский центр онкологии» Министерства здравоохранения Российской Федерации (протокол №5/223 от 06.09.2024). Информированное согласие получено от всех участников исследования.

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Конфликт интересов: автор статьи Кит О. И. является главным редактором журнала «Южно-Российский онкологический журнал»; авторы статьи Дженкова Е. А. и Златник Е.Ю. являются членами редакционной коллегии журнала «Южно-Российский онкологический журнал». Статья прошла принятую в журнале процедуру рецензирования. Об иных конфликтах интересов авторы не заявляли.

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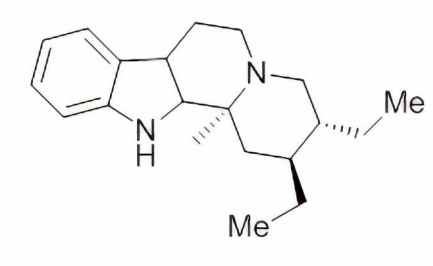
BACKGROUND

Cytostatic agents used in oncology represent an important class of drugs that play a key role in the treatment of various types of cancer, including colorectal cancer (CRC) [1]. According to the World Health Organization (WHO), the incidence of CRC continues to rise, underscoring the need to develop new cytostatic compounds with high antiproliferative activity and low toxicity [2].

In recent years, plant alkaloids have attracted increasing interest due to the established cytostatic properties demonstrated by many representatives of this group. One of the most extensively studied alkaloids, berberine, has shown the ability to inhibit cancer cell proliferation and induce apoptosis [3]. Berberine acts on key protein targets within signaling pathways that regulate cell growth, including phosphatidylinositol-3-kinase (PI3K), protein kinase B (Akt), and the mechanistic target of rapamycin (mTOR) in the PI3K/Akt pathway, as well as Raf kinase, mitogen-activated protein kinase (MEK), and extracellular signal-regulated kinase (ERK) in the MAPK pathway. These mechanisms allow berberine to be considered a promising antitumor agent [4].

However, despite encouraging results, berberine has several limitations, including low bioavailability and potential adverse effects, which may restrict its clinical applicability [5, 6]. In this context, the search for novel plant-derived alkaloids has become particularly relevant.

The compound (P1) investigated in our study belongs to the class of indole alkaloids and is structurally related to alkaloids isolated from plants of the genus *Corynanthe sp*., known for their analgesic and anti-inflammatory properties [7] (Fig. 1)



Chemical Formula: $C_{20}H_{30}N_2$
Molecular Weight: 298,4740

Fig. 1. Structural formula of compound (P1) isolated from *Petasites hybridus (L.)* G.Gaertn., B.Mey. & Scherb.

According to preliminary data, this compound demonstrates notable cytostatic effects against pancreatic cancer cell lines and non-small cell lung adenocarcinoma [8]. Comparing the activity of the novel alkaloid (P1) with berberine on CRC cultures will allow assessment of its efficacy and potential as a therapeutic candidate.

Purpose of the study: to evaluate the antiproliferative properties of the novel alkaloid (P1) against CRC cell lines HT-29, Caco-2, and HCT116.

MATERIALS AND METHODS

Dried and powdered rhizomes of *P. hybridus* (L.) were placed in a Soxhlet extractor; 250 mL of tetrachloroethylene (C_2Cl_4) was added to the extraction flask. Extraction was carried out under heating in the Soxhlet apparatus with a reflux condenser for 24 hours. After extraction, tetrachloroethylene was distilled off from the resulting 250 mL mixture, leaving 5 mL of extract in the distillation flask. The concentrated solution was applied to a chromatographic column packed with silica gel ($SiO_2 \cdot xH_2O$). The eluents used sequentially were C_2Cl_4, CH_2Cl_2, and a CH_2Cl_2/ EtOH mixture at a ratio of 10:1.

The structure of the isolated alkaloid (P1) was confirmed by nuclear magnetic resonance spectroscopy (^1H and ^{13}C NMR) [3]. After purification, the alkaloid was dissolved in dimethyl sulfoxide (DMSO) (Biolot, Russian Federation) to prepare a stock solution at a concentration of 8.8 mmol/L. A stock solution of berberine (25 mmol/L) was similarly prepared in DMSO from dry berberine chloride (Sigma-Aldrich, USA).

The experiment utilized CRC cell lines HT-29, Caco-2, and HCT116 obtained from the Cell Culture Collection of the Institute of Cytology, Russian Academy of Sciences (St. Petersburg, Russian Federation), as well as peripheral blood mononuclear cells (PBMCs) collected from healthy donors. Cancer cell lines were seeded at 5,000 cells per well in 96-well plates using complete culture medium (CCM) DMEM (Servicebio, China) supplemented

with 10 % fetal bovine serum (Gibco, USA), 1 % glutamine (Biolot, Russia), 1 % non-essential amino acids (Biolot, Russia), and 1 % penicillin-streptomycin (Biolot, Russia). After cell adhesion, the medium was replaced with CCM containing the tested alkaloids in serial twofold dilutions: 125 μmol/L to 10.12 μmol/L for berberine, and 44 μmol/L to 0.34 μmol/L for the novel alkaloid (P1). Cells were incubated for 24 and 72 hours at 37 °C in an atmosphere of 5.0 % CO_2. Multiple dilutions of berberine were tested in preliminary experiments, allowing us to determine the optimal concentrations for this study [9, 10].

PBMCs from healthy donors were obtained from venous blood collected in EDTA tubes (MiniMed, Russian Federation). On the day of collection, the blood was diluted 1:1 with RPMI 1640 medium (Servicebio, China) and layered onto Ficoll (Biolot, Russia), followed by centrifugation for 30 minutes at 730 g. The PBMC ring at the phase interface was carefully collected and transferred into a separate tube. The isolated PBMCs were washed once with RPMI 1640, counted, and seeded at 5,000 cells per well in 96-well plates in RPMI 1640 supplemented with 10 % fetal bovine serum. The alkaloid (P1) was added in serial twofold dilutions (44 μmol/L to 0.34 μmol/L), and the cells were incubated for 72 hours at 37 °C in an atmosphere of 5.0 % CO_2.

After incubation, both adherent cancer cell lines and PBMCs were stained using a mixture of nuclear dyes Hoechst 33342 (1 mg/mL) (ThermoFisher, USA) and ethidium bromide (10 mg/mL) (Servicebio, China) for direct counting of live and dead cells. Visualization was performed using a LionheartFX imager (BioTek, USA), and nuclear quantification was carried out using Gen5 software (BioTek, USA). Cell viability was calculated as the percentage of live cells in treated wells relative to untreated controls. Each experimental condition was plated in 8 replicates, and each experiment was repeated three times. Results are expressed as mean ± SD.

Statistical significance between mean viability values was assessed using the Student's t-test with Bonferroni correction. Dose–response curves and half-maximal inhibitory concentration (IC_{50}) values were calculated using the online tool IC_{50} Calculator ("Quest Graph™ IC_{50} Calculator." AAT Bioquest, Inc., 13 Feb. 2025, https://www.aatbio.com/tools/IC50-calculator).

STUDY RESULTS

The study demonstrated that the alkaloid (P1) exhibits a selective antiproliferative effect against the tested malignant cell lines. Incubation with 44 μmol/L of (P1) for 72 hours resulted in a > 30-fold increase in the number of dead cells relative to untreated controls in the HCT116 culture, a 7.55-fold increase in Caco-2, and a 6.37-fold increase in HT-29, which was significantly higher than in PBMCs from healthy donors at the same concentration (2.5-fold) (Fig. 2A). A decrease in the concentration of the tested compound (P1) was accompanied by a reduction in the magnitude of differences between malignant and normal cell cultures. Thus, incubation with 22 μmol/L (P1) resulted in significantly higher levels of cell death in two cultures – HCT116 (7.59-fold) and Caco-2 (3.41-fold) – compared to PBMCs (2.03-fold), whereas no significant difference was observed in HT-29 (2.22-fold). Finally, at 11 μmol/L, a significant difference remained only between PBMCs (1.09-fold) and Caco-2 (1.72-fold).

The cytostatic activity of alkaloid (P1) against CRC cultures varied within a relatively narrow range. After 24 hours of exposure, the half-maximal inhibitory concentration (IC_{50}) was lowest for HCT116 cells (IC_{50} = 51.98 ± 4.8 μmol/L) and highest for HT-29 (IC_{50} = 55.375 ± 7.1 μmol/L). After 72 hours, HCT116 again showed the lowest IC_{50} value (15.73 ± 3.2 μmol/L), whereas the highest value was observed in Caco-2 cells (IC_{50} = 32.505 ± 9.2 μmol/L), with HT-29 showing a similar response (IC_{50} = 29.075 ± 7.4 μmol/L). Across all cell lines, the dose–response curves demonstrated a hormetic effect – an increase in cell viability at low concentrations of alkaloid P1. At 24 hours, hormesis was observed in HCT116 and HT-29 cultures (Figs. 2C, 2D), whereas in Caco-2 cells the effect was more pronounced at 72 hours (Fig. 2B).

The sensitivity of the CRC cell lines to the antiproliferative effects of berberine varied across

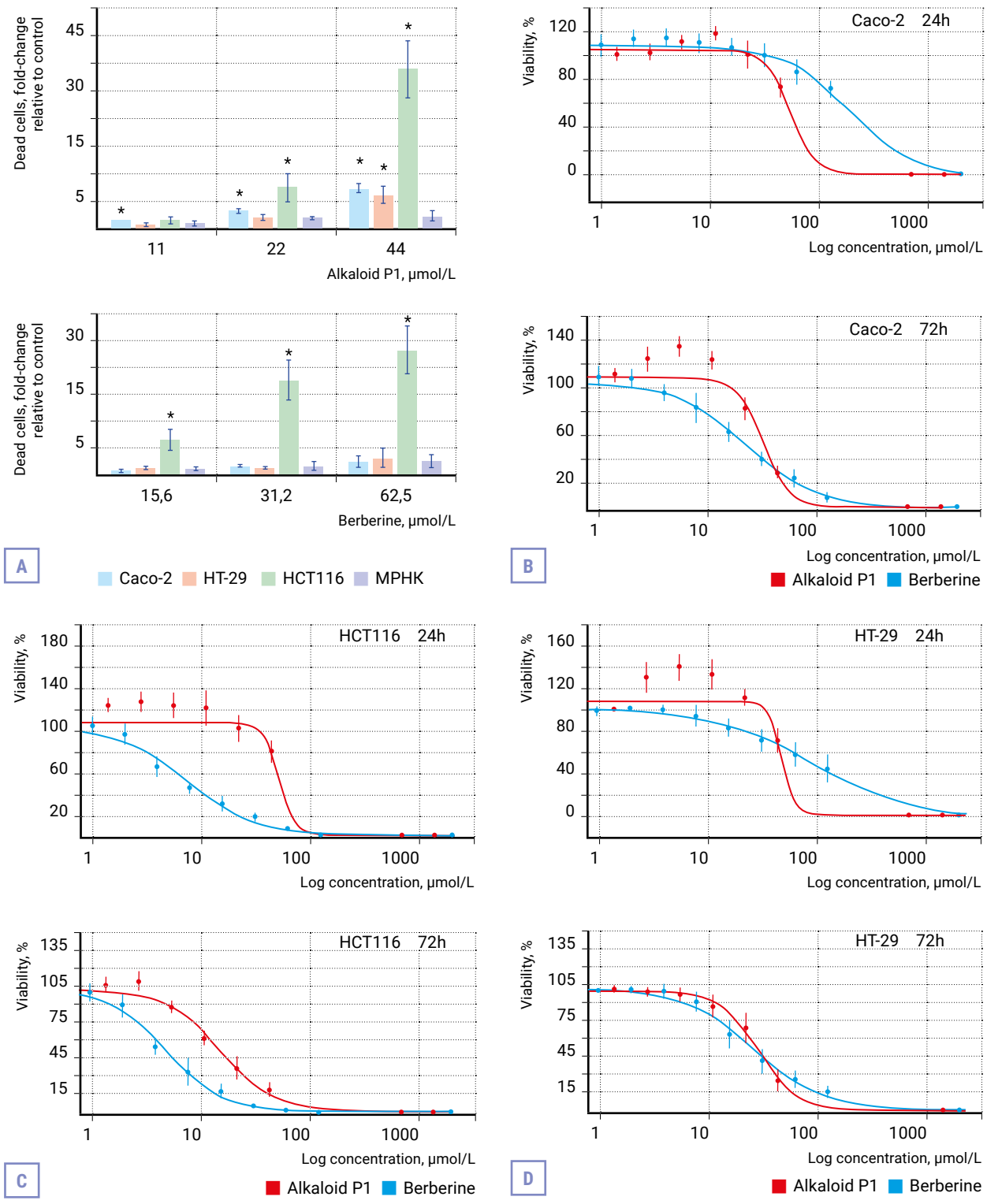


Fig. 2. Antiproliferative and cytostatic effects of alkaloid (P1). A – comparison of the antiproliferative activity of berberine and alkaloid (P1) against CRC cell cultures and PBMCs from healthy donors, 72 h exposure; B – dose–response curves for Caco-2; C – dose–response curves for HCT116; D – dose–response curves for HT-29.
* – the difference between CRC and PBMC values at the corresponding (P1) concentration is statistically significant, $p < 0.05$.
PBMC – peripheral blood mononuclear cells.

a broader range than in the assays with (P1). At 24-hour exposure, the IC_{50} for berberine was lowest in HCT116 (IC_{50} = 7.43 ± 2.4 μmol/L) and highest in Caco-2 (IC_{50} = 193.154 ± 13.1 μmol/L), with intermediate values in HT-29 (IC_{50} = 90.22 ± 8.2 μmol/L). At 72 hours, the lowest IC_{50} remained in HCT116 (IC_{50} = 4.94 ± 1.2 μmol/L), while the highest IC_{50} was observed in HT-29 (IC_{50} = 26.269 ± 4.5 μmol/L), with values in Caco-2 being similar (IC_{50} = 23 ± 3.1 μmol/L).

A direct comparison of the antiproliferative properties of the two alkaloids showed that at 24 h exposure, (P1) exhibited stronger cytostatic activity than berberine in Caco-2
($IC_{50}^{(P1)}$ = 54.489 ± 8.3 μmol/L vs
$IC_{50}^{(berb)}$ = 193.154 ± 13.1 μmol/L) (Fig. 2B)
and HT-29 ($IC_{50}^{(P1)}$ = 55.375 ± 7.1 μmol/L vs
$IC_{50}^{(berb)}$ = 90.22 ± 8.2 μmol/L) (Fig. 2D).
In HCT116, the opposite pattern was observed: berberine demonstrated markedly higher potency, with an IC_{50} nearly one order of magnitude lower
($IC_{50}^{(P1)}$ = 51.98 ± 4.8 μmol/L vs
$IC_{50}^{(berb)}$ = 7.43 ± 2.4 μmol/L).
With prolonged exposure (72 h), IC_{50} values for both alkaloids converged in Caco-2 and HT-29: Caco-2:
$IC_{50}^{(P1)}$ = 32.505 ± 9.2 μmol/L vs
$IC_{50}^{(berb)}$ = 23 ± 3.1 μmol/L.
HT-29: $IC_{50}^{(P1)}$ = 29.075 ± 7.4 μmol/L vs
$IC_{50}^{(berb)}$ = 26.269 ± 4.5 μmol/L.
In HCT116, the higher sensitivity to berberine remained evident
($IC_{50}^{(P1)}$ = 15.73 ± 3.2 μmol/L vs
$IC_{50}^{(berb)}$ = 4.94 ± 1.2 μmol/L).

DISCUSSION

The findings of this study indicate that the alkaloid (P1), isolated from *Petasites hybridus* (L.) G. Gaertn., B. Mey. & Scherb., induces dose-dependent cell death in several colorectal adenocarcinoma cell lines. Its cytostatic effect is influenced not only by concentration but also by exposure time, with the strongest effect occurring after 72 hours of incubation.

Among the tested lines, HCT116 demonstrated higher sensitivity to both (P1) and berberine compared with Caco-2 and HT-29. This highlights the importance of selecting appropriate cell lines for assessing the activity of novel anticancer agents, given the notable inter-line variability in drug response.

The CRC lines studied have distinct molecular and metabolic features that likely underlie their differing sensitivities to (P1) and berberine. For example, HCT116 is more sensitive than HT-29 to FOLFOX chemotherapy and hypoxia, possibly due to the absence of p53 loss in HCT116, whereas HT-29 carries a p53 deficiency and displays microsatellite instability [11]. Caco-2 cells lack activating mutations in KRAS, NRAS, BRAF, and PIK3CA and are resistant to cetuximab [12]. Their capacity for enterocyte-like differentiation is strongest among the three lines and often used in drug absorption studies rather than tumor modeling [13, 14].

HCT116 is highly aggressive, poorly differentiated, and enriched in cancer stem cell-like subpopulations [15]. It exhibits MDR1 overexpression and associated chemoresistance linked to NOX and Nrf2 gene activity [16]. Molecularly: HCT116 harbors KRAS codon 13 mutations; HT-29 expresses KRAS, APC, and BRAF V600E [12, 17].

Thus, differences in response to (P1) and berberine may reflect variations in KRAS, TP53, and MLH gene status, which regulate RAS/RAF/MAPK and PI3K/Akt/mTOR signaling, cell cycle progression, apoptosis, and DNA repair [18, 19].

Berberine, widely recognized for its antitumor activity, also demonstrated substantial cytostatic effects against CRC cells. Its mechanism of action includes inhibition of enzymes involved in glucose and lipid metabolism, as well as modulation of signaling pathways such as the AMPK pathway [20]. Studies have shown that berberine can induce apoptosis through caspase activation and suppression of protein kinases [21].

We compared our findings on berberine with results reported by other international research groups, as the cytostatic activity values (IC_{50}) obtained in our study varied within a broad range. In several studies, depending on the exposure time, IC_{50} values for berberine in HT-29 cells ranged from 34.6 to 52.37 ± 3.45 μM [22, 23], while in HCT116

cells they ranged from 31 to 55.27 μM [24–26]. At these concentrations, berberine reduced the expression of aquaporins 1, 3, and 5 and increased PTEN expression in HT-29, SW-480, and HCT116 cultures. Upregulation of PTEN contributed to suppression of the PI3K, AKT, and mTOR signaling pathways, leading to enhanced apoptosis in CRC cells and reduced migratory capacity – findings consistent with those reported by other researchers [23].

An important aspect relevant to our study is the previously documented sensitivity of HCT116 cells to several plant-derived compounds. For example, their in vitro growth is inhibited by flavopiridol – a compound developed from a natural molecule through substitution of a flavonoid moiety with a nitrogen-containing heterocyclic alkaloid. Flavopiridol has been shown to inhibit CDK9 kinase and exhibit antitumor activity in lymphoproliferative disorders. In recent years, HCT116 cells have also demonstrated sensitivity to a number of additional plant-derived and synthetic compounds [26–28]. In the study by Parry R. A. et al., various fractions obtained from extracts of Alcea rosea were tested, and HCT116 cells exhibited greater sensitivity to these fractions in an MTT assay compared with HT-29 cells [29].

Our findings support the hypothesis that the novel plant alkaloid (P1) exerts markedly greater efficacy against CRC cells compared with berberine.

CONCLUSION

The novel alkaloid (P1), isolated from *Petasites hybridus* (L.) G.Gaertn., B.Mey. & Scherb, demonstrates clear dose-dependent cytostatic activity against colorectal cancer (CRC) cell cultures, while exerting minimal effects on peripheral blood mononuclear cells (PBMCs). Among the tested cell lines, HCT116 exhibited the highest sensitivity to P1. The results of this study indicate that the new plant-derived alkaloid (P1) is markedly more effective and possesses strong antiproliferative potential against CRC cells.

Thus, the findings highlight the relevance of P1 as a promising candidate for the development of new therapeutic strategies against colorectal cancer. However, further research is required to draw definitive conclusions, including comprehensive evaluation of its toxicity, pharmacokinetics, and mechanism of action.

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ORIGINAL ARTICLE

Cytostatic effect of ricobendazole on primary cultures of soft tissue sarcomas *in vitro*

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ABSTRACT

Soft tissue sarcomas (STS) are often resistant to treatment. The search for new antitumor compounds against STS remains an urgent task.

Purpose of the study. To assess the sensitivity of primary STS cultures of various histological subtypes to albendazole sulfoxide (ricobendazole) and doxorubicin, the primary metabolite of albendazole.

Materials and methods. STS tumor samples were used. The enzymatic dissociation method was used using 300 units/ml collagenase I (Thermo Fisher Scientific, USA). Ricasol® (NITA-PHARM, Russia) and Doxorubicin-LENS® (VEROPHARM, Russia) were used as test substances. Sensitivity to ricobendazole and doxorubicin was tested using the MTT test. The cultures were seeded in a 96-well plate at 7,000 cells in DMEM medium with 10 % FSC added. After 24 h, the medium was replaced with PPS with ricobendazole in a series of two-fold dilutions from 35.5 μmol/l to 0.0347 μmol/l or doxorubicin from 10 μmol/l to 0.009 μmol/l. After 72 hours of incubation, the MTT test was performed. The cells were seeded in a 24-well plate and cultured in PPS with 2 μmol/l ricabendazole for 72 h. Hoechst 33342 dye (Life Technologies, USA) was added to the culture at a concentration of 1 μg/ml, and photographs were taken using a LionHeart FX digital automatic microscope (BioTek Instruments Inc., USA).

