ISSN: 2686-9039 Online

PEER-REVIEWED SCIENTIFIC AND PRACTICAL

South Russian Journal of Cancer

РЕЦЕНЗИРУЕМЫЙ НАУЧНО-ПРАКТИЧЕСКИЙ

Южно-Российский онкологический журнал

vol. 4 № 1/2023
том

www.cancersp.com

ISSN: 2686-9039 (Сет.)

РЕЦЕНЗИРУЕМЫЙ НАУЧНО-ПРАКТИЧЕСКИЙ

Южно-Российский онкологический журнал

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 нем публикуются новости медицинского и фармацевтического сообществ, научно-практические статьи для целевой аудитории – врачей-онкологов. Редакция журнала ставит своей задачей популяризацию научно-исследовательских работ и достижений онкологов Южного федерального округа, анализ процесса глубокой реорганизации здравоохранения в России. Редакция приглашает в качестве авторов всех, кто ищет и находит интересные решения многогранных задач, стоящих перед современной медициной, и хочет поделиться своими мыслями и наблюдениями с коллегами.

Цель: способствовать развитию онкологической медицины Юга России и внедрению её достижений в практику.

Задачи: освещать современные достижения онкологической службы Юга России; содействовать обмену опытом и передовыми знаниями между специалистами; информировать читателей об итогах крупных медицинских форумов.

В журнале размещаются публикации различных рубрик: обзоры литературы, мета-анализы, клинические исследования, наблюдения клинических случаев, обсуждения, анонсы и описания новых методов лечения.

Журнал принимает к публикации: оригинальные статьи, организации здравоохранения, лучевой диагностики, обмен опытом, обзоры, клинические наблюдения.

Издатель и учредитель:
Автономная некоммерческая организация «Перспективы онкологии» (АНО «Перспективы онкологии»)

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344037, Россия, Ростов-на-Дону, 14-я линия, д. 63, литер Г, комната 1
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Сайт: www.cancersp.com
Для корреспонденции: 111555, Москва, а/я 3

Журнал зарегистрирован в Роскомнадзоре 28.10.2019 г., ЭЛ № ФС 77-80665 – сетевое издание.
Периодичность: 4 раза в год.

Опубликовано 06.03.2023

ISSN: 2686-9039 (Online)

PEER-REVIEWED SCIENTIFIC AND PRACTICAL JOURNAL

South Russian Journal of Cancer

The journal is included in the list of peer reviewed scientific journals and publications recommended by the Higher Attestation Commission of the Russian Federation for publishing the main scientific results of dissertations for the degree of candidate and Doctor of Sciences.

South Russian Journal of Cancer: professional medical publication. It publishes news from the medical and pharmaceutical communities, scientific and practical articles for the target audience-oncologists. The editorial board of the journal aims to popularize the research works and achievements of oncologists of the Southern Federal District, to analyze the process of deep reorganization of healthcare in Russia. The editorial board invites as authors all those who are looking for and find interesting solutions to the multifaceted problems facing modern medicine and want to share their thoughts and observations with colleagues.

Purpose: to promote the development of cancer medicine in the South of Russia and the introduction of its achievements into practice.
Tasks: to highlight the current achievements of the oncology service in the South of Russia; to promote the exchange of experience and advanced knowledge between specialists; to inform readers about the results of major medical forums.
The journal contains publications of various categories: literature reviews, meta-analyses, clinical studies, observations of clinical cases, discussions, announcements and descriptions of new treatment methods.
The journal accepts for publication: original articles, health organizations, radiation diagnostics, exchange of experience, reviews, clinical case reviews.

Founder and Publisher:
Autonomous Non-profit Organization "Perspectives of Oncology" (ANO "Perspectives of Oncology")

Editorial and publisher address:
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www.cancersp.com
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The journal is registered at the Roskomnadzor on 28.10.2019,
EL No. FS 77-80665 – online.
Frequency: 4 issues per year.

Published 06.03.2023

Subscription: the magazine is subscribed to via the electronic editorial system on the website. The price is free.

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South Russian Journal of Cancer 2023. Vol. 4, No. 1. P. 6-13
https://doi.org/10.37748/2686-9039-2023-4-1-1
https://elibrary.ru/atehfo
ORIGINAL ARTICLE

IMMUNOHISTOCHEMICAL ASSESSMENT OF POSSIBLE ANTICANCER EFFECT MECHANISMS OF 2-(6,8-DIMETHYL-5-NITRO-4-CHLOROQUINOLINE-2-YL)-5,6,7-TRICHLORO-1,3-TROPOLONE IN PDX MODELS OF LUNG CANCER

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ABSTRACT

Purpose of the study. Evaluation of the expression of immunohistochemical tumor markers Ki-67, b-catenin, Bcl-2, P53, connexin 32 and connexin 43 when using 2-(6,8-dimethyl-5-nitro-4-chloroquinoline-2-yl)-5,6,7-trichloro-1,3-tropolone in mice with xenographs of squamous cell lung cancer.

Materials and methods. Subcutaneous PDX models of human squamous cell lung cancer were created in immunodeficient BALB/c Nude mice. A fragment of the patient's tumor (3 × 3 × 3 mm) was implanted subcutaneously in the right thigh of a previously anesthetized mouse. 200 µl of 2-(6,8-dimethyl-5-nitro-4-chloroquinoline-2-yl)-5,6,7-trichloro-1,3-tropolone was administered orally using a probe in 12 doses once every 3 days. All animals were divided into groups depending on the tropolone doses: experimental groups 2–5 with doses of 0.0055, 0.055, 0.55 and 2.75 mg/g, respectively. The control group received 1 % starch gel which was tropolone carrier. The animals were euthanized 36 days after the start of the substance administration, and the tumor tissue was isolated and prepared for the IHC study according to the standard protocol. IHC reactions were performed using antibodies for Ki-67, b-catenin, Bcl-2, P53, connexin 32 and connexin 43.

Results. Higher tropolone doses were associated with decreased expression of Ki-67, b-catenin, and the Bcl-2 protein, but increased expression of the P53 protein. The dosage of tropolone and expression of connexin 43 were directly proportional.

Conclusion. Immunohistochemical analysis of expression of proteins in PDX models of human squamous cell lung cancer when using 2-(6,8-dimethyl-5-nitro-4-chloroquinoline-2-yl)-5,6,7-trichloro-1,3-tropolone showed the changes indicating its antitumor efficacy and suggesting a possible mechanism of action based on the activation of apoptosis.

Keywords:
2-(6,8-dimethyl-5-nitro-4-chloroquinoline-2-yl)-5,6,7-trichloro-1,3-tropolone, squamous cell lung cancer, PDX, Ki-67, b-catenin, Bcl-2, P53, connexin 32 and connexin 43, apoptosis

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The ethical principles presented by the Helsinki Declaration of the World Medical Association (World Medical Association Declaration of Helsinki, 1964, ed. 2013) were observed in the work. The study was approved by the Ethics Committee of the National Medical Research Centre for Oncology (Protocol No. 1/61 of 02/19/2019).
Funding: this work was not funded.
Conflict of interest: authors report no conflict of interest.

For citation:
Komarova E. F., Lukbanova E. A., Dzhenkova E. A., Goncharova A. S., Zaikina E. V., Gurova S. V., Galina A. V., Kurbanova L. K., Mindar M. V., Khodakova D. V., Gusareva M. S., Zinkovich M. S. Immunohistochemical assessment of possible anticancer effect mechanisms of 2-(6,8-dimethyl-5-nitro-4-chloroquinoline-2-yl)-5,6,7-trichloro-1,3-tropolone in PDX models of lung cancer. South Russian Journal of Cancer. 2023; 4(1): 6-13. (In Russ.). https://doi.org/10.37748/2686-9039-2023-4-1-1, https://elibrary.ru/atehfo

The article was submitted 18.06.2022; approved after reviewing 03.11.2022; accepted for publication 06.03.2023.

Южно-Российский онкологический журнал 2023. Т. 4, № 1. С. 6-13
https://doi.org/10.37748/2686-9039-2023-4-1-1
https://elibrary.ru/atehfo
3.1.6. Онкология, лучевая терапия
ОРИГИНАЛЬНАЯ СТАТЬЯ

ИММУНОГИСТОХИМИЧЕСКАЯ ОЦЕНКА ВОЗМОЖНЫХ МЕХАНИЗМОВ ПРОТИВООПУХОЛЕВОГО ДЕЙСТВИЯ 2-(6,8-ДИМЕТИЛ-5-НИТРО-4-ХЛОРХИНОЛИН-2-ИЛ)-5,6,7-ТРИХЛОР-1,3-ТРОПОЛОНА НА PDX-МОДЕЛЯХ РАКА ЛЕГКОГО

Е. Ф. Комарова[1,2]✉, Е. А. Лукбанова[1], Е. А. Дженкова[1], А. С. Гончарова[1], Е. В. Заикина[1], С. В. Гурова[1], А. В. Галина[1], Л. К. Курбанова[1], М. В. Миндарь[1], Д. В. Ходакова[1], М. С. Гусарева[1], М. С. Зинькович[1]

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РЕЗЮМЕ

Цель исследования. Оценить уровень экспрессии иммуногистохимических опухолевых маркеров Ki-67, b-catenin, Bcl-2, P53, коннексина 32 и коннексина 43 при применении 2-(6,8-диметил-5-нитро-4-хлорхинолин-2-ил)-5,6,7-трихлор-1,3-трополона у мышей с ксенографтами плоскоклеточного рака легкого.
Материалы и методы. На иммунодефицитных мышах линии BALB/c Nude были получены подкожные PDX-модели плоскоклеточного рака легкого человека. Фрагмент опухоли пациента размером 3 × 3 × 3 мм имплантировали подкожно в область правого бедра мыши, предварительно наркотизировав животное при помощи ксилазина концентрацией 20 мг/мл и золетила (тилетамин, золазепам основание) концентрацией 22,57 мг/мл. Введение субстанции 2-(6,8-диметил-5-нитро-4-хлорхинолин-2-ил)-5,6,7-трихлор-1,3-трополон, производили перорально при помощи зонда в объеме 200 мкл в 12 приемов с кратностью 1 раз в 3 дня. Все животные были распределены по группам в зависимости от примененной дозы трополона: опытные группы № 2–5 в дозах 0,0055, 0,055, 0,55 и 2,75 мг/г соответственно. Контрольной группе животных вводили 1 % крахмальный гель, который являлся носителем для трополона. После 36 дней от начала введения субстанции животных эвтаназировали, выделяли опухолевую ткань, которую готовили к ИГХ исследованию по стандартному протоколу. Проводили ИГХ реакции с использованием антител для Ki-67, b-catenin, Bcl-2, P53, коннексина 32 и коннексина 43.
Результаты. Было обнаружено, что с повышением дозы трополона уменьшается экспрессия Ki-67, b-catenin, а также происходит снижение уровня экспрессии белка Bcl-2. При этом уровень экспрессии белка P53 нарастает при увеличении дозы примененного вещества. При исследовании влияния трополона на уровень экспрессии коннексина 43 была обнаружена прямо пропорциональная зависимость его экспрессии при повышении дозы трополона.
Заключение. Проведенный иммуногистохимический анализ уровня экспрессии белков в PDX-моделях плоскоклеточного рака легкого человека при применении 2-(6,8-диметил-5-нитро-4-хлорхинолин-2-ил)-5,6,7-трихлор-1,3-трополона обнаружил их изменения, указывающие на его противоопухолевую эффективность и позволяющие предполагать возможный механизм действия изученной субстанции за счет активации апоптоза.

Ключевые слова:
2-(6,8-диметил-5-нитро-4-хлорхинолин-2-ил)-5,6,7-трихлор-1,3-трополон, плоскоклеточный рак легкого, PDX, Ki-67, b-catenin, Bcl-2, P53, коннексин 32 и коннексин 43, апоптоз

Для корреспонденции:
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В работе соблюдались этические принципы, предъявляемые Хельсинкской декларацией Всемирной медицинской ассоциации (World Medical Association Declaration of Helsinki, 1964, ред. 2013). Исследование одобрено этическим комитетом ФГБУ «НМИЦ онкологии» Минздрава России (протокол № 1/61 от 19.02.2019 г.).
Финансирование: финансирование данной работы не проводилось.
Конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

Для цитирования:
Комарова Е. Ф., Лукбанова Е. А., Дженкова Е. А., Гончарова А. С., Заикина Е. В., Гурова С. В., Галина А. В., Курбанова Л. К., Миндарь М. В., Ходакова Д. В., Гусарева М. С., Зинькович М. С. Иммуногистохимическая оценка возможных механизмов противоопухолевого действия 2-(6,8-диметил-5-нитро-4-хлорхинолин-2-ил)-5,6,7-трихлор-1,3-трополона на PDX-моделях рака легкого. Южно-Российский онкологический журнал. 2023; 4(1): 6-13. https://doi.org/10.37748/2686-9039-2023-4-1-1, https://elibrary.ru/atehfo

Статья поступила в редакцию 18.06.2022; одобрена после рецензирования 03.11.2022; принята к публикации 06.03.2023.

INTRODUCTION

Platinum preparations are still used as a chemotherapeutic treatment for lung cancer, and therefore the search for chemical substances with an effective antitumor effect in this nosology continues [1; 2]. In recent years, tropolone alkaloids, which have demonstrated selective toxicity against malignant tumor cells, have been considered as such [3]. The most well–known tropolones of natural origin – colchicine, colhamine and β-tuiplicin (quinocithiol) demonstrate pronounced antitumor properties due to various mechanisms [4].

The natural troponoid colchicine has the effect of inhibiting tumor growth due to binding to tubulin, which disrupts cell division, as well as due to the ability to limit mitochondrial metabolism in malignant cells by inhibiting potential-dependent anion channels of the mitochondrial membrane [5; 6]. The antitumor effect of the modern natural troponoid quinocithiol due to the induction of caspase-dependent apoptosis, autophagy, blocking of the S-phase of the cell cycle, DNA damage and its demethylation was noted in relation to colon cancer cells, lung adenocarcinoma, breast cancer, multiple myeloma, hepatocellular cancer [7; 8].

Unlike natural and synthetic β-substituted tropolones, the mechanisms of antitumor action of α-substituted analogues have been little studied. Some studies have shown the ability of synthesized α-substituted tropolones by induced caspase-dependent apoptosis to suppress the growth of lymphocytic leukemia cells (but not healthy blood cells), as well as cell culture of multiple myeloma [9]. *In vitro* studies have shown a connection between the mechanisms of cytotoxic action of the compounds studied with the induction of apoptosis and changes in the activity of the ERK signaling pathway in ovarian and colon cancer cells [10].

Purpose of the study: to evaluate the expression level of immunohistochemical tumor markers Ki-67, b-catenin, Bcl-2, P53, connexin 32 and connexin 43 when using 2-(6,8-dimethyl-5-nitro-4-chloroquinoline-2-yl)-5,6,7-trichloro-1,3-tropolone in mice with xenografts of squamous cell lung cancer.

MATERIALS AND METHODS

The study used 50 BALB/c Nude mice of both sexes, which were obtained from the nursery of the Russian Academy of Sciences (Novosibirsk) and were kept in standard conditions of the SPF vivarium of the National Medical Research Centre for Oncology Testing Laboratory Center. The study was approved by the Ethical Committee National Medical Research Centre for Oncology (Protocol No. 1/61 of 02/19/2019). Manipulations with animals were carried out in accordance with the ethical principles established by the European Convention for the Protection of Vertebrates Used for Experimental and Other Scientific Purposes.

Subcutaneous PDX models (Patient-Derived Xenograft) of human squamous cell lung cancer in the 4th passage were obtained on immunodeficient BALB/c Nude mice [11]. The donor of the tumor material was patient T., diagnosed with: C34.3 Central cancer of the lower lobe of the right lung, pT3N0M0, st IIB. The histological type of the patient's tumor is squamous cell lung cancer. A fragment of the patient's tumor measuring 3 × 3 × 3 mm was implanted subcutaneously into the right thigh of the mouse, having previously anesthetized the animal with xylazine at a concentration of 20 mg/ml and zoletil (tiletamine, zolazepamine) at a concentration of 22.57 mg/ml [12].

In the experiment, the substance 2-(6,8-dimethyl-5-nitro-4-chloroquinoline-2-yl)-5,6,7-trichloro-1,3-tropolone (hereinafter tropolone) synthesized by the expansion of the o-quinone cycle in SRI of Physical and Organic Chemistry of the Southern Federal University was introduced [13]. The administration of substances began after the tumor nodes reached volumes of 100 mm^3. 1 % starch gel was used as a carrier for the introduction of tropolone. The substances were administered orally using a probe in the volume of 200 μl in 12 doses with a multiplicity of 1 every 3 days, regardless of food and water intake. All animals were divided into groups depending on the applied dose of tropolone (Table 1).