Results. Four primary sarcoma cultures were obtained: SAR-1, SAR-2, SAR-3, and SAR-4. SAR-1 and SAR-4. Cultures demonstrated the most rapid growth, with doubling times of 38 and 27 hours, respectively. The slowest proliferation was observed in the SAR-2 culture (doubling time 156 hours), while SAR-3 showed a doubling time of 45 hours. According to the MTT assay, the IC_{50} values for ricobendazole were 4.54 ± 1.2 μmol/L for SAR-1, 3.31 ± 0.7 μmol/L for SAR-3, and 1.51 ± 0.2 μmol/L for SAR-4, whereas the slowly dividing SAR-2 culture proved to be insensitive to ricobendazole. The cytostatic activity of doxorubicin was higher than that of ricobendazole. The SAR-2 culture was the least sensitive (IC_{50} could not be determined), and SAR-4 (IC_{50} SAR-4 = 0.16 ± 0.01 μmol/l) was the most sensitive to the action of doxorubicin. The IC_{50} value of SAR-1 = 0.64 ± 0.02 μmol/l and IC_{50} SAR-3 = 1.8 ± 0.1 μmol/l. The effect of ricobendazole caused pronounced disturbances in the nuclei of SAR-1 and SAR-4 cultures, in SAR-2 and SAR-3 they were less pronounced.

Conclusion. Ricobenzale had a cytostatic effect on primary STS cultures characterized by rapid cell growth, but the activity was lower than that of doxorubicin. Changes in the morphology of cells and nuclei indicated probable disturbances in the functioning of the spindle and cytoskeleton occurring under the action of this compound. Of particular interest for further research is the combination of ricobendazole with taxanes and other tubulin inhibitors

Keywords: primary cell culture, soft tissue sarcoma, albendazole sulfoxide, chemotherapy, ricobendazole

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Compliance with ethical standards: patients were informed about their participation in the scientific study and provided written informed consent for the collection of biological material. The study was approved by the Local Ethics Committee of the National Medical Research Center for Oncology (Protocol No. 6/1 dated February 10, 2020).

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3.1.6. Онкология, лучевая терапия
ОРИГИНАЛЬНАЯ СТАТЬЯ

Цитостатическое действие рикобензадола на первичные культуры сарком мягких тканей *in vitro*

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РЕЗЮМЕ

Саркомы мягких тканей (СМТ) часто резистентны к лечению. Поиск новых противоопухолевых соединений в отношении СМТ остается актуальной задачей.

Цель исследования. Изучить чувствительность к основному метаболиту альбендазола сульфоксида (рикобендазол) и доксорубицину первичных культур СМТ различных гистологических подтипов.

Материалы и методы. Первичные культуры были получены из образцов СМТ, полученных от ранее не леченых пациентов в ходе хирургического удаления опухоли. В качестве исследуемых веществ использовали Риказол® (НИТА-ФАРМ, Россия) и Доксорубицин-ЛЭНС® (ВЕРОФАРМ, Россия). Чувствительность к рикобендазолу и доксорубицину проверяли с использованием МТТ-теста после культивирования с рикобендазолом в серии двукратных разведений от 35,5 мкмоль/л до 0,0347 мкмоль/л или доксорубицином от 10 мкмоль/л до 0,009 мкмоль/л в течение 72 ч. Для изучения морфологических изменений клеточных ядер клетки культивировали с 2 мкмоль/л рикабендазола в течение 72 ч, после чего проводили окрашивание 1 мкг/мл Hoechst 33342 (Life Technologies, США), и фотографировали в цифровом автоматическом микроскопе LionHeart FX (BioTek Instruments Inc., США).

Результаты. Получены 4 первичные культуры сарком. Культуры SAR-1 и SAR-4 характеризовались наиболее быстрым ростом (время удвоения 38 и 27 ч соответственно). Наиболее медленным ростом характеризовалась культура SAR-2 (время удвоения 156 ч), в культуре SAR-3 время удвоения составило 45 ч. По данным МТТ-теста для рикобендазола для SAR-1 IC_{50}–4,54 ± 1,2 мкмоль/л, для SAR-3–3,31 ± 0,7 мкмоль/л и для SAR-4–1,51 ± 0,2 мкмоль/л соответственно, медленно делящаяся SAR-2 оказалась нечувствительной к рикобендазолу. Цитостатическая активность доксорубицина была выше, чем у рикобендазола. Культура SAR-2 была наименее чувствительной (IC_{50} определить не удалось), а SAR-4 (IC_{50} SAR-4 = 0,16 ± 0,01 мкмоль/л) наиболее чувствительной к действию доксорубицина. Значение IC_{50} SAR-1 = 0,64±0,02 мкмоль/л и IC_{50} SAR-3 = 1,8 ± 0,1 мкмоль/л. Воздействие рикобендазола вызвало выраженные нарушения в ядрах в культурах SAR-1 и SAR-4, в SAR-2 и SAR-3 они были менее выражены.

Заключение. Рикобендазол оказал цитостатическое действие на первичные культуры СМТ, характеризующиеся быстрым клеточным ростом, но активность была ниже, чем у доксорубицина. Изменения в морфологии клеток и ядер свидетельствовали о вероятных нарушениях в работе веретена деления и цитоскелета, происходящих под действием данного соединения. Особенный интерес для дальнейшего исследования представляет комбинирование рикобендазола с таксанами и другими ингибиторами тубулинов.

Ключевые слова: первичная клеточная культура, саркома мягких тканей, сульфоксид альбендазола, химиотерапия, рикобендазол

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BACKGROUND

Soft tissue sarcomas (STS) are rare malignant tumors of mesenchymal origin, accounting for approximately 1 % of all human malignancies. In Russia, about 3,000–3,500 new STS cases are diagnosed annually, with a prevalence of 22.1 cases per 100,000 population [1]. STS are characterized by aggressive behavior, rapid recurrence, early dissemination, and high resistance to treatment. Doxorubicin remains the standard first-line chemotherapeutic drug for advanced and unresectable STS; however, the response rate is only around 15 %.

Thus, the search for new compounds with antitumor activity against STS remains an important task. One promising direction is the exploration of therapeutic approaches already tested in other oncologic diseases. Nitrogen-containing heterocyclic compounds – benzimidazole derivatives – are considered a promising basis for anticancer agents [2, 3]. In addition to synthesizing new compounds, repurposing approved benzimidazole-based agents is of great interest, since their safety profile and pharmacological properties are already known, potentially accelerating their introduction as anticancer agents. Some benzimidazole-based anthelmintic drugs used in veterinary medicine exhibit antitumor activity in animals; among them, albendazole and mebendazole are also approved for treating parasitic infections in humans [4]. These compounds were later shown to have cytostatic activity and selectivity toward human cancer cells. Albendazole, for example, suppresses tumor cell growth *in vitro* and *in vivo* through induction of oxidative stress and inhibition of β-tubulin polymerization, leading to impaired glucose uptake, metabolic starvation, cell-cycle arrest, and apoptosis in malignant cell cultures, while its inhibitory effect on normal cells is less pronounced [5]. Currently, several clinical trials of albendazole and mebendazole in various cancers are ongoing, although the number of enrolled patients remains limited [4]. Researchers note relatively good tolerability, with only occasional symptoms of myelosuppression. In isolated cases of patients receiving mebendazole, a clinical effect manifested as disease stabilization has been observed [5].

Several studies have evaluated the antitumor properties of benzimidazole derivatives in sarcoma cell cultures. Michaelis M. et al. demonstrated high sensitivity of Ewing sarcoma cell lines to flubendazole, with relatively low sensitivity observed in osteosarcoma and rhabdomyosarcoma cultures; other sarcoma histotypes were not investigated [6]. There is also evidence of successful use of albendazole to enhance the antitumor effect of doxorubicin when incorporated into composite nanoparticles against osteosarcoma cell lines [7].

Purpose of the study: to assess the sensitivity of primary STS cultures of various histological subtypes to albendazole sulfoxide (ricobendazole) and doxorubicin , the primary metabolite of albendazole.

MATERIALS AND METHODS

Primary cultures of sarcomas of various histological subtypes, obtained intraoperatively in the Department of Bone, Skin, Soft Tissue, and Breast Tumors of the National Medical Research Center for Oncology (Ministry of Health of the Russian Federation) in 2023–2024, served as the material for this study. The histological diagnosis was confirmed in the Department of Pathology of the same institution. The study included treatment-naive patients with soft tissue sarcomas. Exclusion criteria comprised prior chemoradiotherapy for soft tissue sarcomas as well as the presence of blood-borne infectious diseases.

Tumor samples were transferred from the operating room in Hank's Balanced Salt Solution (HBSS, Gibco, USA) supplemented with 1 % penicillin–streptomycin (Biolot, Russia) at +4–8 °C and delivered to the Laboratory of Cell Technologies within 20 minutes after surgical excision. The tissue was minced with a scalpel into fragments of 1–2 mm³ and placed into a culture tube containing DMEM medium (Gibco, USA) with 1 % gentamicin (Biolot, Russia) and collagenase type I (300 U/mL; Thermo Fisher Scientific, USA). The material was incubated for 2 hours at 37 °C on a shaker.

The resulting cell suspension was passed through a sterile nylon filter (70 μm; Becton Dickinson, USA)

and washed twice with DMEM (Gibco, USA). Cell counting and viability assessment were performed in a Goryaev chamber using 0.4 % trypan blue solution (Biolot, Russia). Primary sarcoma cultures were maintained in complete growth medium based on DMEM (Gibco, USA) supplemented with 10 % FBS (HyClone, USA), 1 % insulin–transferrin–selenium (Biolot, Russia), 1 % NEAA (Gibco, USA), and 1 % gentamicin (Biolot, Russia). At each passage, cells were counted and the doubling time was calculated using the formula $DT = t \times ln(2) / ln(n_1/n_2)$, where DT is doubling time, t is the time interval between two measurements, and n_1 and n_2 are cell numbers at the first and second time points, respectively.

Before conducting the main experiments, the presence of malignant cells in the cultures was confirmed in the Pathology Department of the NMRC for oncology, using standard cytological examination with azure–eosin staining according to the Romanowsky-Giemsa method.

Assessment of the Cytostatic Properties of Ricobendazole and Doxorubicin

As stock solutions of the test compounds, we used the anthelmintic drug Rikazol® (NITA-PHARM, Russia) (ricobendazole, albendazole-sulphoxide, 100 mg/mL) and the antitumor drug Doxorubicin-LENS® (VEROPHARM, Russia) (50 mg/25 mL). The sensitivity of primary sarcoma cultures to ricobendazole and doxorubicin was assessed by constructing dose–response curves using indirect quantification of viable cells via the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide). Cells were seeded into 96-well plates at 7,000 cells per well in complete growth medium. After 24 hours, the culture medium was replaced with medium containing ricobendazole in a series of two-fold dilutions ranging from 35.5 μmol/L to 0.0347 μmol/L, or doxorubicin in a two-fold dilution series from 10 μmol/L to 0.009 μmol/L. The concentration range for ricobendazole was selected to include previously reported IC50 values for the structurally related compound flubendazole obtained for a broad panel of malignant cell lines in the study by Michaelis M. et al. (2015) [6]. Plates were incubated for 72 hours, after which the MTT assay was performed according to the standard protocol [8]. Cell viability was determined as the optical density measured at 570 nm in treated wells relative to control wells, expressed as a percentage. Each experimental condition was assessed in 10 technical replicates, and the experiment was repeated in three biological replicates. Viability values are presented as mean ± SD. Data processing and graph construction were performed in Microsoft Excel. The half-maximal inhibitory concentration (IC50) was determined using the drc package in the R programming language [9]. For curve fitting, a three-parameter logistic model with a fixed lower limit (c = 0) was applied, without imposing constraints on the estimated parameters.

$$y = \frac{d}{(1+exp\,(b(log\,(x)-log\,(e))))},$$

where b – is the slope, d – is the upper limit, and e – is the half-maximal effective dose. In cases where hormesis was observed, data points lying above the upper asymptote were excluded from model fitting. IC_{50} values are presented as the mean ± 95 % confidence interval.

Analysis of Morphological Features of Cells and Nuclei

Cells of the investigated cultures were seeded into 24-well plates and cultured in complete medium supplemented with 2 μmol/L ricobendazole under standard conditions for 72 hours. A single concentration of ricobendazole was chosen to allow direct comparison of culture sensitivity to the compound. Hoechst 33342 dye (Life Technologies, USA) was then added to the cultures at a final concentration of 1 μg/mL, followed by a 20-minute incubation under standard conditions. Subsequently, the cultures were imaged using a LionHeart FX automated digital microscope (BioTek Instruments Inc., USA).

STUDY RESULTS

Four primary soft tissue sarcoma cultures of different histological subtypes were obtained and demonstrated varying doubling times (Table 1). At the time of the experiment, cultures SAR-1 and SAR-4 exhibited the most rapid growth (doubling

times of 38 and 27 hours, respectively). The slowest growth rate was observed in SAR-2 (doubling time 156 hours), while SAR-3 had a doubling time of 45 hours. Consequently, the number of passages completed prior to the experiment differed between cultures, with the faster-growing cultures reaching later passages by the start of testing (Table 1).

The assessment of ricobendazole's impact on the viability of primary soft tissue sarcoma cultures demonstrated that the slow-growing pleomorphic rhabdomyosarcoma culture SAR-2 was insensitive to the compound. In contrast, the remaining three cultures showed a pronounced decrease in viability in response to increasing concentrations of ricobendazole (Fig. 1A). Among them, the undifferentiated pleomorphic sarcoma culture SAR-4 exhibited the lowest half-maximal inhibitory concentration (IC_{50} SAR-4 = 1.51 ± 0.2 μmol/L) and a clear hormesis effect – an increase in cell viability relative to the untreated control under low concentrations of the toxicant. The IC_{50} values for the other two cultures were IC_{50} SAR-1 = 4.54 ± 1.2 μmol/L and IC_{50} SAR-3 = 3.31 ± 0.7 μmol/L.

The cytostatic activity of doxorubicin in the studied cultures was higher than that of ricobendazole; however, the overall response patterns were similar for both compounds. The SAR-2 culture was the least sensitive (IC_{50} could not be determined within the tested concentration range), whereas SAR-4 was the most sensitive to doxorubicin (IC_{50} SAR-4 = 0.16 ± 0.01 μmol/L). The half-maximal inhibitory concentrations for the remaining two cultures were IC_{50} SAR-1 = 0.64 ± 0.02 μmol/L and IC_{50} SAR-3 = 1.8 ± 0.1 μmol/L (Fig. 1B).

Table 1. Characteristics of primary soft-tissue sarcoma cultures

№	Culture ID	Histological diagnosis	Treatment	Passage at the time of experiment	Doubling time at the time of experiment, h
1	SAR-1	Epithelioid sarcoma	Surgical treatment only	Passage 5	38
2	SAR-2	Pleomorphic rhabdomyosarcoma	Surgical treatment only	Passage 3	156
3	SAR-3	Spindle cell/synovial sarcoma	Surgical treatment only	Passage 5	45
4	SAR-4	Undifferentiated pleomorphic sarcoma with infiltrative growth and areas of high-grade extraosseous osteosarcoma	Surgical treatment only	Passage 7	27

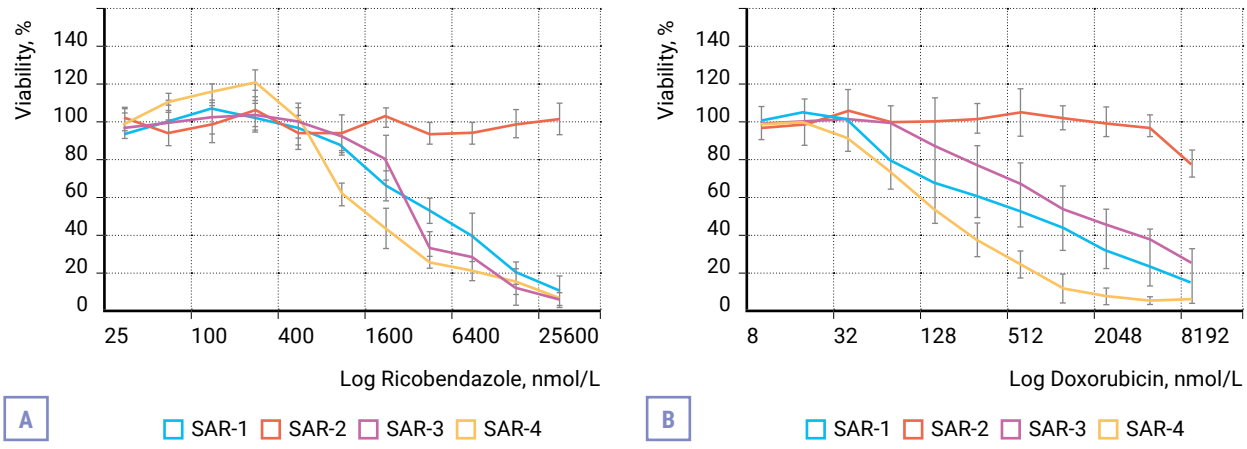

Fig. 1. Cytostatic activity of ricobendazole and doxorubicin against primary sarcoma cultures. A – dose–response curve for ricobendazole; B – dose–response curve for doxorubicin.

A pairwise comparison of the two compounds indicates that in SAR-1 and SAR-4 cultures, the cytostatic activity of ricobendazole was, on average, an order of magnitude lower than that of doxorubicin. In the SAR-3 culture, the difference was less pronounced: the IC_{50} for doxorubicin was only approximately twofold lower than the corresponding value for ricobendazole.

Without exposure to the tested compound, cells of SAR-1 and SAR-2 cultures exhibited an elongated, spindle-shaped morphology (Fig. 2A, C), whereas the SAR-3 and SAR-4 cultures consisted predominantly of cells with an epithelioid appearance (Fig. 2D, G). Cultivation in the presence of 2 μmol/L ricobendazole induced changes in monolayer confluence, cell morphology and the appearance of cytopathic features of varying severity. In the SAR-1 culture, a marked reduction in confluence was observed, along with a decrease in the number of cytoplasmic processes and general compaction of cells (Fig. 2B). A characteristic feature of treated samples was the noticeable increase in the number of rounded cells in metaphase (visible metaphase plate) (Fig. 2B, black arrows). In the SAR-2 culture, ricobendazole exposure induced changes in cell shape – similar to SAR-1, the cells became more compact – however, overall monolayer confluence did not undergo substantial alterations, and no pronounced cytopathic features were observed (Fig. 2B). In the SAR-3 culture, in contrast to the other cultures, a sharp shift in the growth pattern was documented: instead of forming a two-dimensional monolayer, the cells began forming distinct three-dimensional spheroid-like colonies attached to the well bottom (Fig. 2F). Finally, in the SAR-4 culture, in addition to reduced monolayer confluence, all cells demonstrated increased cytoplasmic granularity, a higher number of cells in metaphase (Fig. 2H, black arrows), and an increased proportion of multinucleated cells (Fig. 2H, white arrows).

Ricobendazole exposure induced pronounced nuclear abnormalities in the SAR-1 and SAR-4 cultures. In both cultures, apoptotic features were observed, including hyperchromatic fragmented nuclei (Fig. 3B, H, red arrows).

Alongside these changes, multinucleated cells with polymorphic nuclei and micronuclei were observed in these cultures. Unlike the fragmented nuclei characteristic of late-stage apoptosis, these structures were stained with an intensity typical of normal nuclei, displayed normal chromatin architecture, and had a smooth, rounded shape (Fig. 3B, H, blue arrows). In the SAR-2 and SAR-3 cultures, such features were not detected (Fig. 3D, F).

DISCUSSION

The half-maximal inhibitory concentration values obtained in our study for three of the four primary

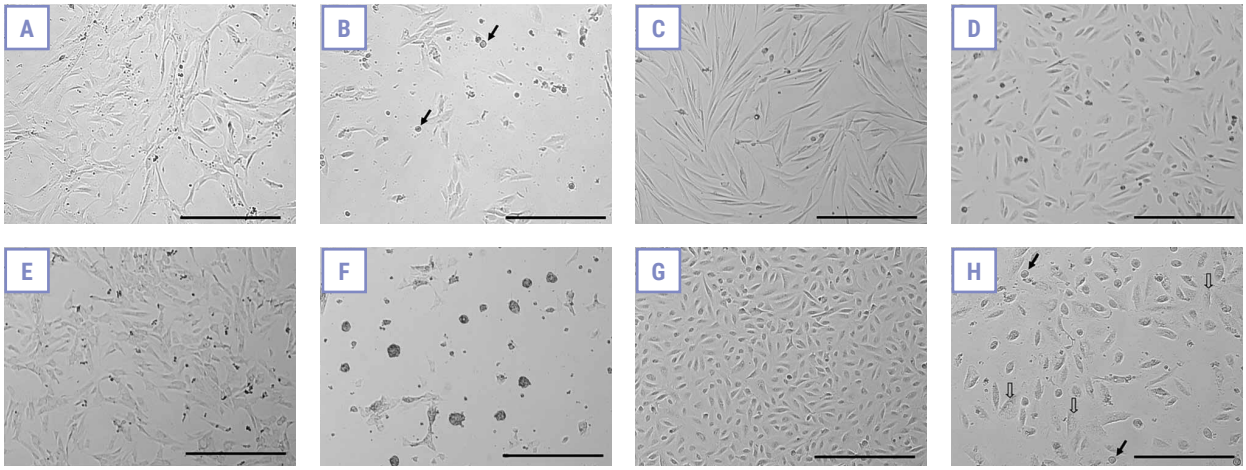

Fig. 2. Effect of ricobendazole at 2 μmol/L on primary sarcoma cultures. Exposure time: 72 h. Objective magnification ×5. A – SAR-1 culture, control; B – SAR-1 culture, ricobendazole; C – SAR-2 culture, control; D – SAR-2 culture, ricobendazole; E – SAR-3 culture, control; F – SAR-3 culture, ricobendazole; G – SAR-4 culture, control; H – SAR-4 culture, ricobendazole.
Annotations: black arrows – cells in metaphase; white arrows – multinucleated cells. Scale bar: 400 μm.

sarcoma cultures ranged from 1.5 to 4.5 μmol/L. According to available data, the maximal blood concentration of albendazole sulphoxide after oral administration of albendazole at a dose of 400 mg (6–8 mg/kg) is 0.16–1.58 mg/L, which corresponds to 0.6–6 μmol/L [10]. Thus, an antitumor effect of ricobendazole in humans may theoretically be achievable at doses considered safe and commonly used for helminthiasis treatment [4].

Based on our findings, it may be hypothesized that sensitivity to ricobendazole, as well as to doxorubicin, is likely more dependent on the proliferation index than on the histotype of soft tissue sarcoma cultures.The observed alterations in cell and nuclear morphology suggest that the target of ricobendazole in primary sarcoma cultures may be the microtubules of the mitotic spindle and cytoskeleton. The action of the compound likely leads to mitotic arrest at metaphase and to unequal segregation of chromosomes, giving rise to micronuclei or mitotic catastrophe and subsequent cell death. Disturbances of the cytoskeleton may also be reflected in the altered cell shape seen across cultures and the shift in growth behavior in the spindle-cell/synovial sarcoma SAR-3 culture – from a two-dimensional monolayer to three-dimensional spheroid-like structures. β-Tubulin has been indicated as a target for benzimidazole derivatives in the literature [5]. The hypothesis that ricobendazole exerts its effect through the cell division machinery is consistent with the observation that the slowly proliferating SAR-2 pleomorphic rhabdomyosarcoma culture was insensitive to the cytostatic action of this compound. However, additional studies are required to verify this hypothesis, as the weak impact on slowly dividing cells is a common feature of all cytostatic agents, regardless of their mechanism of action. For example, doxorubicin, to which SAR-2 was likewise insensitive, inhibits DNA synthesis in proliferating cells without targeting the cell division apparatus.

Tubulin inhibitors such as paclitaxel and vincristine have been well-established anticancer agents since the 1960s. Paclitaxel stabilizes microtubules by shifting the equilibrium toward polymer formation and thereby lowering the critical tubulin concentration, disrupting normal microtubule dynamics essential for spindle function and intracellular transport. Vincristine, in contrast, is a microtubule-destabilizing agent that binds specifically to microtubule plus-ends, preventing polymerization and impairing mitotic spindle assembly and function [11]. Although highly effective, these tubulin inhibitors lack sufficient selectivity for tumor cells, which has stimulated the search for novel agents targeting tubulin isotypes overexpressed in malignant tumors [12, 13]. The β3-tubulin (TUBB3) isotype is most strongly as-

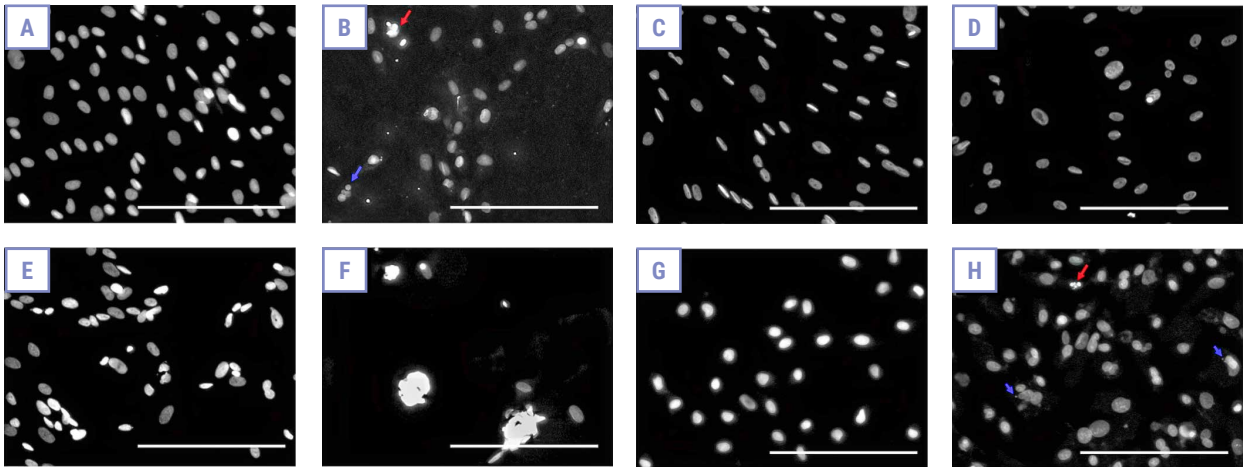

Fig. 3. Effect of ricobendazole at 2 μmol/L on nuclear morphology in primary sarcoma cultures. Exposure: 72 h. Hoechst 33342 staining. Objective magnification ×10. A – SAR-1 culture, control; B – SAR-1 culture, ricobendazole; C – SAR-2 culture, control; D – SAR-2 culture, ricobendazole; E – SAR-3 culture, control; F – SAR-3 culture, ricobendazole; G – SAR-4 culture, control; H – SAR-4 culture, ricobendazole.
Annotations: red arrows – nuclei with apoptotic features; blue arrows – micronuclei. Scale bar: 200 μm.

sociated with tumor progression, metastasis, and chemoresistance [14]. Notably, β3-tubulin overexpression correlates with resistance to eribulin in leiomyosarcoma cells [15]. Its critical role in sarcoma oncogenesis is further underscored by the sensitivity of these tumors to the β3-tubulin inhibitor plocabulin [16]. Current evidence suggests that the pro-oncogenic properties of β3-tubulin are driven by multiple mechanisms. β3-Tubulin-associated activation of the transcription factors Snail and ZEB1 may trigger epithelial-mesenchymal transition [17]. Resistance to taxanes may be linked to the increased dynamic instability of β3-tubulin-containing microtubules, conferring reduced susceptibility to microtubule-stabilizing agents [18]. In addition, β3-tubulin may protect cells from endoplasmic reticulum stress and reactive oxygen species-induced stress, thereby promoting survival during chemotherapy exposure [14, 19]. Possible therapeutic applications of ricobendazole in sarcomas via its potential interaction with β3-tubulin remain an important subject for future investigation.

CONCLUSION

Ricobendazole exerted a pronounced cytostatic effect on primary soft tissue sarcoma cultures characterized by rapid proliferation; however, its activity was lower than that of doxorubicin. Particularly promising is the potential for combining ricobendazole with taxanes and other tubulin-targeting agents in future studies.

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EXPERIENCE EXCHANGE

CHEK2 p.Ile157Thr (c.470T>C) mutation in non-small cell lung cancer: regional experience

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ABSTRACT

Purpose of the study. To determine the frequency and co-occurrence with major somatic drivers of the germline CHEK2 p.Ile157Thr mutation in patients with non-small cell lung cancer (NSCLC) in the Republic of Tatarstan.

Patients and methods. Targeted next-generation sequencing (NGS) of key oncogenes was performed on tumor tissue from 151 patients. A bibliographic search was carried out manually in Google Scholar and PubMed using the terms "CHEK2 I157T," "p.Ile157Thr," "c.470T>C," "NSCLC," "germline," "NGS," among others; search formulations were refined with the aid of artificial intelligence, followed by mandatory manual verification. Statistical analysis was performed in SPSS v18.0. Proportions were compared using Fisher's exact test with a significance threshold of $p < 0.05$.

Results. The p.Ile157Thr variant was identified in 12 patients (7.9 %); all cases (100 %) were histologically confirmed adenocarcinomas. A positive family history of malignant tumors was recorded in 2 patients (16.7 %), and multiple primary malignancies in 2 patients (16.7 %). Concomitant driver mutations were detected in 8 patients (66.7 %): EGFR in 5 (62.5 %), while 3 patients (37.5 %) harbored mutations in KRAS (12.5 %), NRAS, and BRAF, respectively. In 4 patients (33.3 %), p.Ile157Thr was the sole molecular event. In a comparison of carriers versus non-carriers of CHEK2 p.Ile157Thr, no statistically significant differences were observed in stage distribution or in the frequency of co-occurring driver alterations (Fisher's exact test, $p > 0.05$).

Conclusion. The germline CHEK2 p.Ile157Thr (c.470T>C) variant was identified in a subset of patients with lung adenocarcinoma and in several cases was accompanied by somatic driver mutations. The obtained data refine the frequency of this variant in the studied population and describe the molecular characteristics of tumors in carriers, providing a basis for further evaluation of the potential clinical relevance of CHEK2 in NSCLC.

Keywords: CHEK2, p.Ile157Thr, c.470T>C, I157T, lung cancer, non-small cell lung cancer, NGS, next-generation sequencing, germline mutations, hereditary mutations

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Compliance with ethical standards: the study followed the ethical principles set forth by the World Medical Association Declaration of Helsinki, 1964, ed. 2013. The study protocol was reviewed and approved by the Ethics Committee of Kazan Federal University, Kazan, Russian Federation (protocol extract No. 52, August 28, 2025). Written informed consent was obtained from all participants prior to their inclusion in the study.

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ОБМЕН ОПЫТОМ

Мутация CHEK2 p.Ile157Thr (c.470T>C) при немелкоклеточном раке легкого: региональный опыт

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РЕЗЮМЕ

Цель исследования. Определить частоту и сочетание с основными соматическими драйверами герминальной мутации CHEK2 p.Ile157Thr у пациентов с немелкоклеточным раком легкого (НМРЛ) в Республике Татарстан.

Пациенты и методы. Панельное NGS-секвенирование ключевых онкогенов выполнено в опухолевом материале 151 пациента. Библиографический поиск проводился вручную в Google Scholar, PubMed по сочетаниям «CHEK2 I157T», «p.Ile157Thr», «c.470T>C», «NSCLC», «germline», «NGS» и др.; формулировки уточнялись с помощью искусственного интеллекта при обязательной ручной верификации. Статистическую обработку выполняли в SPSS v18.0; для сравнения долей применяли точный критерий Фишера, уровень значимости $p < 0,05$.

Результаты. Вариант p.Ile157Thr выявлен у 12 (7,9 %) пациентов: у 100 % подтверждена гистологически аденокарцинома. Семейный анамнез злокачественных опухолей зафиксирован у 2 (16,7 %) пациентов, множественные первичные новообразования – у 2 (16,7 %). Сопутствующие драйверные мутации обнаружены у 8 (66,7 %): EGFR – у 5 (62,5 %), у 3 (37,5 %) была обнаружена одна из мутаций KRAS (12,5 %), NRAS и BRAF соответственно. У 4 (33,3 %) p.Ile157Thr было единственным молекулярным событием. При сравнении носителей и неносителей CHEK2 p.Ile157Thr по стадиям заболевания и частоте сопутствующих драйверов статистически значимых различий не выявлено (точный критерий Фишера, $p > 0,05$).

Заключение. Герминальный вариант CHEK2 p.Ile157Thr (c.470T>C) выявлен у части пациентов с аденокарциномой легкого и в ряде случаев сочетался с соматическими драйверными мутациями. Полученные данные уточняют его частоту в исследуемой популяции и описывают молекулярные особенности опухолей у носителей, что может быть использовано в дальнейших исследованиях клинического значения CHEK2 при НМРЛ.

Ключевые слова: CHEK2, p.Ile157Thr, c.470T>C, I157T, рак легкого, немелкоклеточный рак легкого, NGS, секвенирование нового поколения, герминальные мутации, наследственные мутации

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Соблюдение этических стандартов: в работе соблюдались этические принципы, предъявляемые Хельсинкской декларацией Всемирной медицинской ассоциации (World Medical Association Declaration of Helsinki, 1964, ред. 2013). Исследование одобрено Комитетом по этике при ФГАОУ ВО «Казанский (Приволжский) федеральный университет», г. Казань, Российская Федерация (выписка из протокола заседания № 52 от 28.08.2025). Информированное согласие получено от всех участников исследования.

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BACKGROUND

Lung cancer is one of the most aggressive malignant neoplasms and remains among the leading causes of cancer morbidity (2nd place – 2.1 million new cases) and mortality (1st place – 1.8 million deaths annually) worldwide [1]. The etiology of the disease represents a complex interplay between exogenous factors and genetic alterations, including both hereditary predisposition and sporadic somatic mutations [2]. Smoking is considered the predominant exogenous driver, accounting for 80–90 % of cases among men and 60–70 % among women [3].

Despite advances in diagnostic and therapeutic strategies, the prognosis of lung cancer remains poor, underscoring the need for novel treatment approaches based on a deeper understanding of the molecular mechanisms of carcinogenesis. In this context, precision medicine, grounded in the identification of tumor-specific genetic characteristics, has emerged as a pivotal direction in modern oncology, transforming therapeutic paradigms and improving the likelihood of long-term remission.

A major breakthrough in the molecular characterization of non-small cell lung cancer (NSCLC) was the discovery of the role of EGFR (epidermal growth factor receptor) mutations in the early 2000s, which not only revealed the pathogenetic heterogeneity of the disease but also laid the foundation for the development of targeted therapies [4]. The introduction of high-throughput next-generation sequencing (NGS) technologies into clinical practice enabled the detection of rare and previously unrecognized genetic variants associated with oncogenesis. This led to the identification of a wide spectrum of driver mutations, including alterations in ALK, ROS1, KRAS, BRAF, MET, RET, and other genes, each defining a unique biological tumor subtype requiring an individualized therapeutic approach [5].

Although most molecular alterations in NSCLC are somatic and associated with exposure to exogenous carcinogens, recent evidence highlights the contribution of hereditary mutations. In a large cohort study by Sorscher S. et al., including 7,788 unrelated patients with lung cancer who underwent uniform NGS testing (panel of up to 159 genes, mean sequencing depth ≈ ×350), pathogenic or likely pathogenic variants were identified in 14.9 % of patients (1,161/7,788). The most frequent mutations included BRCA2 (2.8 %), CHEK2 (2.1 %), ATM (1.9 %), TP53 (1.3 %), BRCA1 (1.2 %), EGFR (1.0 %), APC (0.9 %), and PALB2 (0.5 %). Notably, 61.3 % of all detected variants were found in genes involved in DNA damage repair, and 95 % were classified as clinically significant [6]. In the present review, we focus on germline variants in the CHEK2 (Checkpoint Kinase 2) gene, which plays a key role in maintaining genomic stability and regulating the cell cycle, with specific emphasis on the CHEK2 p.Ile157Thr (c.470T>C) missense variant.

The involvement of CHEK2, a gene encoding the serine/threonine kinase CHK2, a central component of the DNA double-strand break response pathway, was first demonstrated by Bell D. W. et al. in 1999. In their study, a germline 1100delC mutation was identified in a family with Li–Fraumeni syndrome. Loss of CHK2 kinase activity was shown to impair phosphorylation of key proteins (p53, BRCA1, CDC25C), compromising checkpoint activation and DNA repair, and thereby predisposing to early-onset and multiple malignancies. These findings established CHEK2 as a tumor suppressor gene [7].

In 2002, two independent studies extended this concept to a broader population context. Meijers-Heijboer H. et al. demonstrated that 1100delC confers an approximately twofold increased risk of breast cancer in women and nearly a tenfold increase in men in non-BRCA families [8]. Vahteristo P. et al. reported that the same allele accounts for a considerable proportion of familial clusters of breast cancer and is associated with loss of CHK2 expression in tumor tissue [9].

In 2004, both the allelic and nosological spectra were expanded: Kilpivaara O. et al. were the first to associate the missense variant I157T, which retains partial kinase activity, with a moderate increase in breast cancer risk, demonstrating that not only truncating mutations but also functionally defective amino acid substitutions are involved in tumorigenesis [10].

Cybulski C. et al. simultaneously investigated three Polish founder alleles of CHEK2 – the truncating 1100delC and IVS2+1G>A variants, as well as

the missense p.Ile157Thr – and showed that their carriage increases the risk not only of breast cancer but also of malignancies of the colon, prostate, thyroid, and kidney, thereby firmly establishing the gene as a multi-organ, moderate-penetrance cancer susceptibility factor [11].

The best-studied CHEK2 mutations remain p.Ile157Thr and 1100delC, both associated with an increased risk of several malignancies, including breast cancer [12], colorectal cancer [13], and prostate cancer [11]. However, the limited evidence on the role of CHEK2 in lung cancer pathogenesis leaves a substantial gap in understanding the molecular basis of the disease and restricts the potential for applying personalized therapeutic approaches in affected carriers of this genetic alteration.

CHEK2 exhibits pronounced geographic heterogeneity. In Northern Europe, the truncating founder mutation c.1100delCpredominates, with population carrier frequencies approaching approximately 1 % in the United Kingdom and the Netherlands, gradually decreasing toward Southern Europe, where it is detected only sporadically. In Eastern (and partly Central) Europe, the distribution shifts toward the missense variant p.I157T (c.470T>C), with heterozygous carriers comprising around 5 % of the general population in Poland, Latvia, Hungary, and Russia, and 2–3 % in the Czech Republic, Slovakia, and Germany, making it the most common regional CHEK2 variant. In Asian populations, European founder mutations are virtually absent: in a cohort of 8,085 Chinese women, the total frequency of pathogenic CHEK2variants did not exceed 0.3 %, with half represented by a novel nonsense allele p.Y139X. Independent datasets demonstrated an association between the recurrent missense variant p.H371Y and breast cancer susceptibility [14]. Taking into account this regional distribution, the present study focused on the p.Ile157Thr (c.470T>C) variant as the most prevalent and clinically relevant CHEK2 alteration in Eastern Europe.

A neighboring region ethnographically related to the Republic of Tatarstan – the Republic of Bashkortostan – demonstrates a similar Eastern European pattern: in a cohort of 977 breast cancer patients and 1,069 controls, the p.Ile157Thr (c.470T>C) missense variant was the most frequent (~5 % in both cohorts), whereas c.1100delC and the splice mutation c.444+1G>A were detected in only 0.4 % of cases, and a large deletion del5395 occurred in 1.23 % of patients and only 0.09 % of healthy controls [15].

Thus, the rationale for selecting CHEK2 for investigation is determined by its pivotal role in the DNA damage response system and the high prevalence of the p.Ile157Thr variant in the Eastern European population.

Purpose of the study: to determine the frequency and co-occurrence with major somatic drivers of the germline CHEK2 p.Ile157Thr mutation in patients with NSCLC in the Republic of Tatarstan.

PATIENTS AND METHODS

The present study included patients with NSCLC treated at the Republican Clinical Oncology Dispensary of the Ministry of Health of the Republic of Tatarstan named after Prof. M. Z. Sigal. In total, tumor tissue samples from 151 patients were analyzed. The median age was 67 years, and the mean age was 64 years. There were 70 men (46.3 %) and 81 women (53.7 %). The ethnic composition of the cohort was homogeneous (Russians and Tatars), reflecting the demographic structure of the region. The smoking history of the patients demonstrated a typical distribution for an NSCLC cohort and included both current/former smokers and never-smokers.

Tumor samples were analyzed using next-generation sequencing (NGS) on the NextSeq 2000 platform (Illumina) with the KAPA HyperPETE LC Fusion Panel and KAPA HyperChoice panels (Roche Diagnostics).

The genomic analysis included:

A DNA panel comprising 43 genes associated with DNA repair, cell cycle regulation, and oncogenesis:

- *ATM, ATR, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CDK12, CHEK1, CHEK2, EPCAM, FANCL, MLH1, MSH2, NBN, NF1, PALB2, PMS2, RAD51B, RAD51C, RAD51D, RAD54, STK11, TP53, KRAS, NRAS, BRAF, EGFR, ERBB2, PIK3CA, MET (ex14), KIT, POLE, KEAP1, PDGFRA, ESR1, EPCAM, IDH1/2*.

- An RNA panel aimed at detecting transcriptional alterations and fusion transcripts in 17 key oncogenes: *ALK, AXL, BRAF, EGFR, FGFR1–3, MET, NRG1, NTRK1–3, PDGFRA, PDGFRB, PPARG, RET, ROS1*.

Annotation and interpretation of identified variants were performed using the *ClinVar*, *gnomAD*, and *dbSNP* databases, which allowed differentiation between somatic and germline alterations. The *CHEK2 p.Ile157Thr (c.470T>C, rs17879961)* variant was identified as a known germline variant registered in these databases and classified as pathogenic according to ACMG (2015) criteria. Detection of this variant in tumor tissue reflects the presence of an inherited mutation rather than a purely somatic event, which is supported by its annotation in population and clinical variant databases.

The literature search was performed manually in Google Scholar and PubMed using combinations of the following keywords: "CHEK2", "p.Ile157Thr", "c.470T>C", "NSCLC", "germline", "NGS", etc. At the stage of content analysis, interpretation of the articles, and stylistic optimization of the wording, the artificial intelligence system ChatGPT o3 was used, with mandatory manual verification and editing of each fragment.