The duration of the experiment was 36 days, starting from the first administration of the substance. After euthanasia of animals by dislocation of the cervical vertebrae, tumor material was isolated and, after standard preparation, enclosed in paraffin blocks. For the IHC study, sections were made from paraffin blocks using a rotary microtome, which were subsequently dewaxed according to a standard protocol. All stages of the immunohistochemical reaction were carried out in the VENTANA BenchMark ULTRA immunohistostainer of Roche (Switzerland), according

to the protocols of manufacturers attached to the mono- and polyclonal antibodies used. UltraView Universal DAB Detection, manufactured by Ventana Medical Systems, was used as a primary antibody detection system. Antibodies were used for IHC reactions: Ki-67 – Cell Marque, USA, p53, b-catenin, Bcl-2, connexin 32 and –43 – Ventana Medical Systems, USA. The intensity of immuno–staining was assessed using light microscopy (Leica DM3000 microscope).

The normality of the distribution of signs was assessed using the Shapiro-Wilk criterion. Median and interquartile range were calculated for quantitative data. The statistical significance of the differences between the groups was assessed using a pairwise comparative analysis using the Mann-Whitney criterion. The significance level for the methods used was set as $p \leq 0.05$.

RESEARCH RESULTS AND DISCUSSION

In the immunohistochemical study of the expression of the Ki-67 proliferation marker in human lung cancer xenograft tissues, the proportion of immunopositive cells in the 1st control group was 66.3 [61.5–69.3] %, in the 2nd, 3rd, 4th and 5th experimental groups – 64.7 [62.3–65.3] %, 61.4 [60.3–62.3] %, 59.3 [58.1–60.2] % and 55.2 [53.2–57.4] %, respectively, that is, there was a statistically significant decrease in the level of Ki-67 expression as the dose of tropolone increased and compared with the control group ($p < 0.01$) (Fig. 1).

The percentage of positively stained cells when assessing the expression of b-catenin in human lung cancer xenographs was in the experimental groups: in the 2nd – 32.7 [31.2–33.1] %, in the 3rd –24.5 [23.4–26.5] %, 4-th – 22.3 [20.7–23.6] % and in 5-th – 8.4 [7.2–9.3] %, which was statistically significantly lower at $p < 0.05$ relative to the control group (33.7 ± 0.3 %) (Fig. 2). When assessing Bcl-2 expression in human lung cancer xenographs, a statistically significant ($p < 0.01$) decrease in Bcl-2 expression was observed with an increase in the tropolone dose, and the proportion of immunopositive cells was on average in group 1 (control) – 61.1 [57.9–62.4] %, in

Table 1. Study design

Group number	Group naming	Number of animals involved	Injected agent	Agent dose, mg/g
1	Control	10	Starch gel	1 %
2	Study	10	Tropolon	0.0055
3		10		0.055
4		10		0.55
5		10		2.75

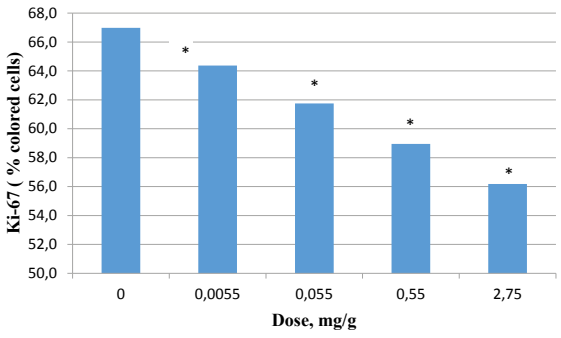

Fig. 1. The relationship between the dose of tropolone and the proportion of immunopositive cells in assessing the expression of Ki-67 in PDF models of human lung cancer in immunodeficient BALB/c Nude mice. 0 – control group, * – statistically significant differences in comparison with the control group ($p < 0.05$).

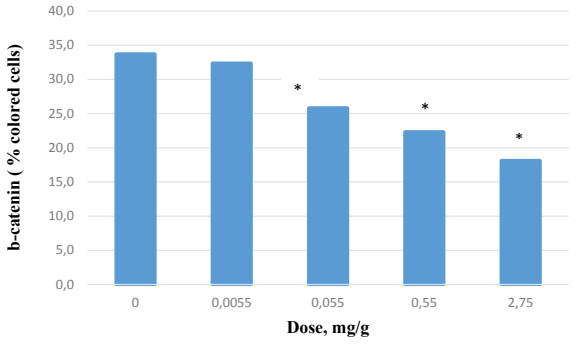

Fig. 2. The relationship between the dose of tropolone and the proportion of immunopositive cells when evaluating the expression of i-catenin in PDF models of human lung cancer in immunodeficient BALB/c Nude mice. 0 – control group, * – statistically significant differences in comparison with the control group ($p < 0.05$).

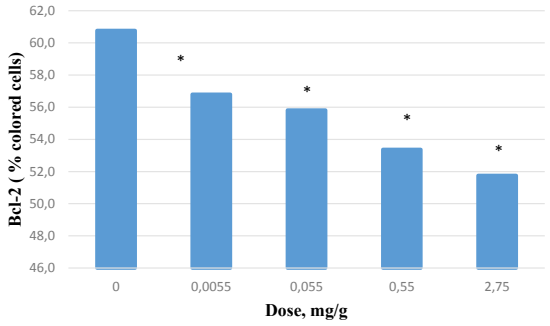

Fig. 3. The relationship between the dose of tropolone and the proportion of immunopositive cells in assessing Bcl-2 expression in PDX models of human lung cancer in immunodeficient BALB/c Nude mice. 0 – control group, * – statistically significant differences in comparison with the control group ($p < 0.05$).

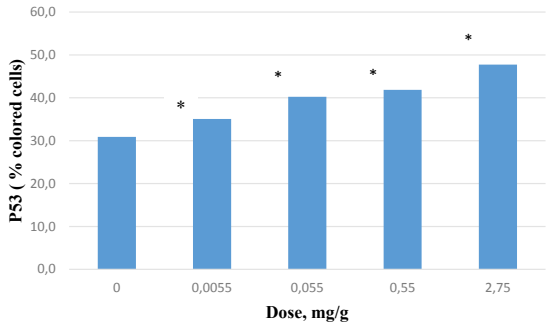

Fig. 4. The relationship between the dose of tropolone and the proportion of immunopositive cells when evaluating the expression of P53 in PDX models of human lung cancer in immunodeficient BALB/c Nude mice. 0 – control group, * – statistically significant differences in comparison with the control group ($p < 0.05$).

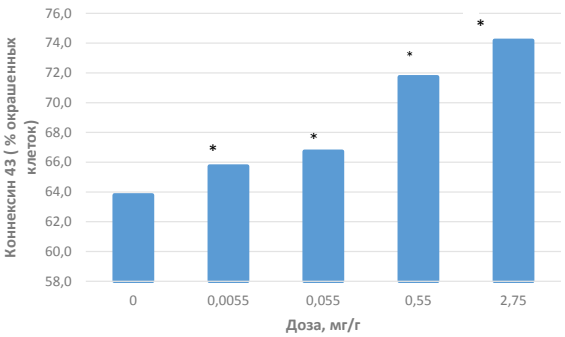

Fig. 5. The relationship between the dose of tropolone and the proportion of immunopositive cells in assessing the expression of connexin 43 in PDX models of human lung cancer in immunodeficient BALB/c Nude mice. 0 – control group, * – statistically significant differences in comparison with the control group ($p < 0.05$).

the 2nd (0.0055 mg/g), 3rd (0.055 mg/g), 4th (0.55 mg/g) and 5th (2.75 mg/g) groups – 56.8 [55.4–58.6] %, 55.5 [53.1–56.3] %, 52.5 [52.9–55.2] % and 50.9* [50.1–53.2] %, respectively (Fig. 3).

When evaluating the expression of the oncosuppressor protein P53, the proportion of immunopositive cells in human lung cancer xenographs averaged 30.9 ± 0.5 % in group 1, in groups 2, 3, 4 and 5 – 35.8 ± 0.4 [32.3–37.5] %, 41.4 ± 1 [39.9–43.1] %, 42.9 ± 1.3 [41.2–44.3] % and 48.1 ± 0.4 [45.9–51.3] %, respectively (Fig. 4). At the same time, a statistically significant ($p < 0.01$) increase in the expression level of P53 was observed with an increase in the dose of tropolone (in 2, 3, 4 and 5groups compared with the control group), which indicates an increase in the processes of apoptosis in the tumor tissue (Fig. 4).

When analyzing the expression of connexin 32 in the tissues of PDX models of lung cancer in all experimental groups of animals, no statistically significant differences ($p > 0.05$) were found with the control group. The percentage of positively colored cells was 62.1 [60.2–63.5] %, 61.1 [59.9–62.9] %, 61.6 [60.1–63.1] %, 61.3 [59.8–62.1] % and 61.7 [60.4–62.9] % for 1–5 groups, respectively.