Statistical analysis

Statistical processing was carried out using SPSS software (version 18.0). Fisher's exact test was applied for comparison of categorical variables. Differences were considered statistically significant at $p < 0.05$. Statistical analysis was limited to internal comparison of clinical and molecular characteristics between carriers and non-carriers of the CHEK2 p.Ile157Thr (c.470T>C) variant.

STUDY RESULTS

A germline CHEK2 mutation was identified in 12 patients (prevalence 7.95 %), and in all cases, the detected variant was c.470T>C (p.Ile157Thr, I157T). Histologically, all patients had lung adenocarcinoma of varying degrees of differentiation. The sex distribution was equal (6 men and 6 women), and the mean age was 65 years. Five individuals (41.7 %) were active or former smokers, whereas 7 patients (58.3 %) reported no history of smoking.

One female patient had been diagnosed in 2006 with right breast cancer (stage III, T3N1M1), for which she underwent radical mastectomy, chemotherapy, and external-beam radiation therapy. In 2024, a second primary tumor was identified a right upper lobe lung adenocarcinoma localized in a region of prior radiation changes. Tumor profiling revealed EGFR exon 19 deletion, a BRCA1 mutation, and the germline CHEK2 p.Ile157Thr (c.470T>C) variant.

Another female patient had been diagnosed in 2009 with papillary thyroid carcinoma (stage II, pT2N0M0) and underwent a thyroidectomy followed by radiation therapy. In 2016, she was diagnosed with left upper lobe lung adenocarcinoma (stage IIA, pT1N1M0), treated with extended lobectomy. In 2024, disease progression was documented, with metastases to the contralateral lung and submandibular salivary gland, along with the diagnosis of a third primary tumor – renal cancer. Molecular analysis of the lung tumor identified an EGFR exon 19 deletion and the germline CHEK2 p.Ile157Thr (c.470T>C) variant.

These two clinical observations demonstrate a phenotypic association between CHEK2 p.Ile157Thr carrier status and the development of multiple primary tumors of distinct localization, including malignancies of the thoracic and genitourinary systems, consistent with previously reported associations in CHEK2-positive individuals.

Analysis of disease stages revealed a distribution typical for NSCLC: early (stage I) and metastatic disease (stage IV) predominated, while intermediate stages (II–III) were less common (Fig. 1). The clinical course corresponded to patterns widely observed in NSCLC cohorts, with most patients presenting with localized or locally advanced disease, and the remainder with metastatic spread.

Additional somatic driver mutations were detected in 8 patients (66.7 %) (Fig. 2). The most frequent alterations involved EGFR, followed by less common changes in KRAS, NRAS, and BRAF. In two patients, EGFR-positive tumors co-occurred with TP53 or BRCA1 variants; a potential germline origin of the latter cannot be excluded because only tumor tissue was sequenced. In four patients (33.3 %), the CHEK2 p.Ile157Thr variant was detected in isolation, without accompanying somatic driver mutations.

The immunohistochemical profile of tumors was

consistent with lung adenocarcinoma: TTF-1 and CK7 positive, p40 negative, with Ki-67 ranging from 15 % to 60 %.

Comparison of disease stage distribution and frequency of somatic driver mutations between carriers and non-carriers of the CHEK2 p.Ile157Thr (c.470T>C) variant revealed no statistically significant differences (Fisher's exact test, $p > 0.05$).

DISCUSSION

The present study is one of the first in the Republic of Tatarstan aimed at identifying the germline CHEK2 p.Ile157Thr (c.470T>C) mutation and its co-occurrence with somatic driver alterations in patients with NSCLC. The analysis was performed using next-generation sequencing (NGS).

In the study by Kazakov A. M. et al., which included 90 patients with stage I–IIIA NSCLC, the authors conducted targeted sequencing of tumor tissue with parallel analysis of paired "normal" lung material, enabling a systematic description of the somatic mutational landscape of two histological subtypes (63 adenocarcinomas and 27 squamous cell carcinomas). The 78-gene panel encompassed both point mutations (e. g., *EGFR*, *KRAS*, *BRAF*) and gene rearrangements (e. g., *ALK*, *ROS1*, *RET*). Adenocarcinomas were dominated by TP53, KRAS, and EGFR alterations, with a range of rare but clinically meaningful events (including BRAF) that were consistent with global estimates. For CHEK2, rare somatic variants were reported predominantly in adenocarcinomas (mainly in exon 2), while germline status was not assessed and the p.Ile157Thr (I157T) allele was not detected. Thus, although the study provides relevant context for the somatic landscape of localized NSCLC and supports the value of expanded panels, our work fundamentally differs by focusing on the germline CHEK2 p.Ile157Thr variant and its prevalence and molecular associations in an Eastern European clinical cohort [16].

Research addressing the CHEK2 p.Ile157Thr (c.470T>C) variant as a hereditary risk factor for lung cancer is represented in the works of Brennan P. et al. [17], Cybulski C. et al. [18], and Wang Y. et al. [19], which demonstrated that I157T is associated with a reduced risk of squamous cell carcinoma of the lung, while no significant protective effect has been confirmed for adenocarcinoma. All three studies were conducted in European populations, where the I157T allele shows a minor allele frequency of approximately 4–5 %. The reported CHEK2 I157T carrier rate among lung cancer patients ranged from 1 % to 3 %, and approximately 5 % in the general population.

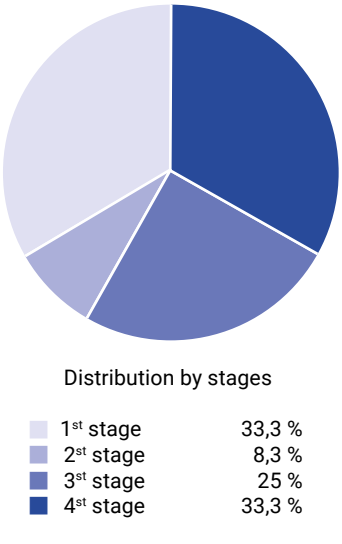

Fig. 1. Percentage distribution of patients with the CHEK2 p.Ile157Thr mutation by stage of lung cancer

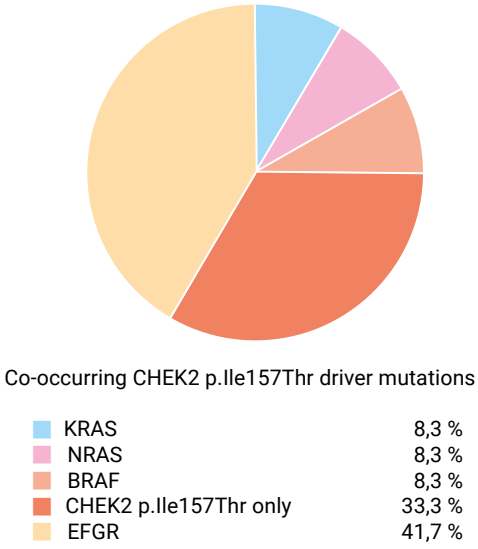

Fig. 2. Percentage distribution of patients with the CHEK2 p.Ile157Thr mutation according to the coexistence of lung cancer with driver mutations.

In our study, the p.Ile157Thr (c.470T>C) mutation was detected in 12 out of 151 patients, corresponding to a carrier frequency of 7.9 % (95 % CI: 4.6–13.4 %). All carriers were heterozygous, with an allele frequency of 3.98 % (95 % CI: 2.3–6.8 %).

All 12 carriers of the CHEK2 p.Ile157Thr (c.470T>C) variant were diagnosed with lung adenocarcinoma; squamous cell carcinoma and small cell carcinoma were not observed in the carrier subset. Historically, CHEK2 p.Ile157Thr has been associated with a protective effect primarily for squamous cell lung cancer, without a significant influence on adenocarcinoma risk. The higher proportion of carriers in the present study may reflect population-specific and histological characteristics, as well as the limited sample size.

Current evidence on the role of CHEK2 p.Ile157Thr (c.470T>C) in lung cancer remains based on a small number of studies, predominantly from 2007–2014. Large European NGS investigations focusing specifically on this variant and its somatic co-alterations have not yet been conducted.

In our cohort, 66.7 % of CHEK2 p.Ile157Thr carriers exhibited additional oncogenic events, most commonly EGFR alterations, and less frequently KRAS, BRAF, and NRAS mutations. This pattern reflects the expected mutation profile of lung adenocarcinoma, summarized in the review by Kharagezov D. A. et al., which discusses common point mutations (EGFR, KRAS) and rare but clinically relevant events (BRAF), whereas germline CHEK2 variants were not considered [20]. The observed co-occurrence of germline CHEK2 with common and rare drivers should be interpreted as hypothesis-generating. Validation requires larger multicenter cohorts, paired tumor–normal testing, and functional assays.

NGS remains essential for identifying such complex interactions. Accumulating data on the relationships between germline predisposition variants and somatic tumor profiles may help refine the biological role of CHEK2 and potentially support its incorporation as a prognostic or stratification marker in NSCLC precision oncology.

To date, only a limited number of large NGS datasets include both germline variants and somatic profiles in NSCLC with reported CHEK2 alterations. In a study by Zhang S. S. et al., 70 patients (1.15 %) carried pathogenic germline CHEK2 variants, and among them, 33 % (*n* = 23) were p.Ile157Thr carriers. Co-occurring somatic drivers included KRAS G12C/G12D (~40 %), EGFR ex19del/L858R (~25 %), and isolated cases of ALK, ROS1, RET, MET exon 14 skipping, and BRAF V600E [21]. However, the somatic spectrum was not stratified by specific CHEK2 alleles, including p.Ile157Thr (c.470T>C), limiting conclusions.

Mezquita L. et al. identified 547 patients (0.62 %) with any pathogenic CHEK2 variant, with somatic driver frequencies of EGFR 34 %, KRAS 21 %, and *ALK/ROS1/MET/BRAF* ~15 % combined; however, specific CHEK2 alleles were not detailed [22].

In a Chinese study by Zhou N. et al., germline CHEK2 mutations were identified in 89 individuals (1.8 %), but p.Ile157Thr (c.470T>C) was not observed, consistent with its low prevalence in Asian populations. Nonsense and truncating alleles (p.Y139*, K373fs) predominated. Among somatic drivers, EGFR alterations dominated (~55 %), KRAS occurred in <10 %, and ALK/ROS1/MET exon 14 events were rare [23].

Thus, CHEK2 I157T is rare in Asian cohorts and more frequent in North American datasets, although the multiethnic composition of these cohorts complicates population-specific interpretation. Without robust ancestry stratification, precise prevalence estimates for Eastern Europe remain uncertain. Importantly, no large NGS study has yet systematically examined the association between germline CHEK2 variants and somatic NSCLC drivers in Europe, particularly with focused attention on p.Ile157Thr (c.470T>C).

Therefore, the present regional NGS-based investigation from an Eastern European population provides the first data generated using contemporary molecular diagnostics on the prevalence of CHEK2 p.Ile157Thr (c.470T>C) and its co-mutation patterns in NSCLC.

At present, germline CHEK2 mutations are known to increase the risk of breast, colorectal, and prostate cancer, which is reflected in NCCN[1] guidelines [24, 25]. Enhanced surveillance protocols are

[1] National Comprehensive Cancer Network (NCCN) [Internet]. Available at: https://www.nccn.org – *Editorial note.*

recommended for carriers. However, in NSCLC, the role of CHEK2 remains poorly defined. CHEK2 is not included among hereditary risk genes in the NCCN NSCLC guidelines or in the low-dose CT lung cancer screening guideline [26, 27]. Only two germline contexts are clearly addressed: exclusion of germline EGFR p.T790M when detected in tumor tissue, and Li–Fraumeni syndrome (germline TP53), discussed in specific recommendations. Other driver loci (ALK, ROS1, MET, BRCA2, RB1, etc.) are considered exclusively therapeutic somatic targets, without guidance for family screening [27].

This creates a dual gap: carriers of CHEK2 variants do not receive lung cancer screening recommendations even in the presence of family history, while families with hereditary NSCLC patterns do not routinely undergo CHEK2 testing despite its relevance in other cancers. This imbalance underscores the need for further research into the contribution of CHEK2 – particularly p.Ile157Thr (c.470T>C) – to NSCLC carcinogenesis.

CONCLUSION

Analysis of this regional cohort of patients with non-small cell lung cancer demonstrates that the germline CHEK2 p.Ile157Thr (c.470T>C) variant occurs more frequently than suggested by international datasets and forms a distinct molecular context within these tumors. All identified carriers had lung adenocarcinoma, and most exhibited co-occurrence of the germline variant with somatic driver alterations typical for this subtype – predominantly EGFR mutations, and less frequently KRAS, BRAF, and NRAS. This indicates that, even in the presence of well-established “classical” somatic patterns, the disease profile may be influenced by the underlying hereditary background. The findings obtained using modern NGS approaches represent the first systematic data on the frequency and molecular environment of p.Ile157Thr in Eastern Europe and highlight an evident gap in the literature.

Comparative analysis of the clinical and molecular features of carriers and non-carriers of CHEK2 p.Ile157Thr did not reveal statistically significant differences; however, the observed combinations underscore the need for deeper investigation into the interplay between germline and somatic events in lung carcinogenesis. Particular attention is warranted for patients with multiple primary malignancies, consistent with the known multi-organ cancer susceptibility associated with pathogenic CHEK2 variants and emphasizing the limited representation of such patients in current NSCLC guidelines.

The practical value of the obtained data lies in their potential to inform regional approaches to genetic counseling and refine the interpretation of NGS profiles. With further data accumulation, it may become possible to determine whether germline CHEK2 variants – especially p.Ile157Thr –serve as prognostic markers, risk-stratification factors, or indirect indicators of the likelihood of specific somatic drivers.

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REVIEW

Fertility preservation in women with BRCA1/2-related cancers: contemporary strategies, international recommendations, and a multidisciplinary approach

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ABSTRACT

Inherited mutations in the BRCA1/BRCA2 genes significantly increase the risk of breast and ovarian cancer in women of reproductive age, posing a clinical and socioeconomic challenge due to loss of fertility during cancer treatment and preventive interventions. The expansion of genetic testing programs is shifting the focus to proactive management of reproductive potential, requiring the integration of oncology, reproductive medicine, and medical genetics. The novelty of this review lies in its comprehensive synthesis of data on the impact of treatment and prevention of BRCA-associated cancer on fertility and a critical assessment of the effectiveness of fertility preservation strategies.

Purpose of the study. To summarize and analyze current advances, clinical guidelines, and unresolved issues related to preserving reproductive function in women carrying BRCA1/BRCA2 mutations.

Materials and methods. A systematic search of PubMed/MEDLINE, Embase, the Cochrane Library, and Web of Science was performed, along with an analysis of international guidelines (ESHRE (European Society of Human Reproduction and Embryology), ASCO (American Society of Clinical Oncology), ASRM (American Society for Reproductive Medicine), NCCN (National Comprehensive Cancer Network), ESMO (European Society for Medical Oncology)). Keywords: “BRCA1,” “BRCA2,” “fertility preservation,” “oocyte cryopreservation,” “embryo cryopreservation,” “ovarian tissue cryopreservation,” “PGT-M,” “PARP inhibitors,” and “chemotherapy gonadotoxicity.”, in the period of 2005–2025. Studies with incomplete data, duplicates, reviews of low methodological quality, and case series with fewer than 10 observations were excluded. Priority was given to meta-analyses, RCTs, large cohorts, and consensus reports.

Results. The included studies included cancer patients before and after treatment, BRCA carriers with and without prophylactic strategies, and IVF/ICSI cohorts with cryopreservation. Alkylating agents and taxanes have been shown to increase the risk of premature ovarian failure, while GnRH agonists partially reduce the risk of ovarian toxicity. The efficacy of oocyte and embryo cryopreservation in BRCA-positive women is comparable to the population-based efficacy with optimized stimulation (GnRH antagonists, letrozole-containing protocols). Ovarian tissue cryopreservation is applicable in urgently needed patients but requires oncoprotective assessment. PGT-M ensures the selection of mutation-free embryos. Multidisciplinary pathways improve the timelines of referrals and the completion rate of fertility preservation programs

Conclusion. Early identification of BRCA-positive women and the integration of a gynecologic oncologist, reproductive specialist, and geneticist enable personalized strategy selection: gamete/embryo cryopreservation, ovarian tissue, pharmacoprotection, and PGT-M. Standardized stimulation protocols and therapy timing, long-term safety and fertility data, and economic access models are needed. Improvements in biotechnology and patient pathways improve reproductive outcomes and quality of life.

Keywords: BRCA1, BRCA2, fertility, breast cancer, ovarian cancer, cryopreservation, oncoreproductology, preimplantation genetic diagnosis, multidisciplinary approach, hereditary cancer, ovarian reserve, reproductive counseling

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3.1.6. Онкология, лучевая терапия
ОБЗОР

Сохранение фертильности у женщин с BRCA1/2-ассоциированными опухолями: современные подходы, международные рекомендации и мультидисциплинарная тактика

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РЕЗЮМЕ

Наследственные мутации в генах BRCA1/BRCA2 существенно повышают риск развития рака молочной железы и яичников у женщин репродуктивного возраста, формируя клинический и социально-экономический вызов из-за потери фертильности на фоне противоопухолевого лечения и профилактических вмешательств.

Цель исследования. Обобщить и проанализировать современные достижения, клинические рекомендации и нерешенные вопросы по сохранению репродуктивной функции у женщин-носителей мутаций BRCA1/BRCA2.

Материалы и методы. Выполнен систематизированный поиск в PubMed/MEDLINE, Embase, Cochrane Library и Web of Science, а также анализ международных руководств Европейского общества репродукции человека и эмбриологии (ESHRE), Американского общества клинической онкологии (ASCO), Американского общества репродуктивной медицины (ASRM), Национальной комплексной онкологической сети (NCCN), Европейского общества медицинской онкологии (ESMO). Ключевые слова: «BRCA1», «BRCA2», «fertility preservation», «oocyte cryopreservation», «embryo cryopreservation», «ovarian tissue cryopreservation», «PGT-M», «PARP inhibitors», «chemotherapy gonadotoxicity». Период: 2005–2025 гг. Исключались работы с неполными данными, обзоры низкого методологического качества, серии случаев <10 наблюдений; приоритет отдавался метаанализам, RCT, крупным когортам и консенсусам.

Результаты. Включенные исследования охватывали онкологических пациенток до начала лечения и после него, носительниц BRCA с профилактическими стратегиями и без них, а также когорты ЭКО/ИКСИ с криоконсервацией. Показано, что алкилирующие агенты и таксаны повышают риск преждевременной недостаточности яичников, тогда как агонисты ГнРГ частично снижают риск овариальной токсичности. Эффективность криоконсервации ооцитов и эмбрионов у BRCA сопоставима с популяционной при оптимизации стимуляции (антагонисты ГнРГ, летрозол-содержащие протоколы). Криоконсервация овариальной ткани применима у срочных пациенток, но требует онкобезопасной оценки. PGT-M обеспечивает отбор эмбрионов без мутации. Мультидисциплинарные маршруты повышают своевременность направления и долю завершенных программ сохранения фертильности.

Заключение. Ранняя идентификация носительниц BRCA и интеграция онкогинеколога, репродуктолога и генетика обеспечивают персонализированный выбор стратегии: криоконсервация гамет/эмбрионов, овариальная ткань, фармакопротекция, PGT-M. Необходимы стандартизованные протоколы стимуляции и тайминга относительно терапии, долгосрочные данные о безопасности и деторождениях, а также экономические модели доступа. Совершенствование биотехнологий и маршрутизация пациентов улучшают репродуктивные исходы и качество жизни.

Ключевые слова: BRCA1, BRCA2, фертильность, рак молочной железы, рак яичников, криоконсервация, онкорепродуктология, преимплантационная генетическая диагностика, мультидисциплинарный подход, наследственный рак, овариальный резерв, репродуктивное консультирование

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BACKGROUND

BRCA mutations are hereditary variants in the BRCA1 (Breast Cancer Susceptibility Gene 1) and BRCA2 (Breast Cancer Susceptibility Gene 2) genes, which play a fundamental role in maintaining genomic stability through highly efficient homologous recombination-mediated DNA double-strand break repair mechanisms [1]. Dysfunction of these genes due to pathogenic variants leads to a significant decline in DNA repair capacity, which, in turn, markedly increases the lifetime risk of developing malignant neoplasms, most notably breast cancer (BC) and ovarian cancer (OC). According to current data, carriers of pathogenic BRCA1 variants have an estimated lifetime BC risk of 65–80 % and OC risk of up to 40–60 % [2], while for BRCA2 mutation carriers these estimates are 45–60 % and 10–20 %, respectively. Importantly, hereditary predisposition associated with BRCA1/2 mutations occurs in both women and men; however, the clinical impact is significantly greater in women due to their substantially higher baseline risk for associated cancers [3].

Identification of a pathogenic BRCA1/2 mutation warrants referral for specialized genetic counseling, which encompasses several key objectives. Primarily, counseling aims to provide the patient and her at-risk relatives with information on mutation-associated cancer risks, the molecular characteristics of the defect, as well as contemporary strategies for individualized surveillance, prevention, and early detection of malignancies. Additionally, when hereditary cancer predisposition is confirmed, counseling supports personalized therapeutic planning, including the discussion of risk-reducing surgical options. An essential component of counseling is evaluation of reproductive considerations and discussion of the risk of transmitting the mutation to future offspring, which requires collaboration with reproductive specialists and assessment of fertility preservation options [4].