The proportion of immunopositive cells in assessing the expression of connexin 43 in human lung cancer xenographs was on average in group 1 (control) – 62.8 [58.3–63.2] %, in the 2nd, 3rd, 4th and 5th groups – 65.3 [64.1–66.5] %, 66.9 [65.3–69.5] %, 71.8 [69.4–72.9] % and 74.8 [72.3–76.4] %, respectively, that is, when exposed to tropolone, there was a statistically significant increase in the level of connexin 43 expression in the experimental groups compared with the control group. ($P_{1-2,1-3} \leq 0.05$; $P_{1-4,1-5} < 0.01$) (Fig. 5).

The immunohistochemical analysis of the expression level of proteins Ki-67, b-catenin, Bcl-2, P53 and connexins 43 and 32 in PDX models of squamous cell lung cancer with the use of 2-(6,8-dimethyl-5-nitro-4-chloroquinoline-2-yl)-5,6,7-trichloro-1,3-tropolona found their changes indicating dose-dependent antitumor activity and suggesting possible mechanisms of action of the studied substance. Thus, it was found that with an increase in the dose of tropolone, the expression of Ki-67, b-catenin decreases, and there is also a decrease in the expression level of the anti-apoptotic protein Bcl-2, while the expression level of the oncosuppressor protein P53 increases with an increase in

the dose of the substance used. The Ki-67 protein is currently considered as the most reliable marker of proliferation, including for lung cancer, and its decrease observed in the experimental groups relative to the indicators in the control group indicates a decrease in the proliferative activity of tumor cells of squamous cell lung cancer when using tropolone [14]. It is believed that b-catenin exhibits various effects in tumor cells, in particular, it activates proliferation, being one of the key molecules of the tumorogenic signaling pathway Wnt/b-catenin and, due to the intersection with other signaling pathways, regulates apoptosis, angiogenesis and cell invasion, and also participates in the shift of cellular metabolism towards oxygen-free glucose oxidation [15]. A decrease in the expression of b-catenin by lung cancer tumor cells in PDX models with the use of tropolone confirms the antitumor effect of this substance, obviously mediated through the Wnt/b-catenin signaling pathway. Studies have shown that overexpression of Bcl-2 protein blocks apoptosis and thus promotes tumor progression [16]. Lee Y.-S. and co-authors (2013) showed a decrease in Bcl-2 expression when exposed to natural tropolone – quinokithiol on subcutaneous xenografts obtained by transplantation of human colon cancer cell cultures HCT-116 and SW-620 [17]. Suppression of the expression of anti-apoptotic protein Bcl-2 in subcutaneous xenograft cells of squamous cell lung cancer in combination with increased expression of oncosuppressor P53, known for its activating effect on pro-apoptotic proteins Bax and Bid, indicates stimulation of apoptosis with the use of 2-(6,8-dimethyl-5-nitro-4-chloroquinoline-2-il)-5,6,7-trichloro-1,3-tropolone.

When studying the effect of tropolone on the expression level of connexin 43, a dependence of its expression was found with an increase in the dose of tropolone. It is known that connexins are tumor suppressors that regulate cell proliferation, apoptosis, chemoresistance, migration and invasion with the help of intercellular communication of the slit junction [18]. Overexpression of connexin 43 in the nucleus correlates with increased aggressiveness of lung tumors, which is associated with its ability to recruit E-cadherin, providing tumor cells with a more invasive phenotype, increasing their ability to migrate, survival, and contributing to the development of distant metastases [19]. However, connexin 43 with its cytoplasmic localization is able to inhibit tumorogenesis [20]. Assuming the overall antitumor efficacy of the tropolone studied, we can talk about the induction of the suppressor function of connexin 43 relative to lung cancer in the PDX model. Some studies show that connexin 32 is overexpressed in lung cancer cells [21]. Knockout or knockdown of the connexin 32 gene leads to an increase in the frequency of chemical and radiation-induced lung tumors, and also enhances epithelial-mesenchymal transition, migration and invasion of tumor cells [22], probably partly due to activation of the MAPK pathway. However, we have not detected the effect of tropolone on the expression of connexin 32, the reason for this is not clear and requires further study.

CONCLUSION

The detected changes in the studied immunohistochemical markers on PDX models, indicating a decrease in the proliferative activity of lung tumor cells, as well as activation of apoptosis processes, indicate the manifestation of the antitumor effect of tropolone. Based on the results obtained, we assume that a possible mechanism of antitumor action of the studied tropolone is activation of apoptosis.

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Dzhenkova E. A. – scientific editing of the article;
Goncharova A. S. – scientific editing of the article;
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Gurova S. V. – design of the bibliography;
Galina A. V. – conducting an experiment;
Kurbanova L. K. – design of the bibliography;
Mindar M. V. – statistical data analysis;
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Zinkovich M. S. – technical editing of the article.

South Russian Journal of Cancer 2023. Vol. 4, No. 1. P. 14-22
https://doi.org/10.37748/2686-9039-2023-4-1-2
https://elibrary.ru/fvybig
ORIGINAL ARTICLE

INFLUENCE OF INDUCED DIABETES MELLITUS ON HORMONAL PROFILE OF LEWIS LUNG CARCINOMA IN BALB/C NUDE MICE

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ABSTRACT

Purpose of the study. The assessment of diabetes mellitus (DM) effect on levels of sex hormones in tumor and peritumoral tissues in BALB/c Nude mice with Lewis lung carcinoma (LLC).
Materials and methods. The study included 42 male and female BALB/c Nude mice aged 8–9 weeks weighing 21–22 g. Alloxan-induced DM was reproduced in mice of the main group, and then LLC was transplanted. Levels of estrone (E1), estradiol (E2), testosterone (T), progesterone (P4) and prolactin (PRL), as well as steroid hormone receptors: estrogens (REα, REβ), androgens (RA), and progesterone (RP4) were measured by RIA and ELISA in samples of tumor and peritumoral tissues. Animals with LLC without DM were used as controls. The statistical analysis was performed using the Statistica 10 program; differences were considered significant at $p < 0.05$.
Results. DM in males was reproduced only after a double injection of alloxan, and was characterized by lower blood glucose levels compared to females. The growth of LLC in animals with alloxan-induced DM was possible only in female BALB/c Nude mice; in BALB/c Nude males, the tumor could not be transplanted either independently or in combination with DM. Females in the main group showed greater average tumor volumes throughout the experiment and reduced survival, compared to the control group. Tumor samples from females with LLC+DM were more saturated with sex steroids, but depleted in steroid hormone receptors, which probably contributed to the ability to avoid the body's regulatory signals.
Conclusion. The growth of LLC in presence of induced DM was sex-dependent, since the tumor could not be transplanted to male mice. DM affected the levels of sex steroids and their receptors tumor tissues in female BALB/c Nude mice.

Keywords:
diabetes mellitus, Lewis lung carcinoma, BALB/c Nude mice, sex steroids, sex steroid receptors

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The work with animals was carried out in accordance with the rules of the "European Convention for the Protection of Animals Used in Experiments" (Directive 86/609/EEC). Protocol of the Ethics Committee No. 7/111 of 02/26/2021.

Funding: this work was not funded.
Conflict of interest: authors report no conflict of interest.

For citation:
Frantsiyants E. M., Bandovkina V. A., Kaplieva I. V., Shikhlyarova A. I., Surikova E. I., Neskubina I. V., Pogorelova Yu. A., Trepitaki L. K., Cheryarina N. D. Influence of induced diabetes mellitus on hormonal profile of Lewis lung carcinoma in BALB/c Nude mice. South Russian Journal of Cancer. 2023; 4(1):14-22. https://doi.org/10.37748/2686-9039-2023-4-1-2, https://elibrary.ru/fvybig

The article was submitted 18.08.2022; approved after reviewing 12.01.2023; accepted for publication 06.03.2023.

Южно-Российский онкологический журнал 2023. Т. 4, № 1. С. 14-22
https://doi.org/10.37748/2686-9039-2023-4-1-2
https://elibrary.ru/fvybig
3.1.6. Онкология, лучевая терапия
ОРИГИНАЛЬНАЯ СТАТЬЯ

ВЛИЯНИЕ ИНДУЦИРОВАННОГО САХАРНОГО ДИАБЕТА НА ГОРМОНАЛЬНЫЙ ФОН КАРЦИНОМЫ ЛЬЮИСА У МЫШЕЙ ЛИНИИ BALB/C NUDE

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РЕЗЮМЕ

Цель исследования. Изучение влияния сахарного диабета (СД) на содержание половых гормонов в опухоли и перифокальной зоне у мышей BALB/c Nude с карциномой Льюиса.