The issue of fertility preservation is particularly relevant for young women of reproductive age who, due to their BRCA mutation status and early cancer diagnosis, may require intensive surgical treatment and/or aggressive systemic therapy. Systemic antineoplastic treatment, as well as risk-reducing salpingo-oophorectomy, is associated with a high risk of ovarian reserve depletion and premature menopause, thereby significantly limiting reproductive potential [5]. Hence, timely and comprehensive discussion of fertility preservation options must begin at the time of diagnosis, prior to initiation of anticancer treatment.

With the increasing number of identified BRCA mutation carriers, advancements in molecular genetic testing, and expanding opportunities in assisted reproductive technologies (ART), fertility preservation has gained substantial clinical and social significance [6]. Addressing this issue requires a multidisciplinary approach that integrates medical, ethical, legal, and psychological aspects, along with further research aimed at improving management strategies and quality of life for young BRCA-positive patients who are facing treatment with potential gonadotoxic effects [7].

International guidelines on fertility preservation in patients with BRCA1/2 mutations

The issue of fertility preservation in women with BRCA1/2 mutations is reflected in multiple international guidelines, including those of the European Society of Human Reproduction and Embryology (ESHRE)[1], the American Society of Clinical Oncology (ASCO)[2], the American Society for Reproductive Medicine (ASRM)[3], as well as leading oncological and genetic professional societies such as the European Society for Medical Oncology (ESMO)[4], the National Comprehensive Cancer Network (NCCN)[5], the International Federation of Gynecology and Obstetrics (FIGO)[6], the European Society of Human Genetics (ESHG)[7], and the American College of Medical Genetics and Genomics (ACMG)[8].

[1] European Society of Human Reproduction and Embryology (ESHRE) [Internet]. Available at: https://www.eshre.eu – *Editorial note.*
[2] American Society of Clinical Oncology (ASCO) [Internet]. Available at: https://www.asco.org – *Editorial note.*
[3] American Society for Reproductive Medicine (ASRM) [Internet]. Available at: https://www.asrm.org – *Editorial note.*
[4] European Society for Medical Oncology (ESMO) [Internet]. Available at: https://www.esmo.org – *Editorial note.*
[5] National Comprehensive Cancer Network (NCCN) [Internet]. Available at: https://www.nccn.org – *Editorial note.*
[6] International Federation on Gynecology and Obstetrics (FIGO) [Internet]. Available at: https://www.figo.org – *Editorial note.*
[7] European Society of Human Genetics (ESHG) [Internet]. Available at: https://www.eshg.org/home – *Editorial note.*
[8] American College of Medical Genetics and Genomics (ACMG) [Internet]. Available at: https://www.acmg.net – *Editorial note.*

The modern multidisciplinary approach requires not only individualized reproductive preservation strategies, but also careful consideration of the specific genetic risks within this patient population [8].

According to the updated ESHRE and ASCO guidelines, all women of reproductive age with confirmed BRCA1/2 mutations, or those at high risk of carrying such mutations due to a burdened family history, must receive timely counseling on the possibilities and limitations of fertility preservation strategies. ASCO emphasizes that fertility preservation counseling should be offered to all patients before the initiation of potentially gonadotoxic therapy, regardless of the woman's ultimate decision regarding the use of preserved gametes or tissues in the future [9].

ESHRE highlights the importance of genetic counseling prior to *in vitro* fertilization (IVF) procedures and oocyte/embryo cryopreservation, as well as discussing the option of preimplantation genetic testing for monogenic disorders (PGT-M) in order to prevent transmission of the mutation to offspring. Both societies also recommend considering oocyte or embryo cryopreservation as the standard and most effective method, whereas ovarian tissue cryopreservation may be proposed only in exceptional cases with mandatory evaluation of the patient's oncological status and the potential risk of reimplanting tissue carrying a pathogenic mutation. ASRM also underscores the limitations of using autotransplanted ovarian tissue specifically in BRCA1/2 mutation carriers due to a theoretically increased risk of malignancy or micro-metastatic disease [10].

Several consensus documents (NCCN, ESMO) emphasize the necessity of integrating fertility preservation counseling into the decision-making process for oncological treatment planning, as well as ensuring multidisciplinary collaboration between oncologists and reproductive specialists.

Fertility preservation protocols should be considered for all reproductive-age patients prior to the initiation of chemotherapy or radiotherapy that may have a gonadotoxic effect, and also before prophylactic bilateral oophorectomy or adnexectomy recommended for BRCA mutation carriers as a preventive measure after the completion of childbearing. It is critically important not to miss the window of opportunity between diagnosis and the start of treatment.

Women carrying BRCA1/2 mutations differ not only in their baseline strategies of cancer treatment and prevention, but also in fertility preservation approaches. This patient group frequently demonstrates a reduced ovarian reserve already at the time of diagnosis, which necessitates early counseling and rapid decision-making regarding fertility preservation.

Methods for preserving reproductive potential in BRCA-associated diseases

Modern oncofertility offers a number of effective strategies for fertility preservation in women with BRCA mutations, which is particularly relevant given the unfavorable prognosis for natural reproductive function when aggressive treatment is required. The choice of method is individualized based on the oncological diagnosis, the time available before starting primary treatment, patient age, ovarian reserve, and reproductive plans [11].

Oocyte cryopreservation is a modern and widely accepted method of fertility preservation for women of reproductive age. The procedure involves controlled ovarian stimulation with gonadotropins, followed by transvaginal ultrasound-guided retrieval of mature oocytes and vitrification (ultra-rapid freezing), which ensures maximum preservation of the structural and functional potential of oocytes during long-term storage. This method is preferable for patients without a permanent partner or those not ready for fertilization at the time of diagnosis. Oocyte cryopreservation is considered effective and safe, and it is not associated with an increased risk of malignant transformation in BRCA mutation carriers [12].

In a study by Cobo A. et al., a comparative analysis of oocyte cryopreservation by vitrification was conducted in two groups: 5289 healthy women and 1073 women with cancer. Significant differences

were found between the cohorts. The post-thaw oocyte survival rate in healthy women was 91.4 %, whereas in cancer patients it was substantially lower at 81.2 %. Clinical pregnancy rates also differed: 65.9 % among healthy women versus 42.8 % among oncologic patients. The authors showed that in women aged up to 35 years inclusive, reproductive outcomes were significantly higher in the healthy cohort; however, after age 35 no statistically significant differences were observed, suggesting the dominant influence of age in the older group [13].

Regarding patients carrying BRCA1/2 mutations, a meta-analysis by Corrado G. et al. included six studies assessing mutation impact on fertility preservation outcomes. A total of 1848 patients were analyzed, 265 of whom carried BRCA mutations. Results were inconsistent: several studies reported reduced ovarian reserve and poorer response to stimulation, reflected by fewer retrieved oocytes and embryos, while others found no significant differences between carriers and non-carriers [14].

Another study by Corrado G. et al. compared *in vitro* maturation (IVM) outcomes between 57 patients with BRCA-mutation carriers and 277 controls. No significant differences in oocyte maturation were found. The mean maturation rate was 68.4 % in BRCA-positive patients versus 71.2 % in the control group (p = 0.287). Oocyte morphology quality was also comparable (82.1 % vs 84.5 %, p = 0.412). Subgroup analysis revealed no impact of mutation type (BRCA1 vs BRCA2) or age. Time to reach metaphase II was similar in both groups (24–26 hours), suggesting that BRCA mutations may not impair *in vitro* maturation, which is encouraging for fertility preservation programs [14].

Embryo cryopreservation is the method of choice for patients with a stable partner and/or defined reproductive plans. Embryos created via IVF are cryopreserved and stored until pregnancy is desired. This approach demonstrates the highest efficiency among assisted reproductive technologies but may require additional time, which is not always feasible in urgent cancer treatment settings [15, 16].

Ovarian tissue cryopreservation is an innovative and rapidly developing method, suitable for patients requiring immediate treatment or those for whom controlled stimulation is contraindicated. The technique involves laparoscopic retrieval of cortical ovarian tissue followed by cryostorage and potential autotransplantation after treatment. This may restore both fertility and endocrine function. However, in BRCA mutation carriers, the risk of reintroducing malignant cells remains a concern. Therefore, international guidelines consider ovarian tissue cryopreservation a limited option that requires careful individualized risk assessment and multidisciplinary counseling [17].

Use of gonadotropin-releasing hormone agonists (GnRHa) during chemotherapy is aimed at temporary suppression of ovarian function, reducing cytotoxic damage to follicles. GnRHa induce transient suppression of the hypothalamic-pituitary-gonadal axis, placing ovaries in a pharmacological “resting” state. Multiple meta-analyses confirm reduced risk of premature ovarian insufficiency and higher rates of spontaneous post-treatment pregnancy. Nevertheless, this method does not replace established cryopreservation protocols and is considered an adjunctive gonadoprotective strategy [18, 19].

Another option is the use of donor oocytes, which is considered a reserve strategy for women who have lost ovarian reserve due to treatment or who initially have a low likelihood of successful IVF with their own oocytes. Donor oocyte use enables the possibility of gestation and childbirth even in cases of complete loss of endogenous fertility, although it is associated with psychological, ethical, and legal considerations that must be discussed with prospective parents. In addition, the use of donor gametes fully eliminates the risk of transmitting a BRCA mutation to the offspring.

The development and creation of artificial or bioengineered ovaries represent one of the most promising areas of research. An artificial ovary is defined as a three-dimensional biocompatible scaffold populated with the patient’s own follicular cells and immature oocytes, or with donor-derived material.

Such constructs theoretically address the risk of malignant cell reintroduction and may allow restoration of both endocrine function and fertility. In the future, novel molecular targets for the pharmacological protection of the female reproductive system are being explored, and new agents are being developed that can selectively block apoptotic signaling pathways or exert cytoprotective effects directly on ovarian follicles [20].

It should be emphasized that timely referral of the patient to a reproductive specialist and the selection of an individualized fertility preservation strategy are essential components of the modern multidisciplinary approach to the management of women with BRCA mutations, helping to safeguard their reproductive autonomy and improve long-term quality of life.

Genetic screening for BRCA mutations to prevent transmission to offspring

The BRCA1 and BRCA2 genes are highly penetrant tumor suppressors. Mutations in these genes are inherited in an autosomal dominant pattern. This means that each pregnancy of a woman carrying a pathogenic mutation in BRCA1 or BRCA2 is associated with a 50 % risk of transmitting this mutation to the child, regardless of the child's sex. The mutations are transmitted equally through both the maternal and paternal lineage; the hereditary predisposition is determined by a homologous defective allele, and even a single altered gene copy substantially increases the risk of developing BRCA-associated malignancies [21].

Currently, a key tool for reducing the risk of transmitting a pathogenic BRCA mutation to offspring is preimplantation genetic testing for monogenic disorders (PGT-M). This method is used within IVF programs. At the blastocyst stage, biopsy of several trophectoderm cells is performed, followed by DNA analysis, which allows precise identification of the presence or absence of BRCA1/2 mutations in each embryo [22]. Based on these results, only embryos that have not inherited the mutation are selected for uterine transfer [23].

Another approach is analysis of BRCA mutations using umbilical cord blood. Cord blood is considered a potential source of DNA, especially in the neonatal period. It contains a sufficient number of nucleated cells to isolate genetic material for screening of known hereditary mutations, including BRCA1 and BRCA2. This approach can be applied, for example, to determine mutation carriage in a newborn as part of family genetic evaluation or by neonatology indications. In addition, cord blood testing has the advantage of non-invasive collection immediately after birth, ensuring both high safety and diagnostic value [24, 25].

Reproductive counseling for patients carrying BRCA mutations should include a discussion of the risk of transmitting the pathogenic allele to offspring, and information about modern possibilities of preimplantation genetic testing as an ethically and clinically meaningful method of interrupting autosomal-dominant transmission of cancer predisposition [26]. This provides families with the right to make an informed decision regarding the planning of healthy offspring.

Reproductive loss associated with BRCA-related therapy

Comprehensive treatment of malignant diseases in patients with BRCA mutations has a pronounced negative effect on reproductive function, primarily reflected in the state of the ovarian reserve and hormonal activity. Major components of contemporary anticancer therapy – chemotherapy, radiotherapy, and surgery – individually and collectively significantly increase the risk of premature ovarian insufficiency.

Chemotherapy is one of the leading causes of ovarian reserve depletion. Agents used in the treatment of breast and ovarian cancer have marked gonadotoxic effects. Toxicity is driven by direct damage to proliferating granulosa cells and induction of apoptosis in growing follicles [27]. The development of treatment-related amenorrhea (TRA) in oncology patients is determined by several interrelated factors, among which the most significant are patient

age, baseline ovarian reserve, and the presence of pathogenic BRCA variants.

Chemotherapeutic regimens containing cyclophosphamide, doxorubicin, and taxanes demonstrate high gonadotoxicity, inducing TRA in 83.6 % of cases, accompanied by a characteristic decline in anti-Müllerian hormone (AMH) levels, which in many patients shows partial recovery within three years after therapy completion. Age-stratified analysis of TRA risk reveals a clear correlation: patients over 35 years have a threefold higher probability of developing TRA. Recovery of reproductive function after TRA is generally favorable: menstrual cycles resume in about 70 % of patients within one year and in 90 % within two years after treatment. BRCA mutation carriers demonstrate additional features of reproductive aging, including earlier menopause by 1–3 years compared to the general population [28].

The baseline AMH level in BRCA carriers is of particular interest for ovarian reserve assessment. Numerous studies have attempted to determine whether women with germline pathogenic BRCA1/2 variants and diagnosed breast cancer have lower baseline AMH and/or a reduced response to controlled ovarian stimulation compared to non-carriers. However, available data show significant heterogeneity, contributing to difficulties in forming uniform clinical recommendations [29].

A major dilemma in contemporary oncology is the potential conflict between preserving fertility in young women and the urgency of initiating treatment. Fertility preservation procedures require time for planning and implementation and may delay chemotherapy, which could theoretically worsen oncological outcomes.

A retrospective study by Greer A. et al. involving 272 patients with stage 0–III breast cancer demonstrated that fertility preservation in 123 patients caused a mean treatment initiation delay of 10 days compared with controls (149 patients). Despite this delay, oncological outcomes were comparable: progression-free survival at three years was 85.4 % vs. 79.4 % (p = 0.411) and overall survival was 95.5 % vs. 93.5 % (p = 0.854) [30].

In modern reproductive practice, Random-Start ovarian stimulation is used, enabling initiation at any point in the menstrual cycle, which is particularly relevant for cancer patients requiring urgent treatment. In women with hormone-receptor–positive breast cancer, stimulation is combined with aromatase inhibitors to avoid supraphysiologic estradiol levels that may adversely affect disease progression. Thus, aromatase inhibitors are started concurrently with gonadotropins and continued for seven days after oocyte retrieval until estradiol falls below 50 pg/mL. The “double trigger” technique (combined hCG + GnRH agonist) is frequently used to optimize final oocyte maturation, especially in patients at risk for poor ovarian response due to BRCA-associated reduced reserve [31].

Hormone therapy usually does not directly damage the ovaries or suppress ovarian reserve. However, the standard treatment duration of 5–10 years significantly reduces reproductive potential due to age-related decline in fertility, limiting the likelihood of future pregnancy after therapy completion [32].

Radiation therapy, particularly irradiation of the pelvic or abdominal region, also adversely affects reproductive function. The ovaries are highly sensitive to ionizing radiation, and even relatively low doses may lead to irreversible follicular reserve damage. Ovarian dysfunction manifests as decreased estrogen levels, elevated FSH, and clinical signs of iatrogenic menopause. The nature and severity of damage depend on radiation dose and the volume of irradiated tissue [33, 34].

Surgical treatment of BRCA-associated malignancies often involves radical procedures such as bilateral oophorectomy (removal of the ovaries) or salpingo-oophorectomy (removal of the ovaries and fallopian tubes). Even when performed prophylactically, such procedures result in the immediate loss of ovarian function with the development of iatrogenic menopause, secondary amenorrhea, and inability to conceive naturally. Additionally, prophylactic mastectomy deserves particular attention. Although this intervention does not directly lead to loss of reproductive capacity, the breast plays

an important biological role in human reproduction. It is not only a symbol of femininity but also a key organ for breastfeeding, which directly affects mother–infant bonding, nutrition, and neonatal immune protection [35]. The loss of breast tissue is also frequently associated with significant psycho-emotional distress related to body-image alteration and perception of feminine identity, which may negatively influence future decisions regarding motherhood. The question of the feasibility and timing of preventive surgical interventions in women with identified pathogenic BRCA1/BRCA2 mutations, who have not yet completed their reproductive plans and have no clinical signs of malignancy, remains the subject of active scientific discussion and requires an individualized approach. Prophylactic mastectomy in carriers of pathogenic BRCA1/BRCA2 variants reduces the lifetime risk of breast cancer by approximately 90–95 %. Prophylactic bilateral salpingo-oophorectomy reduces the risk of ovarian and fallopian tube cancer by more than 80–90 % [36].

International clinical guidelines, based on consensus statements of leading oncological and genetic societies (NCCN, ESMO, SGO, ASCO, etc.), confirm that prophylactic bilateral mastectomy and/or bilateral salpingo-oophorectomy significantly reduce the risk of breast and ovarian cancer in BRCA mutation carriers. However, the optimal timing of such procedures, particularly for young women, remains a matter of debate. Early preventive surgery undoubtedly minimizes cumulative cancer risk but is associated with pronounced consequences for quality of life, fertility potential, psycho-emotional well-being, and hormonal balance. Several guidelines (NCCN, ESMO) indicate the possibility of delaying preventive surgery until childbearing is completed–typically until 35–40 years of age for BRCA1 carriers and 40–45 years for BRCA2 carriers, provided that stringent oncological surveillance is maintained [37].

Thus, all major components of the comprehensive treatment of malignant neoplasms in patients with BRCA mutations are associated, to varying degrees, with a risk of significant impairment of reproductive function. This necessitates early discussion, even before the initiation of specific anticancer treatment, of fertility preservation and individualized reproductive planning for each patient in this category.

CONCLUSION

The issue of fertility preservation in women with pathogenic BRCA1 and BRCA2 mutations is one of the most critical challenges at the intersection of oncology, reproductive medicine, and medical genetics. These patients belong to a population with an extremely high oncological risk, and modern treatment modalities – including surgical interventions, chemotherapy, and radiotherapy – are associated with the threat of ovarian reserve depletion and the development of premature menopause, which effectively leads to infertility, disrupts hormonal balance, and reduces long-term quality of life.

Comprehensive assessment of reproductive risks, thorough genetic counseling, and targeted patient education on available fertility preservation options constitute essential components of a multidisciplinary management approach. Current clinical guidelines emphasize the critical importance of early referral to reproductive specialists: timely optimization of diagnostic and therapeutic planning enables the implementation of effective oocyte or embryo cryopreservation strategies, providing the possibility of having a biologically related child even after completion of anticancer therapy. Furthermore, the active integration of preimplantation genetic testing for monogenic disorders (PGT-M) significantly reduces the risk of transmitting pathogenic BRCA mutations to offspring, which is ethically and clinically important for families with a burdened hereditary cancer history. A key direction for further development includes the improvement of biotechnological and pharmacological approaches to ovarian protection, including the creation of artificial ovaries and pharmacologic prevention of gonadotoxic effects associated with systemic therapy. The efficacy and long-term safety of these innovative strategies are currently the focus of intense scientific investigation,

highlighting the need for continued fundamental and clinical research.

Another essential task is the optimization of decision-making regarding risk-reducing surgeries (mastectomy, salpingo-oophorectomy) in BRCA mutation carriers who have not yet developed cancer. Current data support the possibility of delaying radical preventive interventions until after completion of reproductive plans, combined with dynamic specialized surveillance, which allows preservation of reproductive potential without a clinically significant increase in oncological risk, provided that individualized screening protocols are strictly followed.

Thus, the practical implementation of a multidisciplinary and personalized approach to the management of women with BRCA1/2 mutations, aimed not only at the prevention and timely treatment of malignant tumors but also at the preservation and restoration of reproductive health, must become a priority in modern clinical practice. Only such a comprehensive strategy – integrating medical, genetic, psychological, and ethical-legal aspects – can substantially improve quality of life and ensure informed reproductive decision-making for patients, supporting their right to motherhood and the well-being of future generations.

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REVIEW

Application of dendritic cell vaccine immunotherapy in gynecologic malignancies

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ABSTRACT

The development of antitumor strategies aimed at restoring systemic and local immune regulation is considered one of the most promising directions. Technologies based on dendritic cell vaccines (DCVs), characterized by minimal toxicity and alignment with fundamental immunological mechanisms of antitumor resistance, are of particular interest.

Purpose of the study. Is to evaluate the effectiveness of immunotherapeutic approaches for gynecologic malignancies using DCVs and to outline promising directions for further development.

Materials and methods. A literature search was conducted in the bibliographic registers MEDLINE, ClinicalTrial.gov., eLIBRARY and CyberLeninka, using the search systems PubMed, Google Scholar. The vast majority of the identified sources are indexed in Scopus and Web of Science. The review includes more than 60 publications in Russian and English, over 50 % of which were published within the past five years.

Results. The analysis summarizes data on the clinical outcomes of DCV-based therapy in advanced cervical cancer, endometrial cancer, and ovarian cancer. Reported beneficial effects include temporary disease stabilization, improved overall survival and quality of life in advanced malignancies, enhanced efficacy of subsequent chemotherapy, and occasional cases of partial or complete remission. The review also addresses potential reasons for the limited efficacy of DCVs, as well as possible combinations of this technology with other immunotherapeutic modalities and traditional anticancer treatments. The currently modest therapeutic effectiveness of DCVs in gynecologic cancers may be attributed both to the insufficient maturity of the technology and to inherent mechanisms of tumor immune evasion.

Conclusion. The therapeutic potential of DCVs has not yet been fully realized. Advances in immunotherapy, molecular biology, nanotechnology, and strategies for activating systemic and local antitumor resistance mechanisms provide a foundation for defining future research priorities aimed at improving the efficacy of DCVs as an important component of multimodal treatment for gynecologic malignancies.

Keywords: immunotherapy, dendritic cell vaccines, tumor-specific immune responses, cervical cancer, endometrial cancer, ovarian cancer

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3.1.6. Онкология, лучевая терапия

ОБЗОР

Использование методов иммунотерапии с применением дендритноклеточных вакцин в онкогинекологии

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РЕЗЮМЕ

Разработка методов противоопухолевого лечения, направленных на восстановление системной и локальной иммунной регуляции, рассматривается в качестве наиболее перспективной стратегии в современной онкологии. Большой интерес представляют технологии с использованием дендритноклеточных вакцин (ДКВ), отличающиеся отсутствием токсичности и соответствующие фундаментальным иммунным механизмам противоопухолевой резистентности.

Цель исследования. Изучить эффективность методов иммунотерапии онкогинекологических заболеваний с использованием ДКВ и перспективные направления их развития

Материалы и методы. Проведен поиск литературы в библиографических реестрах MEDLINE, ClinicalTrial.gov., eLIBRARY и КиберЛенинка, с использованием поисковых систем PubMed, Google Scholar. Подавляющее большинство источников включены в базы данных Scopus и WoS. В настоящем обзоре рассмотрено более 60 работ на русском и английском языках, более 50 % которых опубликованы в течение последних пяти лет.

Результаты. Проанализированы сведения о результатах применения ДКВ при терапии распространенных форм рака шейки матки, рака эндометрия и рака яичников. Положительные эффекты ДКВ включают временную стабилизацию заболевания, увеличение продолжительности и качества жизни при распространенном злокачественном процессе, повышение эффективности химиотерапии после ДКВ, отдельные случаи частичной и полной ремиссии. Рассматривают причины недостаточной эффективности ДКВ, варианты сочетания данной технологии с другими методами иммунотерапии и традиционным противоопухолевым лечением. Невысокая эффективность ДКВ в отношении онкогинекологических заболеваний на современном этапе может быть обусловлена недостаточной разработанностью технологии и объективными сложностями преодоления механизмов уклонения опухоли от иммунного надзора.

Заключение. Потенциал ДКВ как метода противоопухолевого лечения в настоящее время не реализован. Анализ современных достижений в области иммунотерапии, молекулярной биологии, нанотехнологий и подходов к активизации системных и локальных механизмов противоопухолевой резистентности позволяет определить направление дальнейших исследований, нацеленных на повышение эффективности ДКВ как важного компонента комплексного лечения онкогинекологических заболеваний.

Ключевые слова: иммунотерапия, дендритноклеточные вакцины, опухолеспецифические иммунные реакции, рак шейки матки, рак эндометрия, рак яичников

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INTRODUCTION

The ability of malignant cell systems to suppress immune surveillance, together with their unrestricted proliferative activity, distinct metabolism that ensures preferential access to the host's energy and biosynthetic resources, and the loss of contact inhibition, represents one of the most significant pathogenetic characteristics of malignant tumors [1]. Despite the widespread introduction and ongoing refinement of radiation therapy, systemic anticancer treatments, and the expanding panel of plant-derived cytotoxic agents (including taxanes and others) [2], the search for therapeutic approaches aimed at restoring systemic and local immune regulation of cellular life cycles and tissue development is increasingly regarded as one of the most promising strategies in fundamental and clinical oncology. In this context, the development of novel and effective methods of tumor immunotherapy continues to generate considerable interest and high expectations.

Purpose of the study is to evaluate the effectiveness of immunotherapeutic approaches for gynecologic malignancies using dendritic cell vaccines and to outline promising directions for further development.

General background on dendritic cell vaccines

It is well established that various immune system cell types are capable, in one way or another, of directly damaging malignantly transformed cells. Such reactions have been described for natural killer cells [3], B-lymphocytes [4], neutrophils [5], monocytes [6], and tissue basophils [7]. Activation of these cells can be achieved not only through cytokines but also through systemic regulatory influences on the central components of the integrated neuroendocrine-immune system [8], mediated by factors of various origins, including phytoimmunomodulators [2, 9], weak electromagnetic radiation, and biologically active fluids [10]. Approaches aimed at mobilizing these processes are classified as nonspecific immunotherapy and hold significant theoretical and practical value.

At present, multiple directions in tumor immunotherapy are being developed, focusing on direct or indirect stimulation of the effector arm of the immune system, whose activity is suppressed under conditions of malignant growth. Historically, the first variant of tumor immunotherapy involved bacterial vaccines [11], initiated over a century ago with William Coley's vaccine, which was successfully applied to soft-tissue sarcomas. A widely used contemporary immunotherapeutic approach is antibody-dependent cytotoxicity, implemented through monoclonal antibody therapy capable of enhancing antitumor cytotoxic responses via Fc-receptor interactions of not only T-lymphocytes but also other immune system elements [12]. Additional strategies include cytotoxic lymphocytes activated *in vitro* through various methods [13], as well as tumor-infiltrating lymphocytes extracted from a patient's tumor tissue, expanded *ex vivo*, and reinfused into the same patient (TIL therapy) [14].

More recent immunotherapy modalities demonstrating high clinical efficacy in recent years include the use of genetically modified T-cells engineered to express CAR-T or TCR-T receptors, which enhance their ability to selectively destroy tumor cells [15]. CAR-T therapy is primarily applied in hematologic malignancies and targets surface antigens, whereas TCR-T approaches can be effective in selected solid tumors by recognizing intracellular antigens. For several cancers, favorable outcomes have been achieved through the inclusion of immune checkpoint inhibitors (ICIs) in multimodal treatment regimens [16].

However, the priority goal of tumor immunotherapy should be the ability to orchestrate robust tumor-specific immune responses – i. e., the activation of tumor antigen (TA) recognition and presentation, generation of highly active tumor-specific cytotoxic T-cells, and massive destruction of transformed cells through apoptosis, enabling rapid and economical clearance of cellular debris without the toxic sequelae characteristic of necrosis [17]. In this context,

methods employing dendritic cell vaccines (DCVs) attract particular attention, since dendritic cells (DCs) are the most effective antigen-presenting components of the immune system. DCs originate from bone marrow hematopoietic progenitors, form reticular cellular networks widely distributed throughout the organism, and play a key role in immune surveillance [18]. These professional antigen-presenting cells require minimal amounts of antigen to stimulate cytotoxic lymphocyte proliferation and can induce lymphoproliferative responses using antigen quantities approximately 100-fold lower than those required for macrophages or B-lymphocytes. Importantly, DCs can migrate into lymph nodes and initiate the formation of tumor-specific cytotoxic T-cells [19].

Mobilization of DCs occurs under the influence of tumor antigens, which are internalized by phagocytosis; DCs then migrate to the lymph nodes, where the antigens are processed into peptides and presented in complex with major histocompatibility complex (HLA) molecules to T-cells.

In addition to their pathogenetic mechanism of action, personalized DCVs are characterized by low toxicity and relative technical simplicity of production [18, 19]. DCV preparation generally includes: (1) isolation of precursor cells from blood (monocytes) or bone marrow (CD34+ hematopoietic stem cells); (2) stimulation of their maturation and differentiation into activated DCs using cytokine cocktails and autologous tumor antigens *ex vivo*; and (3) reinfusion of mature DCs into the patient. After administration, activated DCs migrate to lymph nodes and present tumor antigens to CD4+ and CD8+ T-cells, initiating an adaptive immune response. A less common approach involves *in vivo* expansion of circulating DCs via hematopoietic growth factors such as Flt3L and granulocyte colony-stimulating factor (G-CSF).

The use of precursor populations rather than endogenous mature DCs is explained by the heterogeneity and immunosuppressive phenotype of endogenous DCs in the tumor microenvironment, as well as the relative ease of obtaining enough monocytes and bone-marrow progenitors [20]. A major challenge is the selection of tumor antigens and maturation cocktails to generate highly immunogenic DCs optimally targeted toward malignant cells. Mature DCs differ significantly from immature forms in their molecular profiles, morphology, and functional activity [21]. To enhance immunogenicity, a variety of adjuvants are used, including bacterial and viral components, gangliosides, recombinant proteins, immunogenic peptides, anti-idiotype monoclonal antibodies, mucins (notably MUC1), genetically or chemically modified tumor cells, tumor lysates, and others [18–20]. Adjuvant selection depends on tumor type and localization. Common elements across DCV protocols include the route and schedule of administration (intradermal or subcutaneous, at least 3–4 injections at 1–2-week intervals) and typical dosing (10^6–10^7 DCs per injection).

Despite the clear objective of achieving complete tumor regression through DC-based activation of antitumor immunity, this goal has not yet been realized. Complete responses have been most frequently observed in melanoma, one of the most aggressive and highly immunogenic tumors [19, 22], usually accounting for no more than 3–7 % of cases in specific cohorts. More favorable long-term outcomes – complete remission in one-third of melanoma patients – were reported with DCV administration after primary tumor resection and removal of macrometastases, with follow-up exceeding six years [23]. Studies of DCV efficacy in cutaneous melanoma have also been conducted by domestic researchers [24, 25]. Complete tumor regression in other malignancies has been documented far less frequently [26].

To date, despite their excellent safety profile and minimal toxicity, current DCV formulations have not demonstrated sufficiently strong or consistent antitumor activity [19, 27]. Limited efficacy is attributed primarily to the low immunogenicity of tumor antigens used for DC stimulation, immunosuppressive influences of the tumor microenvironment, and negative selection of cytotoxic T-cells in the thymus.

To mitigate these obstacles and improve therapeutic outcomes, DCVs are increasingly combined with other immunotherapeutic modalities. Promising combinations include DCVs with ICIs, TIL therapy, and TCR/CAR-T-based cellular therapies [18].

Dendritic cell vaccines in the immunotherapy of gynecologic malignancies

The question of whether tumor immunotherapy can be effectively applied to gynecologic cancers carries considerable scientific and practical significance. Persistently high incidence and mortality rates, which have shown little reduction over more than a decade, together with the limited therapeutic effectiveness for women with malignant tumors of the reproductive system, remain among the most urgent challenges in modern oncology. Although cervical cancer (CC) is the only gynecologic malignancy with a well-established etiologic factor – oncogenic human papillomavirus (HPV) subtypes – and despite the existence of effective screening programs and primary/secondary prevention strategies, the incidence of CC continues to rise both in Russia and worldwide. A trend toward a younger age at diagnosis has been observed, and first-year mortality exceeds one-tenth of newly diagnosed cases [28, 29]. This situation is further complicated by insufficiently optimized protocols for preoperative radiotherapy, which represents one of the main treatment modalities for patients with advanced CC [30].

Endometrial cancer (EC) is the most prevalent gynecologic malignancy in developed countries. In Russia, EC accounted for 8 % of all newly diagnosed cancers among women in 2023, exceeding the incidence of cervical cancer and ovarian cancer (OC) by 1.8-fold or more [28]. The incidence of EC continues to increase due to population aging and obesity-related factors. The greatest therapeutic challenges arise in advanced-stage disease. Significant molecular-genetic and histological heterogeneity in EC results in substantial variations in clinical outcomes and prognosis, prompting the development of an additional molecular classification system as an essential prerequisite for creating algorithms for personalized treatment – an approach that remains insufficiently established to date [31].

Mortality associated with OC is the highest among gynecologic cancers [28, 32]. Platinum resistance – developing in 75–90 % of patients after repeated chemotherapy courses – rapid progression of metastases in the omentum and pelvic organs due to exfoliation of tumor cells into serous fluid, and the predominance of high-grade serous carcinoma, the most aggressive OC subtype, severely limit the chances of achieving even temporary disease control and account for the high lethality in this patient population.

Evidence of the immunogenicity of malignant tumors of the female reproductive system [33, 34] provides an additional rationale for pursuing effective immunotherapeutic approaches for gynecologic cancers, including those based on dendritic cell vaccines (DCVs).

Cervical cancer

At the present stage, dendritic cell vaccines (DCVs) are used only as an adjunct modality in the treatment of malignant tumors localized in the uterus, and their clinical application remains limited. In cervical cancer (CC), considerably more is known about the integration of passive immunotherapy approaches – particularly immune checkpoint inhibition [35] and TIL therapy [36] – into multimodal treatment regimens. These methods may exert a meaningful therapeutic effect in selected disseminated forms of CC.

In Russia, experimental and clinical studies involving DCVs for the treatment of CC were conducted at the National Medical Research Center of Oncology (Rostov-on-Don). A DCV was developed based on monocytes derived from the peripheral blood of CC patients, using GM–CSF, IL-4, and TNF-α for maturation, and loaded with HeLa cell lysate to generate mature activated DCs [37]. This vaccine was subse-

quently evaluated as part of a combined treatment strategy for CC patients with different extents of disease [38].

In five patients with CC stage T4aN1M1 (bladder and distal ureter invasion), bilateral nephrostomies, multiple distant metastases, severe endogenous intoxication, grade III anemia, and cachexia, DCV was administered with palliative intent as the only available treatment option. Disease stabilization for 6–12 months was achieved, with a mean overall survival of 14.8 months. Among eleven patients with progressive CC following standard therapy, DCV combined with palliative polychemotherapy (PCT) resulted in stabilization in approximately half of the cases. Progression-free survival and mean overall survival in this group reached 15.8 and 32 months, respectively. In three patients with CC T2bN1M0 whose tumors remained unresectable after standard chemoradiation, DCV administered alongside second-line PCT induced complete tumor regression. However, the authors noted that in 18 % of cases the DCV-based treatment produced no meaningful effect and failed to halt disease progression [39].

In incurable and progressive CC, DCV use resulted in significant improvements in quality of life compared with chemoradiation alone, largely owing to marked analgesic and anti-inflammatory effects. Pain relief typically occurred after 2–3 DCV administrations. Immunologic and biochemical indicators in DCV-treated patients demonstrated improved systemic homeostasis after at least six DCV cycles, including restoration of previously reduced NK-cell and CD8+ T-cell levels, increased Tm/Th0 ratios ("memory" / "naive" T-cells) among CD4+ and CD8+ subsets, and normalization of albumin functionality, medium molecular weight molecules, and blood redox parameters [39].

Earlier reports by Santin A. D. and colleagues from the University of Arkansas for Medical Sciences similarly described improved therapeutic outcomes and patient status following DCV administration in CC [40, 41]. Their work examined DCVs produced from monocytes stimulated with GM–CSF and loaded with HPV E6 and E7 oncoproteins – antigens frequently expressed in HPV-associated tumors. These oncoproteins were considered suitable targets for therapeutic vaccination against HPV-infected cancer cells. In a study of 18 patients with advanced CC, clinical benefit was observed in four cases: disease stabilization for one year in two patients, and complete tumor regression following PCT administered after vaccination in another two [40].

In a phase II study evaluating DCV in 14 patients with advanced or recurrent CC, stabilization was documented in five patients for up to eight months after four DCV administrations, accompanied by immunologic evidence of activated cytotoxic T-cell responses. The same report described a case of widespread chemoresistant CC with multiple pulmonary macrometastases, where repeated DCV administration produced prolonged stabilization exceeding one year and partial regression of a major lung metastasis. In another setting, DCV combined with low-dose recombinant IL-2 produced temporary disease control in two of four heavily pretreated CC patients with metastatic or recurrent disease, increasing survival from 5 to 13 months after treatment initiation.

In many of these studies, clinical responses correlated with delayed-type hypersensitivity reactions, activation of CD8+ cytotoxic T-cells, and other effector components of the antitumor immune response. The authors concluded that the limited efficacy of DCVs in advanced refractory CC is likely related to immunosuppressive effects of prior chemotherapy and radiotherapy, creating significant barriers to DCV effectiveness. They emphasized the need for trials in earlier stages of CC and earlier treatment windows.

A later trial investigated DCV in patients with CC stage Ib and IIa after radical surgery, using escalating doses of DCV generated through stimulation with recombinant HPV16/18 E7 antigens and keyhole limpet hemocyanin (KLH) as an immunologic marker. After five DCV doses administered at three-week intervals, all participants demonstrated CD4+ T-cell

and B-cell responses. The authors concluded that DCV was safe, immunogenic, and potentially beneficial for CC patients with limited tumor burden or high risk of recurrence [41]. This conclusion was partly supported by subsequent studies conducted at Shanghai University Hospital and Suzhou University Hospital [42]. In patients with squamous cell or adenocarcinoma of the cervix (mostly stage IIa or IIb), postoperative adjuvant treatment consisted of either cisplatin-based chemotherapy alone or chemotherapy combined with DCV. In the DCV group, the vaccine formulation included co-cultured DCs and T-killers rather than isolated antigens. The combined therapy yielded significantly improved immune parameters, a two-fold reduction in cumulative three-year recurrence rate, and an increase in three-year survival from 56.4 % to 80 %.

Isolated reports of successful treatment of disseminated CC with distant metastases following DCV – similar to the case reported by Santin A. D. et al. [40] – have also been documented by other researchers. One study from the Chennai Cancer Institute (India) described a complete clinical response after vaccination with DCs loaded with autologous tumor lysate followed by cisplatin chemotherapy, with no signs of recurrence for more than six years [43]. The reasons for such selective responsiveness to DCV-containing treatment regimens in advanced CC remain unclear.

Recent studies on DCVs for CC have focused on strategies to enhance their therapeutic efficacy, including identification of highly immunogenic CC antigens or methods to improve their presentation [44], development of nanoscale technologies to improve immune effector targeting within tumor tissue, and optimization of the CC tumor microenvironment [45]. At present, these investigations remain predominantly experimental.

Endometrial cancer

The use of dendritic cell vaccines (DCVs) in patients with endometrial cancer (EC) is currently even less common than in cervical cancer. Much more frequently, passive immunotherapy with immune checkpoint inhibitors (ICIs) is considered a promising treatment option for EC [35, 46], owing to the relatively high effectiveness of ICIs in this setting. It has been shown that EC with microsatellite instability (MSI-positive subtype) is highly sensitive to ICIs, with objective response rates to pembrolizumab exceeding 50 %. Even in MSI-negative EC, the use of pembrolizumab is considered appropriate when combined with the multikinase inhibitor lenvatinib.

The focus of active immunotherapy using DCVs on restoring fundamental defense mechanisms of immune surveillance, the high efficacy of tumor antigen (TA)-dependent cytotoxic T-cell responses (where preserved), and the favorable safety profile of DCVs have naturally attracted considerable interest among researchers developing antitumor strategies for EC. This interest has also been driven in part by the limited treatment options for uterine sarcomas and recurrent carcinomas of the uterus, particularly serous endometrial carcinoma [47, 48]. By 2014, fewer than ten studies on DCVs in EC had been published, each including only 1–6 patients [47]. The most systematic work in this area was conducted by Santin A. D. and colleagues at the University of Arkansas for Medical Sciences. In one study, the authors reported outcomes in a 65-year-old patient with progressive, chemoresistant serous endometrial carcinoma and hepatic metastases that increased significantly in size over the three weeks preceding treatment initiation [49]. After three DCV administrations at 3–4-week intervals, immunologic monitoring revealed signs of T-cell cytotoxic responses, while computed tomography demonstrated stabilization of liver metastases. The authors attributed this relatively modest effect to the inability of activated T-cells to adequately penetrate a bulky tumor mass.

Subsequently, the same group published data on the immunogenic effects of autologous DCs stimulated with tumor lysate, showing that DCVs were capable of inducing tumor-specific T-cell responses against autologous uterine cancer in three EC pa-

tients, although clinical efficacy was not evaluated in that study [50].

In work by Coosemans A. and colleagues from the Leuven Cancer Institute (Belgium), which used the Wilms' tumor gene 1 (WT1) product – a known immunogenic antigen in EC – as a TA, emphasis was likewise placed on the feasibility and safety of DCV use in EC rather than on clearly demonstrated clinical benefit [51, 52]. In a 46-year-old patient with terminal-stage serous EC, four weekly DCV injections were well tolerated, accompanied by a 2.5-fold increase in WT1-specific T-cells and a reduction in CA-125 levels. In a comprehensive review published in 2014 [47], the authors concluded that DCV-based immunotherapy in EC remained in its infancy due to insufficient knowledge of local and systemic immune features in this disease. At the same time, recognizing the evident negative impact of immunosuppressive elements within the tumor microenvironment on DCV efficacy, they proposed that the most promising immunotherapeutic strategy for EC might involve combining DCVs with ICIs.