Материалы и методы. В работе использовали 42 мыши линии BALB/c Nude обоего пола, 8–9 недельного возраста с массой тела 21–22 г. У мышей основной группы с помощью инъекций аллоксана индуцировали СД, на фоне которого перевивали карциному Льюиса (LLC). В образцах опухолей и их перифокальных зон радиоиммунным методом (РИА) и иммуноферментным методом (ИФА) определяли уровень эстрона (Е1), эстрадиола (Е2), тестостерона (Т), прогестерона (Р4) и пролактина (ПРЛ), а также рецепторов стероидных гормонов: эстрогенов (REα, REβ), андрогенов (RA), и прогестерона (RP4). В качестве контроля изучали животных с самостоятельным ростом LLC. Статистический анализ проводили с использованием программы Statistica 10, значение $p < 0,05$ рассматривалось как показатель статистической значимости.

Результаты. Сахарный диабет у самцов воспроизводился только после двухкратного введения аллоксана и характеризовался более низкими показателями глюкозы крови, по сравнению с самками. Рост карциномы Льюиса на фоне индуцированного аллоксаном сахарного диабета оказался возможным только у самок мышей линии BALB/c Nude, у самцов линии BALB/c Nude опухоль не перевивалась ни в самостоятельном, ни в сочетанном с СД вариантах. У самок основной группы установлены большие средние объемы опухолей на протяжении всего эксперимента и сокращение продолжительности жизни, по сравнению с группой контроля. При этом, образцы опухоли у самок с развитием злокачественного процесса на фоне СД хотя и были более насыщены половыми стероидами, оказались обеднены рецепторами стероидных гормонов, что, вероятно, способствовало возможности избегать регуляторных сигналов организма.

Заключение. Рост карциномы Льюиса на фоне индуцированного СД имел половую зависимость, опухоль не перевивалась самцам мышей. СД оказал влияние на уровень половых стероидов и их рецепторов в злокачественной опухоли у самок мышей BALB/c Nude.

Ключевые слова:
сахарный диабет, карцинома Льюиса, мыши линий BALB/c Nude, половые стероиды, рецепторы половых стероидов

Для корреспонденции:
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Работа с животными осуществлялась в соответствии с правилами «Европейской конвенции о защите животных, используемых в экспериментах» (Директива 86/609/EEC). Протокол этического комитета № 7/111 от 26.02.2021 г.

Финансирование: финансирование данной работы не проводилось.
Конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

Для цитирования:
Франциянц Е. М., Бандовкина В. А., Каплиева И. В., Шихлярова А. И., Сурикова Е. И., Нескубина И. В., Погорелова Ю. А., Трепитаки Л. К., Черярина Н. Д. Влияние индуцированного сахарного диабета на гормональный фон карциномы Льюиса у мышей линии BALB/c Nude. Южно-Российский онкологический журнал. 2023; 4(1):14-22. https://doi.org/10.37748/2686-9039-2023-4-1-2, https://elibrary.ru/fvybig

Статья поступила в редакцию 18.08.2022; одобрена после рецензирования 12.01.2023; принята к публикации 06.03.2023.

INTRODUCTION

The interaction between diseases was recognized as a key factor influencing the natural course of diseases, including the development of the malignant process [1; 2]. Synergetic relationships between diseases co-existing in the population can influence the dynamics of the epidemic, which leads to noticeable differences in health outcomes compared to the disease occurring in isolation [3]. The concept of disease interaction in the epidemiology of diabetes mellitus (DM) is well documented both at the population and individual level. There is convincing evidence of an association between DM and other macro- and microvascular diseases, such as hypertension, coronary heart disease and congestive heart failure, as well as immunodeficiency [4; 5].

Other studies have also shown a significant association between DM and several oncological diseases, such as colorectal cancer, pancreatic cancer, endometrial and ovarian cancer, stomach, kidney and thyroid cancer, as well as leukemia [6]. Moreover, cancer patients with diabetes mellitus or with abnormal plasma glucose levels have a more unfavorable prognosis. In malignant cells that are exposed to excess physiological glucose concentrations, a change in intracellular signaling is detected, which leads to more aggressive phenotypes [7].

The mechanisms underlying the high aggressiveness of glucose-induced cancer are different for each type of cancer. Glucose can activate many signaling pathways, for example, ERK, STAT3 and NsF-κB, including cell proliferation, metastatic activity and chemoresistance of cancer cells. Activation of these intracellular pathways regulates the transcription of their specific downstream target genes, which contribute to the development of aggressive phenotypes. In addition, it was found that the long-term clinical outcome and survival are worse in cancer patients with diabetes mellitus, whose plasma glucose levels are poorly controlled [8]. Therefore, high glucose content has been proposed and identified as a mechanism by which diabetes mellitus is associated with cancer progression [9].

The purpose of this study was to study the effect of diabetes mellitus on the content of sex hormones in the tumor and perifocal zone in BALB/c Nude mice with Lewis carcinoma.

MATERIALS AND METHODS

Experimental studies were carried out on 42 mice of both sexes of the BALB/c Nude lines, 8–9 weeks of age with a body weight of 21–22 g. Mice of the BALB/c Nude lines were purchased at the Laboratory Animal Nursery in Pushchino, Russian Federation. The animals were kept in an environment free of specific pathogens (SPF), with a 12-hour light/dark cycle. The choice of BALB/c Nude mice for this study is associated with defects in the immune system caused by the absence of a thymus gland and a deficiency of T cells [10], thanks to which the introduction of alloxan in experimental animals was able to induce diabetes mellitus and cure Lewis carcinoma.

All mice were kept in autoclave micro-insulator cages equipped with sterilized pine wood chips at a constant (24 h) temperature (22 °C) and humidity (40–70 %). Throughout the experiment, the mice were fed sterilized food and water. All manipulations were carried out in sterile conditions at a laminar flow workstation. All studies were conducted in accordance with the requirements and conditions set out in the "International Recommendations for conducting Biomedical research using animals" and the Order of the Ministry of Health of the Russian Federation No. 267 dated 06/19/03 "On approval of the rules of laboratory practice". Work with animals was carried out in accordance with the rules of the "European Convention for the Protection of Animals Used in Experiments" (Directive 86/609/EEC). Protocol of the Ethics Committee No. 7/111 of 02/26/2021.

The females of the BALB/c Nude lines were divided into the following groups: the control group – mice with Lewis carcinoma (LLC) (n = 14) and the main group of mice with Lewis carcinoma on the background of DM (n = 14). Of these, 7 animals with independent growth LLC and 7 animals with LLC+The dynamics of tumor growth and life expectancy were observed, and the remaining animals were guillotined 19 days after the tumor was transplanted in order to study sex hormones and their receptors in tissues.

Males were also divided into groups of 7 animals: mice that were transplanted with Lewis carcinoma in an independent variant; mice that were transplanted with Lewis carcinoma on the background of DM.

Diabetes was reproduced in females by a single intraperitoneal injection of alloxan at a dose of 350

mg/kg; in males – by a double intraperitoneal injection of alloxan at a dose of 350 mg/kg.

To transplant Lewis carcinoma in an independent version, 0.5 ml of LLC tumor suspension containing 0.5 million tumor cells was injected under the skin of the back just below the right shoulder blade. Animals of the main groups, for the development of the tumor process against the background of DM, the tumor was transplanted in a similar independent way: females on the 5th day after the introduction of alloxan and a steady increase in blood glucose levels, males on the 12th day from the first injection of alloxan. Measurements of tumor nodes were carried out in 3 dimensions, the volume of the tumor was calculated using the formula (R1 + R2 + R3) [10]. In groups with tumor grafting to study the life expectancy of animals, tumor measurements were carried out weekly until the death of animals. To determine hormones and their receptors, tumor samples, their perifocal zones, as well as the pancreas were taken on ice. 1 % cytosolic fractions prepared on 0.1M potassium phosphate buffer pH 7.4 containing 0.1 % Twin-20 and 1 % BSA were obtained from the tissues. The level of estrone (E1), estradiol (E2), testosterone (T), progesterone (P4), prolactin (PRL), as well as the content of insulin and C-peptide were determined by RIA (Immunotech, Czech Republic), the concentration of steroid hormone receptors: estrogens REa and REß, androgen receptors RA and progesterone RP4 were determined The ELISA method (Cloud-Clone Corp. China), blood glucose by biochemical method (Olveix Diagnosticum),

Statistical processing of the results was carried out using the Statistica 10.0 program. The data are presented as an average value ± standard error of the average. The correspondence of the distribution to the normal was evaluated using the Shapiro-Wilk criterion. The significance of the differences between independent samples was assessed using the Student's t-test and the Mann-Whitney test ($p < 0.05$).