It must be acknowledged that the deficit of knowledge regarding local and systemic immune processes in EC has not yet been overcome. This situation is further complicated by considerable molecular-genetic and histological heterogeneity, which underlies substantial variability in prognosis and hampers the development of personalized treatment algorithms [31, 33]. Analysis of the available literature suggests minimal progress in DCV-based immunotherapy for EC. International treatment guidelines for EC do not currently mention active immunotherapy as a therapeutic option [53]. At the same time, isolated reports have emerged describing combinations of chemotherapy (CT) and DCVs in EC. For example, investigators at the Radboud University Medical Center in Nijmegen (Netherlands) conducted an exploratory study evaluating carboplatin/paclitaxel combined with DCVs loaded with MUC1 and survivin in patients with metastatic EC [54]. Given the severity of disease, the primary positive endpoint was the ability to complete the full treatment schedule without severe complications. This endpoint was achieved in five of seven patients. Antigen-specific immune responses were documented in only two cases.

These findings indicate that DCVs may be a promising component of multimodal therapy in EC and support the need for further development of effective algorithms for their use.

Ovarian cancer

In ovarian cancer (OC), the gynecologic malignancy with the highest mortality and a pronounced tendency toward chemoresistance – the search for new strategies to inhibit tumor growth and induce regression is of particular urgency. As in CC and EC, ICIs are the most widely used immunotherapeutic agents in OC [35, 55]. Tumor sensitivity to PD-1/PD-L1 pathway inhibition is tightly linked to the presence of microsatellite instability; however, MSI-positive advanced OC accounts for less than 10 % of cases, a considerably lower proportion than in CC and EC [35]. This highlights the pressing need to explore additional immunotherapeutic approaches for OC.

A positive correlation between intratumoral densities of mature DCs and CD8+ cytotoxic T-lymphocytes and survival in advanced OC [34, 56] further strengthens interest in DCV-based strategies in this disease. Indeed, more DCV-related studies have been conducted in OC than in CC or EC.

As in most solid tumors, a complete characterization of the OC-associated TA repertoire is not yet available, but several antigens with significant immunogenic potential have been identified. These include cdr2, HER-2/neu, mesothelin, cancer–testis antigens such as NY-ESO-1, melanoma-associated antigens of the MAGE family expressed in OC, the surface protein Sp17, mucins (MUC16 and MUC1), the cancer antigen CA-125, and universal tumor antigens such as survivin [56]. In a randomized open-label phase I/II trial conducted at the Ovarian Cancer Research Center, University of Pennsylvania (USA), DCs loaded

with Her2/neu, hTERT, and PADRE peptides were administered to 11 patients with advanced OC who were in remission following standard therapy. DCVs were delivered either alone or in combination with low-dose intravenous cyclophosphamide; all patients also received pneumococcal vaccination [57]. The DCVs containing immunogenic peptides produced heterogeneous outcomes: two patients experienced relapse during the vaccination course; nine completed all four DCV injections. Among these nine, three developed recurrences at 6, 17, and 26 months, whereas six remained disease-free for at least three years after treatment. Overall three-year survival reached 90 %, which was interpreted as a favorable result. Slight improvements in survival were observed in the cyclophosphamide group compared with controls. However, immunologic analyses revealed only weak peptide-specific immune responses and a substantial immunosuppressive effect of pneumococcal vaccination, underscoring the need to optimize the combinatorial treatment strategy.

In a study by the Dendritic Cell Vaccine Working Group of the Japanese Society of Innovative Cell Therapy (J-SICT), 56 patients with advanced OC, previously treated with standard therapy, received DCs loaded with synthetic peptides [58]. The investigators confirmed DCV safety and immunogenicity but reported only modest clinical benefit. Similarly modest results were obtained in another study using WT1 peptide-loaded DCVs [59], where only one of three patients with chemoresistant recurrent OC experienced disease stabilization and improved quality of life.

More pronounced clinical effects were observed with autologous tumor lysate–based DCVs and combinations of DCVs with other antitumor modalities. Chemical modification of tumor lysates has been explored as a means of increasing TA immunogenicity and, consequently, overall DCV effectiveness. A notable example is a single-center phase I trial in 22 patients with recurrent OC, in which DCVs were generated from DCs stimulated with lysates of autologous tumor cells oxidized by hypochlorous acid (HOCl) [60]. The resulting DCVs were administered intranodally over extended periods, until disease progression or exhaustion of the immune response, in three regimens: DCV alone, DCV plus bevacizumab, or DCV plus bevacizumab and low-dose cyclophosphamide. Half of the patients mounted T-cell responses to autologous tumor antigens (as indicated by increased IFN-γ production); these patients experienced the most pronounced clinical benefits. Two patients achieved partial responses, and 13 experienced disease stabilization, with a median duration of 14 months. Two-year survival was 100 % in patients with detectable immune responses to DCVs, compared with only 25 % in those without such responses. The best outcomes were obtained with the combination of DCVs, bevacizumab, and cyclophosphamide.

These examples illustrate the principal results of DCV-based therapy in OC. Unfortunately, the observed benefits are not clearly superior to those achieved with other immunotherapeutic approaches in advanced OC – including cytokine therapy [56, 61], ICIs [35, 55, 58], TIL therapy, and TCR/CAR-T-based cellular therapies [56]. Nonetheless, the pathogenetic alignment of DCVs with fundamental mechanisms of antitumor resistance, together with isolated reports of robust tumor-specific immune responses and complete remission of advanced OC under DCV-based regimens [62], indicate that the therapeutic potential of DCVs in OC remains largely unrealized.

Critical analyses of accumulated clinical data [63, 64] suggest that objective response rates to DCVs in OC and other tumor types do not exceed 15 %. Phase III trials of DCVs are lacking, and most information on clinical activity derives from phase I/II studies employing short-term endpoints. Moreover, antitumor vaccine trials frequently enroll patients with stage IV disease and prior failure of standard therapy, i. e., the most challenging patient population, which substantially limits assessment of DCV potential. Objective comparisons are also

complicated by marked variation in DCV strategies – including DC subtype, manufacturing processes, antigen source, route of administration, and concomitant treatments – hindering robust cross-trial evaluation. Further difficulties arise from the absence of reliable predictive biomarkers for assessing true therapeutic effectiveness of DCVs. Some authors also point to experimental evidence suggesting functional superiority of DCs derived from bone-marrow progenitors over those generated from peripheral blood monocytes, which are far more commonly used in clinical trials [65].

Recent comprehensive reviews on DCV use in OC have focused on analyzing the existing experimental and clinical data and exploring strategies to enhance DCV efficacy. Particular attention has been devoted to the immunogenicity of TAs used for DC activation, methods to obtain TA sets that best represent the mutanome (i. e., the specific pattern of somatic mutations defining the antigenic landscape of an individual tumor), and selection of rational combinations of DCVs with other immunotherapies, with emphasis on pairing DCVs with ICIs to alleviate the immunosuppressive tumor microenvironment and, where appropriate, integrating CT and targeted therapy [64, 65].

Prospects for the Use of Dendritic Cell Vaccines in the Treatment of Gynecologic Malignancies

The main strategies for improving DCV effectiveness in gynecologic cancers largely parallel those pursued for tumors of other localizations. As repeatedly noted, key issues include the generation of personalized, highly immunogenic TA sets; in-depth comparative evaluation of DCVs based on peripheral blood monocytes versus bone-marrow progenitors; selection of optimal adjuvants to enhance TA and DC properties; methods to overcome the immunosuppressive tumor microenvironment that attenuates tumor-specific cytotoxic T-cell activity; rational design of combination regimens incorporating DCVs with other immunotherapies, targeted agents, and chemoradiotherapy; identification of biomarkers predictive of DCV efficacy; optimization of the immune microenvironment; and criteria for appropriate patient selection and timing of DCV administration within multimodal treatment algorithms to maximize therapeutic benefit [15, 19, 64].

In recent years, several next-generation DCV platforms have been developed, including biomaterial-based DC vaccines that employ implantable biocompatible scaffolds for localized antigen delivery and DC activation; immunogenic cell death–inducing DC vaccines loaded with fragments of tumor cells undergoing immune-mediated death; mRNA-pulsed DC vaccines encoding tumor antigens; DC small extracellular vesicle (sEV)-based vaccines; tumor sEV-based DC vaccines derived from cancer stem cell exosomes; and other DCV formats [18, 20]. In addressing the challenge of combining DCVs with other modalities to counteract tumor heterogeneity, suboptimal activity of *ex vivo*–matured DCs, the immunosuppressive tumor microenvironment, cytokine therapy–related toxicities, and additional obstacles, it is logical to consider the rapidly evolving field of nanotechnology [66]. Nanoparticulate liposomal RNA vaccines encoding highly immunogenic neoantigens and adjuvants may enable precise targeting of effector immune cells, the tumor microenvironment, and distinct tumor subregions characterized by marked molecular-genetic and proliferative heterogeneity. Such approaches also offer the possibility of modulating DCs *in vivo* under near-physiologic conditions, thus supporting the initiation of tumor-specific immune responses through controlled, sustained release of active components.

In our view, another promising avenue involves combining DCVs with strategies that activate non-specific immune mechanisms via neuroendocrine-immune regulatory centers or through interactions between tumor-specific processes and innate lymphoid cell systems [10, 67].

CONCLUSION

Active tumor immunotherapy based on dendritic cell vaccines remains a field whose therapeutic potential has not yet been fully realized. The absence of systemic toxicity and the pathogenetic congruence of this technology with fundamental mechanisms of antitumor resistance support its consideration as a promising and safe approach to cancer treatment. The high prevalence of gynecologic malignancies, their substantial incidence and mortality, rapid asymptomatic progression and metastasis, high rates of recurrence and drug resistance, and the inherent immunogenicity of tumors of the female reproductive system all underscore the need to develop novel treatment modalities incorporating immunotherapeutic strategies.

The currently modest efficacy of DCVs in gynecologic cancers reflects both the limited maturity of the technology and the objective difficulties associated with overcoming tumor immune evasion. Critical analysis of DCV use in cervical, endometrial, and ovarian cancers, together with current advances in immunotherapy, molecular-genetic technologies, nanotechnology, and strategies for activating systemic and local antitumor resistance mechanisms, provides a foundation for future research aimed at enhancing the effectiveness of DCVs as an important component of multimodal treatment for gynecologic malignancies.

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CLINICAL CASE REPORTS

Proximal epithelioid sarcoma of the vulva

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ABSTRACT

Epithelioid sarcoma (ES) is an extremely rare disease that, according to morphological data, can be divided into proximal and distal types. The proximal type of ES (PES) of the vulva arises from the superficial and deep layers of the external genital organs, manifests as single or multiple soft tissue tumor nodules with areas of necrosis and hemorrhage, is characterized by aggressive behavior, and has an unfavorable prognosis due to its high tendency for local recurrence and hematogenous metastasis. Differential diagnosis is performed with various benign and malignant neoplasms, cysts or abscesses of the Bartholin gland, and inguinal and femoral hernias. The final diagnosis and histological type of the tumor are established based on morphological, immunohistochemical, and molecular genetic studies. PES is characterized by solid tumor growth composed of large and pleomorphic epithelioid cells with large vesicular nuclei and distinct eosinophilic nucleoli. Immunohistochemically, loss of SMARCB1 (INI1, BAF47) expression is observed, along with positive expression of EMA, vimentin, and cytokeratins; a positive reaction for CD34 staining is often noted in the absence of expression of other endothelial markers such as CD31 and FLI-1. The clinical presentation and disease characteristics may suggest PES and justify the expansion of the immunohistochemical marker panel. Timely and accurate detection of this tumour plays a key role in improving treatment outcomes, enhancing patients' quality of life, and reducing mortality. However, due to its rarity, PES of the vulva presents objective diagnostic difficulties in both clinical and morphological aspects. Surgical intervention remains the main method of treatment for this pathology, and to date, there are no clearly established recommendations regarding the optimal management strategy for patients with PES of the vulva. This paper presents a clinical case of a 65-year-old patient whose atypical clinical presentation of recurrent vulvar tumor served as the basis for expanding the panel of immunohistochemical markers, which allowed for the diagnosis of PES of the vulva.

Keywords: vulva, sarcoma, proximal epithelioid sarcoma, immunohistochemical study, surgical treatment

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КЛИНИЧЕСКОЕ НАБЛЮДЕНИЕ

Проксимальная эпителиоидная саркома вульвы

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РЕЗЮМЕ

Эпителиоидная саркома (ЭС) является крайне редким заболеванием, в соответствии с морфологическими данными может быть разделена на проксимальный и дистальный тип. Проксимальный тип ЭС (ПЭС) вульвы возникает из поверхностных и глубоких слоев наружных половых органов, проявляется одиночными или множественными опухолевыми узлами мягких тканей с участками некроза и кровоизлияния, отличается агрессивным течением и имеет неблагоприятный прогноз, обусловленный высокой склонностью к развитию местных рецидивов и гематогенных метастазов. Дифференциальный диагноз проводится с различными доброкачественными и злокачественными новообразованиями, кистой или абсцессом бартолиновой железы, паховыми и бедренными грыжами. Окончательный диагноз и гистологический тип опухоли устанавливается на основании морфологического, иммуногистохимического и молекулярно-генетического исследований. Для ПЭС характерен солидный рост опухоли из крупных и плеоморфных эпителиоидных клеток с крупными везикулярными ядрами и легко различимыми эозинофильными ядрышками, при иммуногистохимическом исследовании отмечается потеря экспрессии SMARCB1 (INI1, BAF47), а также позитивная экспрессия EMA, виментина и кератинов, нередко наблюдается положительная реакция на окрашивание CD34 при отсутствии экспрессии других маркеров эндотелия, таких как CD31 и FLI-1. Клиническая картина и особенности течения заболевания позволяют заподозрить ПЭС и расширить панель иммуногистохимических маркеров. Своевременное и точное выявление данной опухоли играет ключевую роль в улучшении результатов лечения, повышении качества жизни пациентов и снижении уровня смертности. Однако из-за своей редкости при ПЭС вульвы имеют место объективные диагностические трудности в клинических и морфологических аспектах. Хирургическое вмешательство остается главным методом лечения этой патологии, и до настоящего времени нет четко установленных рекомендаций по оптимальной тактики лечения больных с ПЭС вульвы. В данной работе представлен клинический случай лечения 65-летней пациентки, у которой нетипичная клиническая картина при рецидиве опухоли вульвы явилась основанием для расширения применяемых для иммуногистохимического исследования маркеров, позволившего диагностировать ПЭС вульвы.

Ключевые слова: вульва, саркома, проксимальная эпителиоидная саркома, иммуногистохимическое исследование, хирургическое лечение

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Соблюдение этических стандартов: в работе соблюдались этические принципы, предъявляемые Хельсинкской декларацией Всемирной медицинской ассоциации (World Medical Association Declaration of Helsinki, 1964, ред. 2013). От пациента получено письменное добровольное согласие на публикацию описания клинического наблюдения.

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Конфликт интересов: все авторы заявляют об отсутствии явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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BACKGROUND

Vulvar sarcomas are rare malignant mesenchymal tumors characterized by rapid growth, necrotic changes, and a high potential for hematogenous metastasis. According to various authors, sarcomas account for no more than 1–3 % of malignant vulvar neoplasms [1, 2]. Epithelioid sarcoma (ES) is one of the rarest types of vulvar sarcomas and is characterized by aggressive clinical behavior. According to morphological data, ES can be divided into proximal and distal types. This article describes a rare clinical case of proximal epithelioid sarcoma (PES) of the vulva, the clinical course and treatment approaches of which remain insufficiently studied due to the small number of reported cases and the short follow-up period [3, 4].

Description of the clinical case

A clinical case of PES of the vulva in patient S., born in 1959, who underwent treatment at the N. N. Blokhin National Medical Research Center of Oncology. The analysis included data from the medical history, physical examination, laboratory and instrumental studies, morphological and immunohistochemical findings, and long-term treatment outcomes.

The patient presented to the outpatient department of the N. N. Blokhin National Medical Research Center of Oncology in May 2019 with complaints of a mass in the vulvar region. According to the anamnesis, in March 2019 the patient noticed an induration in the area of the left labium majus. At a local medical institution, a fine-needle biopsy was performed, and the cytological picture was interpreted as adenocarcinoma with areas of squamous cell carcinoma. Upon review of the cytological slides at the N. N. Blokhin National Medical Research Center of Oncology, the observed changes were interpreted as melanoma. The patient reported no family history of cancer, menopause since age 52, and two pregnancies in the anamnesis: one full-term delivery and one medical abortion. On physical examination, a painless, mobile, firm-elastic tumor measuring up to 2 cm in diameter was detected, localized in the middle third of the left labium majus.

According to the decision of the oncological council, the patient was hospitalized in the Department of Oncogynecology of the N. N. Blokhin National Medical Research Center of Oncology for surgical treatment. In June 2019, surgical intervention was performed – wide local excision of the vulvar tumor in the form of a left-sided hemivulvectomy and sentinel lymph node biopsy using a radioisotope method.

Macroscopically, the tumor appeared as a mass measuring 2.5 × 2 × 1.5 cm, consisting of confluent nodules of pinkish-gray and yellowish-gray color, soft to firm-elastic in consistency, with whitish fibrous strands. Microscopically, the tumor consisted of solid proliferations of large polymorphic epithelioid cells (Fig. 1), consistent with the structure of melanoma.

Simultaneously with the removal of the vulvar tumor, bilateral excision of the sentinel lymph nodes was performed. To exclude metastatic involvement of the latter, immunohistochemical (IHC) analysis with Melan A, HMB45, and tyrosinase was carried

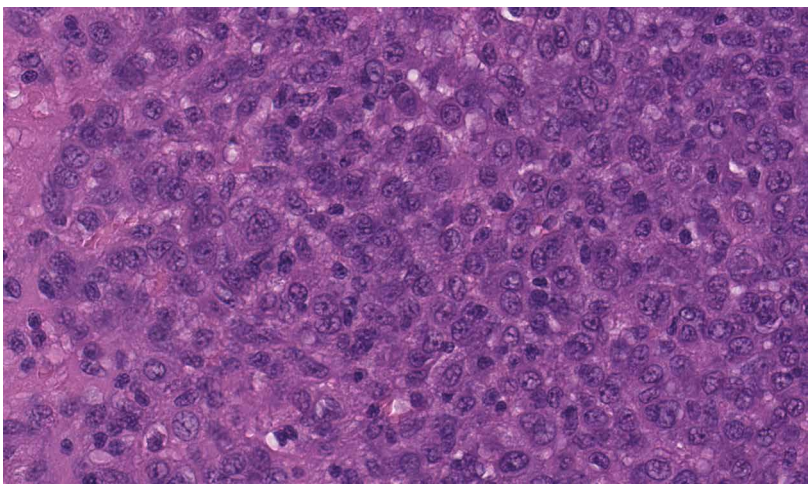

Fig. 1. Pleomorphic epithelioid tumor cells with eosinophilic cytoplasm and enlarged vesicular nuclei with prominent nucleoli (H&E, ×40)

out. No expression of the above-mentioned markers was detected.

Thus, the diagnosis of vulvar melanoma without metastatic involvement of the sentinel lymph nodes was established. Considering the localized nature of the tumor, it was decided to carry out close dynamic follow-up, including quarterly examinations, ultrasound/MRI/CT of the pelvic organs, abdominal cavity, and chest with intravenous contrast enhancement, or whole-body PET-CT.

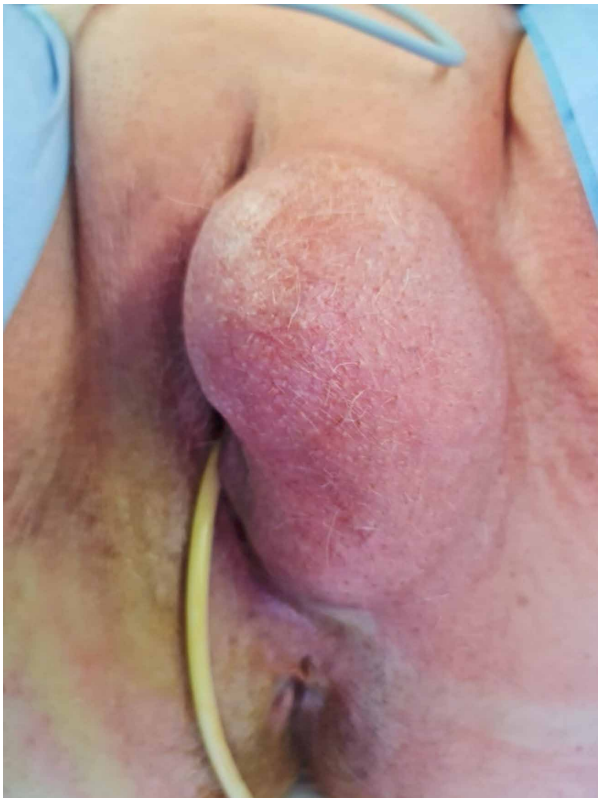

Fig. 2. Recurrent vulvar tumor

In August 2020, the patient noticed the appearance of a nodular mass in the projection of the postoperative scar of the perineal soft tissues. The oncologist at her place of residence referred her for consultation to the N. N. Blokhin National Medical Research Center of Oncology; however, due to the development of coronavirus pneumonia, the patient sought medical attention only in November 2020.