RESEARCH RESULTS

First of all, we studied the development of diabetes mellitus in mice of different sexes. It was found that in all female mice on the 5th day after the introduction of alloxan, the blood glucose level was 26.94 ± 1.2 mM/L versus 5.8 ± 0.7 in intact animals and remained at this level with slight fluctuations throughout the entire duration of the experiment until the death of the animals (Table 1).

At autopsy in females with diabetes macroscopically: the kidneys are enlarged, the pancreas is almost 2 times smaller than in intact animals, it looks "mucous". The level of insulin and C peptide in the pancreas in the females of the main group was 5.4 times and 1.6 times higher, respectively (44.2 ± 3.8 mcIU/g of tissue and 2.95 ± 3.1 pm/g of tissue, respectively, in the main group versus 8.15 ± 0.8 mcIU/g of tissue and 1.86 ± 0.17 pm/g of tissue in intact animals), which indicates the development of insulin resistance in these animals.

The results of measurements of average tumor volumes in female mice with and without diabetes mellitus are presented in the table 1.

Table 1. Lewis carcinoma volume and blood glucose levels in female mice

Days after starting the experiment	8 Days	11 Days	15 Days	19 Days	22 Days
Tumor V cm^3 Main group	0.36 ± 0.018*	4.07 ± 0.13*	7.76 ± 0.18*	10.32 ± 0.4*	-
Blood glucose (mM/L) in the main group	26.91 ± 0.56*	26.41 ± 0.59*	26.36 ± 0.81*	26.48 ± 0.69*	-
Tumor V cm^3 in the control group	0.1 ± 0.011	0.31 ± 0.012	0.94 ± 0.07	2.98 ± 0.15	4.71 ± 0.14
Blood glucose (mM/L) in the control group	5.78 ± 0.12	5.36 ± 0.17	5.63 ± 0.11	5.9 ± 0.12	5.63 ± 0.14

Note: * – statistically significant differences in comparison with the control group, $p < 0.05$.

It is obvious that in the group of female mice with Lewis carcinoma, reproduced against the background of diabetes mellitus, the tumor developed more intensively: on day 8 it was 3.6 times larger than in animals of the control group, on day 11 – almost 13 times, on day 15–8.3 times and before the death of mice on day 19–3.5 times. The greatest growth of the tumor occurred in the period of 11–15 days. The life expectancy of mice with Lewis carcinoma on the background of diabetes mellitus was 19–20 days, whereas with isolated Lewis carcinoma – 28–29 days ($p < 0.05$).

In all male mice, after 1 administration of alloxan, the glucose level didn't significantly rise, so we performed 2 injections at the same dose 8 days after the 1st one. The glucose level is shown in the table 2.

The next day after repeated administration of alloxan, the skin of all mice acquired a bluish hue. Blood for measuring glucose levels was taken with difficulty. Lewis carcinoma was transferred to male mice on day 12 from the 1st administration of alloxan and on day 4 from the repeated administration of alloxan. 4 days after the transfer of the tumor suspension, white spots with a diameter of about 5 mm without signs of tumor growth were observed at the injection site. All the mice had dry atrophic skin. No tumors were detected 30 days after the transplant.

Thus, female mice were more susceptible to the reproduction of diabetes mellitus and Lewis tumors

Table 2. Blood glucose levels in male mice with alloxan-induced diabetes

Days after starting the experiment	Inital	7 Days	8 Days	9 Days	12–19 Days
Glucose levels in DM group (mM/L)	6.6 ± 0.12	7.80 ± 0.11*	9.2 ± 0.23*	9.47 ± 0.12*	14.46 ± 0.21*

Note: * – statistically significant differences in comparison with the initial levels, $p < 0.05$.

Table 3. The content of hormones and receptors in the tumor and its perifocal zone during various processes in female mice

Indicators	Tumor		Perifocal zone	
	LLC	DM + LLC	LLC	DM + LLC
E1 (pg/t)	47.7 ± 2.39**	101.8 ± 2.3*	517.0 ± 2.8	97.0 ± 2.14*
E2 (pg/g t)	62.3 ± 1.37**	84.7 ± 2.62*,**	127.3 ± 1.44	55.5 ± 1.46*
T total (ng/g t)	0.21 ± 0.02**	0.7 ± 0.06*,**	1.0 ± 0.076	0.2 ± 0.01*
P4 (ng/g t)	1.5 ± 0.064**	5.0 ± 0.38*,**	3.7 ± 0.06	3.8 ± 0.177
PRL (ng/g t)	3.0 ± 0.306**	13.2 ± 0.28*,**	12.5 ± 0.42	34.9 ± 1.33*
REα (ng/g t)	2.2 ± 0.099**	1.2 ± 0.11*,**	10.3 ± 0.33	6.6 ± 0.3*
REβ (ng/g t)	4.6 ± 0.05**	2.9 ± 0.25*,**	12.3 ± 0.4	7.4 ± 0.25*
RA (ng/g t)	0.33 ± 0.008**	0.3 ± 0.0056**	2.5 ± 0.06	1.4 ± 0.10*
RP4 (ng/g t)	0.33 ± 0.02**	0.21 ± 0.009*,**	3.7 ± 0.076	1.4 ± 0.11*

Note: statistically significant differences in comparison with: * – the respective samples in the control group; ** – perifocal zone of the entire group ($p < 0.05$).

in them, and Lewis carcinoma on the background of diabetes mellitus grew more intensively. The males turned out to be quite resistant to the reproduction of diabetes mellitus and not at all susceptible to transplantation of Lewis carcinoma, either in an independent variant or against the background of diabetes mellitus.

The question arose: is this related to the level of sex hormones and, especially, to the activation of tumor growth under the influence of sex hormones?

Thus, we studied the level of sex hormones and their receptors in the tumor tissue of female mice with various growth variants, as well as in the tissue surrounding the tumor, I.e. the perifocal zone.

It was found that the tumor tissue growing against the background of diabetes mellitus contained more estrogens: E1–2.1 times and E2–1.4 times relative to the tumor tissue growing in an independent variant (Table 3). At the same time, the level of these hormones in the perifocal zone was lower than in the corresponding samples with independent growth of Lewis carcinoma: E1–5.3 times and E2–2.3 times. The T content in the tumor tissue during its growth against the background of diabetes mellitus turned out to be 3.3 times higher, and in its perifocal zone 5 times lower, compared with similar samples of the control group. In the tumor tissue growing against the background of diabetes mellitus, the level of P4 and PRL was increased by 3.3 times and 4.4 times, respectively, in the tissue of its perifocal zone, only the level of PRL was increased by 2.8 times, while P4 had no significant differences from the corresponding region with independent growth of Lewis carcinoma.

The content of estrogen and progesterone receptors in the females of the main group was reduced in the tumor tissue and its perifocal zone: REα – by 1.8 times and 1.6 times, respectively, REβ – by an average of 1.6 times and RP4 – by 1.5 and 2.6 times, respectively, relative to the indicators in the animals of the control group. The androgen receptor in the tumor tissue had no significant differences between the indicators on the background of diabetes mellitus and without it, and in the tissue of the perifocal zone of the tumor in the animals of the main group was reduced by 1.8 times. It should be noted that, despite the decrease in the level of sex hormone receptors in the tissue of the perifocal zone of the tumor in diabetes mellitus, the indicators remained significantly higher than their level in the tumor tissue.

DISCUSSION

On the background of the ongoing epidemic of both obesity and diabetes, there is great interest in understanding the impact of these conditions on a wide range of cancers. DM is known to be associated with an increased risk of breast cancer, colorectal cancer, endometrial cancer, pancreatic cancer, liver and bladder cancer, but not prostate cancer [11]. In our study, it was shown that in male mice of the BALB/c Nude line, unlike females, it was possible with the help of alloxan injections to cause diabetes at a later date and with lower blood glucose levels. In males, it was not possible to transfer Lewis carcinoma either in an independent version or against the background of diabetes mellitus, which we associate with the protective role of the hormonal background, in particular androgens.

At the same time, it was found that throughout the experiment, in females with an increase in Lewis carcinoma on the background of DM, the blood glucose level was more than 4 times higher than in animals of the control group. At the same time, a malignant tumor on the background of DM developed much faster than in an independent variant, the volume of tumors increased rapidly, the life expectancy of females was less. Experimental studies of gestational diabetes have shown that in response to an intrauterine environment with a high glucose content, the fetus undergoes a number of adaptive changes, such as acceleration of catabolism and glucose utilization, which affects its growth and development [12]. Drawing a certain parallel between the growth of a malignant tumor and pregnancy, when both physiological and pathological processes are "protecting someone else's in their own", it can be assumed that a malignant tumor, receiving additional glucose resources, could significantly accelerate its proliferative potential.

However, there is a growing understanding that the direct contribution of glucose to the metabolism of cancer cells may be greater than Warburg assumed. The effect of diabetes on cancer development is associated with complex biology, including not only insulin resistance and elevated glucose levels, but also inflammation, effects on immunity and hormonal background [11].

Sex hormones are naturally produced in the body and are of fundamental importance for controlling

functions as the oldest growth factors and as bioactive substances capable of exerting genomic effects by binding directly to nuclear receptors. Steroid hormones can also perform non-genomic functions by binding to or near the plasma membrane, causing rapid changes in cellular physiology [13]. Their production is finely regulated, because even a small change in their quantity can cause serious consequences for the whole organism [14]. Among the many functions performed by hormones, some are also associated with cell proliferation and may affect the development of cancer [15].

In our study, it was found that the tumor samples in the animals of the main group contained much higher concentrations of all steroid hormones – E2, E1, T and P4, as well as prolactin. It is obvious that in addition to high glucose concentrations, sex steroids, as well as prolactin, which can act as a growth factor, stress hormone and influence the immune system by suppressing the production of interleukin-6, were necessary for accelerated proliferation of tumor tissue. At the same time, attention is drawn to the fact that if in animals of the control group a higher content of sex steroids was found in the perifocal zone, then in animals of the main group, on the contrary, directly in the neoplasm.

The fact that we found an increase in the local content of sex hormones in the females of the main group is not unexpected. Estradiol levels are known to have been elevated in menopausal women with DM-2, suggesting that excess estrogen may also have played a role in the risk of developing insulin resistance. The link between excess testosterone and diabetes in women has been known for almost a century. In postmenopausal women, higher plasma levels of estradiol and testosterone were associated with an increased risk of diabetes mellitus [16]. In women, hyperandrogenic conditions, such as polycystic ovary syndrome, are associated with insulin resistance, glucose intolerance and subsequent diabetes mellitus. High testosterone levels cause insulin resistance due to a decrease in insulin-stimulated glucose uptake in healthy pre- and postmenopausal women [17; 18]. That is, insulin resistance has a pronounced relationship with hormonal background.

It is known that sex hormone receptors, such as androgen receptors (RA), estrogen receptors (RE) (for example, REα, REß) and progesterone receptors (RP), are a group of steroid receptors that are activated by binding ligands, androgens, estrogens and progestogens, respectively. Recent results indicate the vital role of sex hormone receptor signals in the pathogenesis of urothelial cancer, which may be the main cause of sex differences in bladder cancer. In particular, RA activation has been implicated in urothelial oncogenesis, whereas there are conflicting results regarding the effects of estrogen, which may depend on the functional activity of REα compared to REß in malignant cells [19].

It is also known that REα, REβ and RA are important receptors involved in glucose metabolism in peripheral tissues. They promote glucose and energy homeostasis during androgen/estrogen binding in these tissues by reducing lipogenesis and increasing insulin secretion and sensitivity [20].

It was interesting to find a lower content of steroid hormone receptors both in the tumor samples and in the perifocal zone in the females of the main group, compared with the control, despite an increase in the concentrations of sex steroids. This can be attributed, on the one hand, to the predominance of rapid non-genomic reactions of sex steroids, which stimulate tissue proliferation, and on the other hand, to the departure of the tumor from organizational control by regulatory authorities. Thus, it is known that triple-negative breast cancer, characterized by the complete absence of steroid hormone receptors in the tumor tissue, is a more aggressive biological subtype, unlike luminal BC [21; 22].

CONCLUSION

Thus, in our study it was shown that the growth of Lewis carcinoma against the background of alloxan-induced diabetes mellitus was possible exclusively in female mice of the BALB/c Nude line and was characterized by a large average volume of tumors throughout the experiment and a reduction in the life expectancy of animals. At the same time, tumors in females with the development of a malignant process on the background of diabetes, although they were more saturated with sex steroids, were depleted of steroid hormone receptors, which probably contributed to the possibility of avoiding regulatory signals of the body.

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Cheryarina N. D. – RIA tests, manuscript analysis.
All authors have contributed equally to the preparation of the publication.

South Russian Journal of Cancer 2023. Vol. 4, No. 1. P. 23-33
https://doi.org/10.37748/2686-9039-2023-4-1-3
https://elibrary.ru/gwlsqd
ORIGINAL ARTICLE

INDICES OF INSULIN-LIKE GROWTH FACTORS FAMILY IN THE LUNG TISSUE OF PATIENTS WITH NON-SMALL CELL LUNG CANCER AFTER COVID-19 OF VARIOUS SEVERITY

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ABSTRACT

Purpose of the study. An analysis of levels of IGF and their carrying proteins in lung tissues of cancer patients depending on the severity of the previous COVID-19 infection.

Patients and methods. The study included 60 patients with histologically verified non-small cell lung cancer (NSCLC) $T_{1-3}N_xM_0$ receiving treatment at the Thoracic Department, National Medical Research Centre for Oncology, in 2020–2021. The control group included 30 NSCLC patients after asymptomatic or mild COVID-19 disease (15 males and 15 females); the main group included 30 (15 men and 15 women) patients after severe or moderate to severe COVID-19 infection. The mean age of patients was 59.11 ± 2.89 years; no significant differences were noted between the control and main groups. All participants gave their informed consent prior to the study approved by the Ethics Committee of National Medical Research Centre for Oncology. Qualitative assessment of IGF-I, IGF-II and IGFBP-1,2,3 levels in the tissues of the tumor, peritumoral area and resection line were measured by ELISA (Mediagnost, Germany). The statistical analysis was performed in the Statistica 10 program, the differences were considered statistically significant at $p < 0.05$.

Results. Regardless of the gender, levels of IGF-I and IGF-II in tumor and resection line samples in patients of the main group were higher than in the control group on average by 1.5–2.2 times, and IGFBP-1 in the tumor was lower by 1.3 times in men and by 5 times in women. The ratio of IGF and IGFBP-1-3 in patients of the control group in perifocal tissues changed towards the parameters in the tumor tissue. IGF/IGFBP-1-3 in men of the main group were lower or did not differ from the indices in the intact tissue, while in women they increased, similarly to the tumor tissue.

Conclusion. An increase in the ratio of IGF and carrier proteins in the tumor tissue of patients in the main group indicated an excessive accumulation of IGF in it, which may contribute to more aggressive growth of malignant tumors. The most pronounced disorders in the system of insulin-like growth factors were found in the tissues of the tumor and intact lung of patients with previous severe and moderate to severe COVID-19.

Keywords:
non-small cell lung cancer, COVID-19, IGF-I, IGF-II, IGFBP

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The work followed the ethical principles set forth by the Helsinki Declaration of the World Medical Association (World Medical Association Declaration of Helsinki, 1964, ed. 2013). The study was approved by the Ethics Committee of the National Medical Research Centre for Oncology (Protocol No. 6 of 02/17/2022). Informed consent was obtained from all participants of the study.

Funding: this work was not funded.
Conflict of interest: authors report no conflict of interest.

For citation:
Kit O. I., Frantsiyants E. M., Kharagezov D. A., Bandovkina V. A., Cheryarina N. D., Pogorelova Yu. A., Lazutin Yu. N., Milakin A. G., Leyman I. A., Stateshny O. N. Indices of insulin-like growth factors family in the lung tissue of patients with non-small cell lung cancer after COVID-19 of various severity. South Russian Journal of Cancer. 2023; 4(1): 23-33. https://doi.org/10.37748/2686-9039-2023-4-1-3, https://elibrary.ru/gwlsqd

The article was submitted 19.08.2022; approved after reviewing 09.01.2023; accepted for publication 06.03.2023.

Южно-Российский онкологический журнал 2023. Т. 4, № 1. С. 23-33
https://doi.org/10.37748/2686-9039-2023-4-1-3
https://elibrary.ru/gwlsqd
3.1.6. Онкология, лучевая терапия
ОРИГИНАЛЬНАЯ СТАТЬЯ

ПОКАЗАТЕЛИ СЕМЕЙСТВА ИНСУЛИНОПОДОБНЫХ ФАКТОРОВ РОСТА В ТКАНИ ЛЕГКОГО БОЛЬНЫХ НЕМЕЛКОКЛЕТОЧНЫМ РАКОМ ЛЕГКОГО, ПЕРЕНЕСШИХ COVID-19 РАЗЛИЧНОЙ СТЕПЕНИ ТЯЖЕСТИ

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РЕЗЮМЕ

Цель исследования. Изучить содержание IGF и их белков-переносчиков в тканях легкого больных немелкоклеточным раком легкого (НМРЛ) в зависимости от тяжести перенесенного COVID-19.