During gynecological examination, deformation of the external genital organs was observed due to the presence of a mass within the soft tissues of the left side of the vulva, of firm consistency, with limited mobility, measuring up to 12 cm in diameter. The skin over the mass was unchanged, and it appeared that the tumor was not associated with the surgical scar (Fig. 2). Examination with vaginal specula was not possible due to compression of the vaginal introitus by the tumor. On palpation, no infiltration of the rectovaginal septum was detected; the rectal mucosa in the projection of the tumor appeared unchanged, and no enlargement or change in the consistency of the inguinal lymph nodes was noted.

According to ultrasound examination of the perineal soft tissues, a lesion with clear, smooth borders and blood flow was visualized within the left labium majus, measuring 11 × 6 cm. MRI of the pelvic organs with contrast enhancement revealed a multinodular lesion of heterogeneous structure

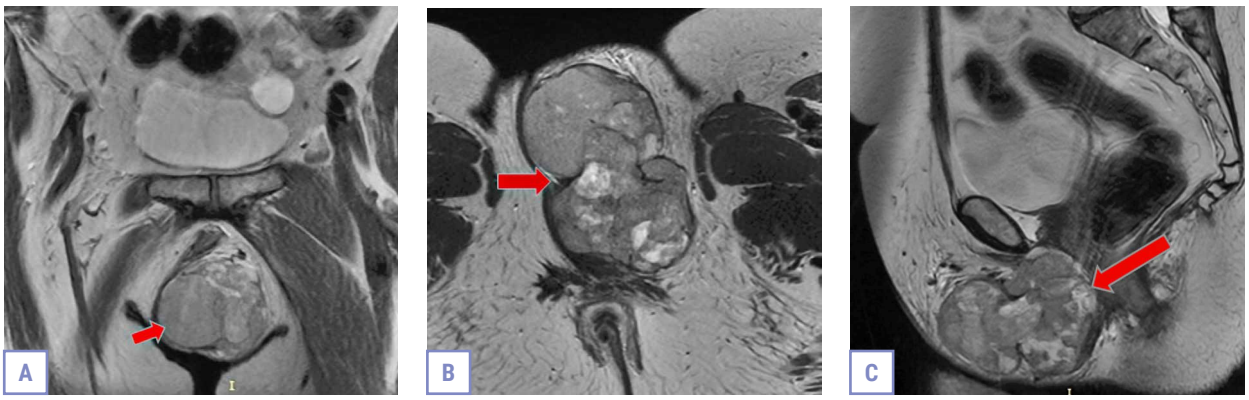

Fig. 3. MRI of the pelvic organs with intravenous contrast enhancement in three projections (A, B, C): a multinodular lesion of heterogeneous structure was detected in the left labium majus (indicated by arrows), measuring up to 55 × 78 × 99 mm, adjacent to the left puborectal muscle and the distal part of the urethra without signs of its invasion.

with well-defined contours, overall dimensions up to 55 × 78 × 99 mm, showing restricted diffusion and active heterogeneous contrast accumulation. The lesion was adjacent to the left puborectal muscle with suspected invasion, to the distal portion of the urethra without signs of invasion, and displaced and deformed the lower third of the vagina without clear evidence of invasion. The pelvic and inguinal lymph nodes were not altered (Fig. 3).

A core biopsy of the vulvar tumor was performed. Histological examination revealed an epithelioid cell tumor, which did not contradict the diagnosis of recurrent epithelioid cell melanoma. However, taking into account the nature of the disease course and the clinical presentation of a mesenchymal tumor without epidermal involvement, additional immunohistochemical (IHC) analysis was performed on the material from the primary tumor using the following markers: S100, HMB45, synaptophysin, chromogranin A, SOX10, CK18, panCK, MelanA, CK7, CD34, BerEP4, Ki67, vimentin, CK20, CD31, desmin, EMA, and FVIII.

Tumor cells showed diffuse and strong expression of vimentin, CD34, and EMA; weak focal expression of CD31, chromogranin A, CK18, panCK, and Melan A. No expression of S100, HMB45, synaptophysin, SOX10, CK7, BerEP4, CK20, FVIII, or desmin was detected in tumor cells. The proliferation index (Ki67) was 24 %. IHC staining with the INI1 marker was not performed at the N. N. Blokhin National Medical Research Center of Oncology laboratory at that time due to technical reasons. However, the co-expression of vimentin, CD34, and EMA, combined with the absence of diagnostically significant expression of markers characteristic of other malignant neoplasms with similar histological structure, allowed the presented morphoimmunophenotype of tumor cells to be interpreted as most consistent with PES.

Comprehensive examination of the patient, including PET-CT, revealed no evidence of regional or distant metastases. Discussion at the oncological multidisciplinary council concluded that, given the local spread of the recurrent tumor, its histological type, and locally aggressive behavior, surgical treatment with a wider margin from the visible tumor boundary was indicated. In December 2020, excision of the recurrent tumor was performed within visually healthy tissues, with a planned margin of at least 2 cm (Fig. 4). The procedure was completed by excision of the labia on the contralateral side to correct the pronounced postoperative deformation of the vulvar region.

The surgical specimen consisted of a fragment of the vulva with adjacent soft tissues measuring

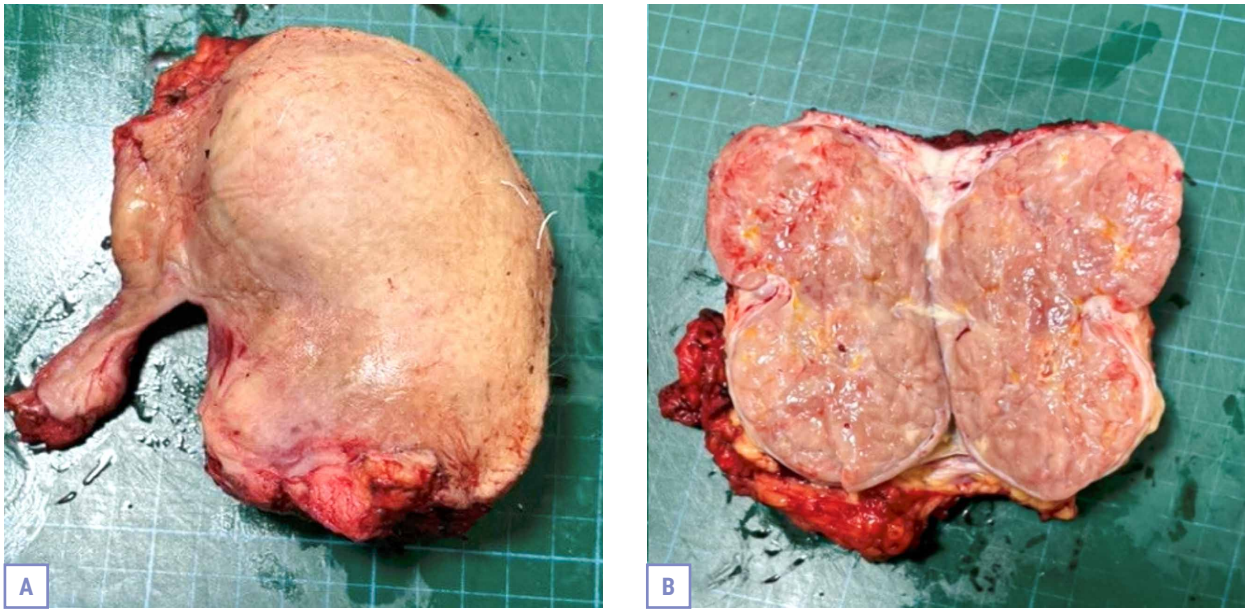

Fig. 4. Gross specimen: A – excised recurrent vulvar tumor; B – tumor on section.

14 × 14.5 × 8.0 cm in total. Within the soft tissues, a subepidermally located tumor nodule measuring 10.5 × 6 × 6.5 cm was identified, with rounded and relatively well-defined borders, composed of confluent lobules of grayish-brown tissue with yellowish streaks and firm-elastic consistency. Microscopically, the tumor was composed of confluent nodules of large epithelioid cells separated by fibrous septa with marked lymphocytic infiltration. Foci of necrosis were observed both in the center and at the periphery of the lobular structures. Mitotic activity reached 20 f. m./10 HPF. The tumor invaded the reticular dermis but showed no invasion of the epidermis and no signs of angiolymphatic or perineural invasion. The morphological pattern was consistent with recurrent PES. No tumor growth was detected at any of the lateral resection margins of the vulva or at the deep margin in the subcutaneous tissue.

Considering the results of the pathological examination of the surgical specimen, the clinical course of the disease, and the absence of evidence of metastatic lesions based on PET-CT findings, the oncological multidisciplinary board decided to continue patient follow-up under the supervision of an oncologist at her place of residence. In December 2023, ultrasound examination revealed a cystic lesion up to 0.5 cm in diameter within the soft tissues in the projection of the left gluteal region. Cytological examination of the aspirate showed no tumor elements. PET-CT with 18F-FDG detected no foci of metabolically active tumor tissue. At present, the patient remains under observation with no signs of recurrence or disease progression.

DISCUSSION

According to the World Health Organization (WHO, 2020) histological classification of tumors, vulvar sarcomas comprise a heterogeneous group of malignant neoplasms differing in clinical course, histological, and immunohistochemical profiles. These include alveolar soft part sarcoma, rhabdomyosarcoma, epithelioid sarcoma, leiomyosarcoma, liposarcoma, dermatofibrosarcoma protuberans, and others [5, 6].

ES is an extremely rare malignant soft tissue tumor with aggressive behavior, which can be divided into proximal and distal types. The proximal type of ES (PES) more commonly occurs in the trunk and pubic area, whereas the distal type typically arises in the upper and lower extremities [7]. PES of the vulva may originate from both superficial and deep tissues, presenting as single or multiple nodules with foci of necrosis and hemorrhage. PES of the vulva is considered a more aggressive form [8]; however, the clinical course of vulvar ES remains poorly understood due to the small number of reported cases and short follow-up periods.

This tumor shares numerous clinical and morphological features with various benign and malignant lesions, including granuloma annulare, melanoma, and epithelioid vascular neoplasms, and may be clinically misdiagnosed as a benign lesion such as a Bartholin gland cyst or abscess, inguinal or femoral hernia, or other benign and malignant soft tissue tumors [9, 10]. In particular, in the present case, the primary tumor was initially interpreted as melanoma, and the clinical presentation did not contradict that diagnosis.

The final diagnosis relies exclusively on pathomorphological examination. PES is characterized by solid growth of large and sometimes pleomorphic epithelioid (carcinoma-like) cells with large vesicular nuclei and distinct eosinophilic nucleoli. Patchy areas of necrosis are often present, but unlike the distal subtype, PES does not form the characteristic pseudogranulomatous pattern. In the distal type, cells show less nuclear atypia, although they may appear more pleomorphic in recurrent or metastatic lesions [11, 12]. Cells with rhabdoid features can occur in both forms but are more frequently seen in the proximal subtype. Immunohistochemical (IHC) analysis serves as a critical tool in the differential diagnosis of ES, with diffuse loss of SMARCB1 (INI1, BAF47) expression and positive co-expression of vimentin, EMA, and cytokeratins being the key diagnostic markers [13]. In a study by Guillou L. et al., more than half of ES cases showed positive staining for CD34 [14], whereas other endothelial markers, such as CD31 and FLI-1, are usually negative [15, 16].

The choice of IHC markers for diagnosis may be of crucial importance. When planning IHC testing, it is essential to consider the clinical presentation

and features of the disease course, which can help expand the marker panel used. In the present clinical case, the atypical appearance of the recurrent lesion, its localization, and the timing of its development after initial treatment prompted the use of an expanded panel of IHC markers, which ultimately allowed for the correct diagnosis.

Cytogenetic studies have revealed chromosomal abnormalities involving the long arm of chromosome 22 in patients with PES, demonstrating inactivation of the tumor suppressor gene SMARCB1/INI1 located at 22q [17, 18].

Chokoeva A. et al. reported that among vulvar sarcomas, ES showed the most aggressive course, while liposarcomas had the most favorable prognosis [19]. According to several studies, unfavorable prognostic factors for ES include primary tumor size greater than 2 cm, deep location, presence of necrosis, high-grade histology, vascular and lymphovascular invasion, early metastasis, and non-radical surgical excision [7, 20, 21]. In a study by Lee N. et al. (2006), an elevated serum CA-125 level was identified as a potentially useful tumor marker for the diagnosis and clinical monitoring of ES [22].

The poor prognosis of patients with vulvar PES is associated with a high tendency for local recurrence, lymph node, and/or distant metastasis. Unlike most other malignant mesenchymal tumors, vulvar ES can also spread via lymphatic and implantation routes to noncontiguous areas of skin, underlying soft tissues, fascia, and bone, which necessitates wider surgical excision [23, 24].

According to a clinical study by Ulutin H. et al., wide excision in the form of vulvectomy prevented local recurrence even without adjuvant therapy. The authors demonstrated that vulvectomy with a wide margin from the visible tumor border provides excellent local control of vulvar ES [25]. However, many authors prefer wide local excision over radical vulvectomy. In a study by Curtin J. et al., only one of seven patients developed local recurrence after surgery alone. Thus, wide excision with a margin of at least 2 cm from the visible tumor border should be performed, as the width of negative resection margins is a major factor influencing local recurrence risk [26, 27]. In the presented clinical case, timely diagnosis of vulvar PES might have allowed for a wider surgical margin during the initial operation, potentially preventing disease recurrence.

Dash B. et al. demonstrated that wide excision remains the preferred treatment for localized PES, while radiotherapy and chemotherapy may be used in unresectable or metastatic forms; however, their role in the adjuvant setting has not been established [28].

Thus, PES represents a pathology associated with objective diagnostic difficulties in both clinical and pathomorphological aspects, which leads to a high rate of diagnostic errors and delays in establishing the correct diagnosis.

CONCLUSION

PES of the vulva is characterized by an aggressive clinical course, necessitating a comprehensive approach to the diagnosis and treatment of this disease. Understanding the morphological, molecular-genetic, and clinical features of vulvar PES contributes to the development of effective therapeutic strategies and improvement of patient prognosis. The presented clinical case clearly demonstrates the importance of timely diagnosis of PES, including immunohistochemical examination, which made it possible to establish the correct diagnosis and plan appropriate treatment.

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Shevchuk A. S. – critical revision with the inclusion of valuable intellectual content, final approval of the version to be published.
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ANNIVERSARY

On the 70th Birthday of Elena Yu. Zlatnik

On December 21, 2025, Elena Yu. Zlatnik, Doctor of Medical Sciences and Professor, celebrates her 70th anniversary.

A prominent scientist in the field of oncoimmunology, Dr. Zlatnik was born into a family of physicians. After graduating with honors from the Rostov Medical Institute in 1979, she began her career at the Department of Microbiology, later working as a junior researcher at the Rostov Institute of Epidemiology, Microbiology and Hygiene, where she completed her PhD thesis on local immunity during the use of lactoglobulin. She successfully defended her dissertation in 1987.

From 1988 to 1993, she worked as a junior researcher in the Department of Immunity and Allergy at the Central Research Laboratory of the Rostov State Medical University. Her primary scientific interests included experimental, laboratory and clinical immunology, immunodiagnostics, and immunotherapy. In 1993, Elena Yuryevna joined the Rostov Research Institute of Oncology as a senior researcher in the Laboratory of Experimental Hormone Therapy of Tumors. Since 1995, she worked as a leading researcher, and in 2006 became the chief researcher of the Pathology Department with Cytology and the Tissue Culture Group, later continuing her work in the Laboratory of Tumor Immunophenotyping. Dr. Zlatnik studied immune status in cancer patients undergoing various methods of chemotherapy, immunotherapy, magnetotherapy and other treatments, as well as the immunological mechanisms underlying these therapeutic approaches. Based on these studies, she completed and successfully defended her doctoral dissertation in 2003 entitled "The Role of the Immune System in the Effects of Chemotherapy on Autologous Liquid Tissues in Cancer Patients." In 2006, she was awarded the academic title of Professor.

As a leading scientist of the National Medical Research Centre for Oncology, Dr. Zlatnik conducts experimental and clinical-diagnostic studies, evaluates immune status and local immunity factors in cancer patients, investigates tumor immunology, and studies the role of immunotherapeutic agents in the multimodal treatment of malignancies. Her research also includes tumor-host regulatory interactions, immunodiagnostics and immunotherapy of tumors, and evaluation of antitumor and immunomodulatory activity of nanoscale particles and other novel anticancer agents under exposure to physical factors in experimental settings.

Her extensive fundamental research is reflected in more than 800 scientific publications. She is a co-author of 64 patents, one of which was recognized among the "100 Best Inventions of Russia – 2012".

During her 32 years at the National Medical Research Centre for Oncology of the Ministry of Health of Russia, Dr. Zlatnik has become a true professional, rising from senior to chief researcher. Throughout these years, she has provided invaluable support to clinicians preparing the immunological components of their PhD and doctoral theses. She has served as the scientific advisor or consultant for seven PhD and one doctoral dissertation, as well as numerous coursework and graduation projects. Professor Zlatnik participates in the education of medical residents and delivers lectures in immunology. She is a member of the Dissertation Council for

defending doctoral and PhD theses, a member of the Academic Council of the Centre, a member of the Ethics Committee, and an active member of the Immunological Society. Elena Yuryevna is a regular participant in scientific conferences, congresses, and symposia.

Colleagues know Dr. Elena Zlatnik as a determined, thoughtful, experienced, and deeply dedicated scientist, possessing exceptional knowledge, erudition, and an outstanding ability to analyze and integrate information.

The staff of the National Medical Research Centre for Oncology
and the Editorial Board of the South Russian Journal of Cancer
extend their heartfelt congratulations to Professor Elena Zlatnik on her anniversary!
You have found the shortest path to success – a sincere love for your work and genuine joy in every accomplishment. We wholeheartedly wish you strong health, enduring optimism, well-being, and many more years of creative scientific endeavor.

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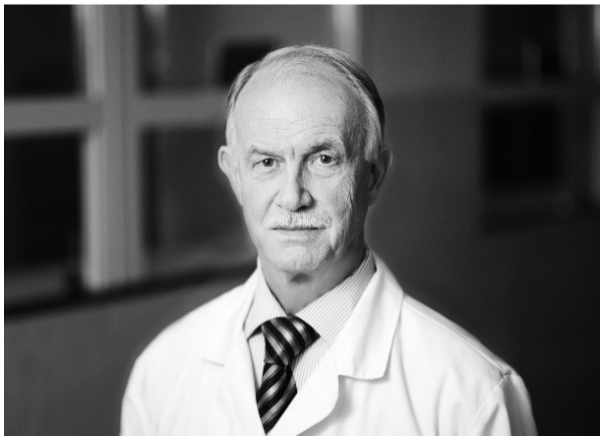
IN MEMORY OF A COLLEAGUE

Professor Pavel Viktorovich Svetitsky of the NMRC for Oncology of the Ministry of Health of Russia Has Passed Away



On 7 December 2025, our colleague, a Senior Research Fellow of the Head and Neck Tumour Department at the National Medical Research Centre for Oncology, Doctor of Medical Sciences, Professor, and Honoured Physician of the Russian Federation, Pavel Viktorovich Svetitsky passed away.

Pavel Viktorovich Svetitsky was born in 1942 in Tashkent. He graduated from the Tashkent State Medical Institute in 1966. He initially worked as a general surgeon and later as an otolaryngologist at Clinical Hospital No. 1 in Tashkent.

In 1972, he defended his Candidate of Sciences thesis entitled “On the Hormonal Origin of Laryngeal Papillomas.” In 1973, he joined the Tashkent Municipal Oncology Dispensary as an otolaryngologist, where he later headed Uzbekistan’s first Head and Neck Tumor Department. In 1975, Pavel Viktorovich became an Assistant Professor at the Department of Oncology at the Tashkent Medical Institute

In 1978, he joined the Research Institute of Oncology and Radiology of the Ministry of Health of the Uzbek SSR as a Senior Research Fellow and was later appointed Head of the Clinical Department. Under his leadership, the Head and Neck Tumor Department became the clinical and methodological centre of the Uzbek SSR for the study and development of modern diagnostic and treatment approaches for head and neck malignancies. In 1985, Pavel Viktorovich Svetitsky defended his Doctor of Sciences thesis entitled “Comprehensive Treatment for Patients with Malignant Tumors of the Head and Neck Using Local Hyperthermia.”.

In 1989, he relocated to Rostov-on-Don and joined the Rostov Research Oncology Institute as a Leading Research Fellow. In 1996, he was appointed Head of the Head and Neck Tumor Department. In 1991, he was awarded the academic title of Professor, and in 2008 he received the honorary title “Honored Physician of the Russian Federation.” Pavel Viktorovich devoted more than 60 years to medicine, including over 35 years at the Rostov Oncology Centre. He served healthcare and science with complete dedication and made an enormous contribution to the advancement of modern oncology. For colleagues and patients alike, Professor Svetitsky was a highly skilled professional, a sensitive and attentive physician, a passionate researcher, a responsible colleague, and a remarkable human being

Pavel Viktorovich was a respected authority among colleagues and numerous trainees both in Russia and abroad. Professor Svetitsky authored more than 500 publications in national and international journals.

The staff of the National Medical Research Centre for Oncology and the editorial board of the South Russian Journal of Cancer mourn his passing and extend their sincere condolences to the family and loved ones of Professor Pavel Svetitsky.
In loving memory of a colleague, mentor, friend, and a truly kind-hearted person.

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