Пациенты и методы. В исследование включены 60 больных с гистологически подтвержденным НМРЛ стадии $T_{1-3Nx}M_0$, проходивших лечение в торакальном отделении ФГБУ «НМИЦ онкологии» Минздрава России с 2020 по 2021 гг. В контрольную группу вошли 30 больных раком легкого с бессимптомными или легкими случаями COVID-19 (15 мужчин и 15 женщин), в основную группу – 30 больных (15 мужчин и 15 женщин), перенесших болезнь в тяжелой или среднетяжелой форме. Средний возраст больных составил 59,11 ± 2,89 года, значимых отличий между контрольной и основной группами не отмечали. Перед началом исследования от участников было получено письменное информированное согласие, одобренное советом по этике ФГБУ «НМИЦ онкологии» Минздрава России. Количественную оценку содержания в ткани опухоли, перифокальной зоне и линии резекции IGF-I, IGF-II и IGFBP-1,2,3 выполняли методом иммуноферментного анализа (ИФА методом (Mediagnost, Германия)). Статистический анализ проводили с использованием программы Statistica 10, значение $p < 0,05$ рассматривалось как показатель статистической значимости.

Результаты. У больных основной группы, по сравнению с контрольной группой, вне зависимости от пола, в образцах опухоли и линии резекции уровень IGF-I и IGF-II был выше в среднем в 1,5–2,2 раза, а IGFBP-1 в опухоли был ниже в 1,3 раза у мужчин и в 5 раз у женщин. Соотношение IGF и IGFBP-1-3 у больных контрольной группы в ткани перифокальной зоны изменялись в сторону показателей ткани опухоли. В основной группе у мужчин IGF/IGFBP-1-3 оказались ниже или не отличались от условно интактной ткани, а у женщин повышались, как и в ткани опухоли.

Заключение. Повышение соотношения IGF и белков-переносчиков в ткани опухоли больных основной группы свидетельствовало об избыточном накоплении в ней IGF, что может способствовать более агрессивному росту злокачественной опухоли. Наиболее выраженные нарушения в системе инсулиноподобных факторов роста мы обнаружили в ткани опухоли и интактного легкого больных, перенесших COVID-19 в тяжелой и среднетяжелой форме.

Ключевые слова:
немелкоклеточный рак легкого, COVID-19, IGF-I, IGF-II, IGFBP

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В работе соблюдались этические принципы, предъявляемые Хельсинкской декларацией Всемирной медицинской ассоциации (World Medical Association Declaration of Helsinki, 1964, ред. 2013). Исследование одобрено этическим комитетом ФГБУ «НМИЦ онкологии» Минздрава России (протокол № 6 от 17.02.2022 г.). Информированное согласие получено от всех участников исследования.

Финансирование: финансирование данной работы не проводилось.
Конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

Для цитирования:
Кит О. И., Франциянц Е. М., Харагезов Д. А., Бандовкина В. А., Черярина Н. Д., Погорелова Ю. А., Лазутин Ю. Н., Милакин А. Г., Лейман И. А., Статешный О. Н. Показатели семейства инсулиноподобных факторов роста в ткани легкого больных немелкоклеточным раком легкого, перенесших COVID-19 различной степени тяжести. Южно-Российский онкологический журнал. 2023; 4(1):23-33. https://doi.org/10.37748/2686-9039-2023-4-1-3, https://elibrary.ru/gwlsqd

Статья поступила в редакцию 19.08.2022; одобрена после рецензирования 09.01.2023; принята к публикации 06.03.2023.

INTRODUCTION

At the end of 2019, a new coronavirus infection (COVID-19) spread rapidly around the world, causing more than 105 million cases of the disease and more than 2.3 million deaths [1]. The destruction of lung cells caused by COVID-19 infection triggers a local immune response, recruiting macrophages and monocytes, releasing chemokines and pro-inflammatory cytokines, thereby triggering adaptive immune responses of T- and B-lymphocytes [2]. In most patients with COVID-19, the recruited cells clear the lungs of infection, then the immune response decreases, and patients carry the disease asymptomatically or in a mild form that does not require hospitalization. On the other side, some patients have a severe course of the disease and even die. At the same time, the deterioration of the condition is often associated with unbridled inflammatory damage caused by a cytokine storm, an uncontrolled immune response leading to acute respiratory distress syndrome (ARDS) [3; 4]. In addition, there is evidence that patients who have undergone COVID-19 with a severe course have a significant transcriptional shift, including the genes of the G protein-linked receptor family, *DNAJB1*, *IGF*, *EGFR* and *HDGF*, which can lead to tissue remodeling, mitochondrial dysfunction, and serious systemic disorders [1; 2].

Among all types of cancer during the pandemic, lung cancer patients are of particular interest, since the lungs are the organs most involved in the initial focus of infection, with a high risk of pneumonia and, in severe cases of ARDS, often with irreversible scarring of lung tissue and respiratory problems that persist largely after recovery [2; 4]. In fact, lung cancer is one of the most common types of cancer among COVID-19 patients, due to a local violation of immunity [5; 6].

It is known that the neuroendocrine system plays an important role in the regulation of immune responses [7]. Steroid and peptide hormones, growth factors, including insulin-like factors are synthesized and secreted by various immune cells, and are able to modulate the humoral and cellular immune response by stimulating and proliferating immunocompetent cells [8].

Determination of the important role of the components of the insulin-like growth factor (IGF) family in the carcinogenesis of a number of tumors, including lung cancer, is based on numerous epidemiological and preclinical studies, *in vivo* and *in vitro* experiments and attempts to use drugs that affect the IGF axis [9; 10]. It is known that in lung cancer, the copyicity of genes responsible for the regulation of apoptosis, proliferation, DNA repair, as well as the expression of a number of growth factors changes [11].

Previous studies have confirmed IGF activity in lung tissue. In other words, IGF signaling plays an essential role in lung pathology. Dysregulation of the IGF axis has been demonstrated at all stages of lung carcinogenesis, ranging from dysplastic lesions of the bronchial epithelium to advanced forms of cancer. In addition, IGF-I has been shown to be involved in various diseases, including metabolic disorders, congenital disorders, inflammation, fibrosis, cancer, acute lung injury and ARDS. Additional studies have shown that high IGF-I and IGF-II expression, as well as IGFBP-3 aberrations, are associated with poor prognosis, metastases and progression of malignant diseases [11; 12]. IGF-I is a biomarker in patients with hyperoxia-induced lung damage. IGF-I levels are elevated in lung biopsy samples in ARDS compared to those in healthy people [11]. Serum levels of IGF-I and growth factor-3 binding protein (IGFBP-3) were found to be elevated in patients with early respiratory distress syndrome when epithelial cells are damaged and die. However, their level was low in the late stages of ARDS [13]. In a prospective case-control study, it was shown that the initial plasma levels of IGF-I and IGFBP-3 were significantly lower in cases of ARDS than in the control [14]. Among ARDS cases, IGF-I and IGFBP-3 levels were lower in patients who did not survive than in survivors, and both groups were negatively associated with the risk of 60-day mortality [14].

Although the role of IGF-I in COVID-19 has not been fully determined, it is known that it modulates influenza A-mediated lung damage in rats [15]. Elevated concentrations of inflammatory cytokines, such as IL-6, TNF-α, are considered as one of the main causes of ARDS in patients infected with COVID-19. Therefore, effective suppression of the cytokine storm is important to prevent deterioration and reduce mortality from COVID-19 [3]. It is assumed that patients with lung cancer are at a higher risk of this severe form of COVID-19 [5; 6]. Recent studies have shown that the mortality rate of lung cancer patients is higher than that of the general population when infected with COVID-19 [16].

The accuracy of treatment prescriptions in patients with non-small cell lung cancer depends on the exact histological classification of the tumor, analysis of specific markers and genetic mutations, which allows choosing the most effective individual method of therapy, excluding the use of empirical treatment and the associated risk of side effects [11; 12].

Given the role of the IGF system in lung development and its involvement in immune responses, the assessment of IGF levels and their binding proteins may further shed light on the mechanisms underlying the pathogenesis of lung cancer against the background of COVID-19.

The purpose of the study: to examine the system of insulin-like growth factors and their carrier proteins in the lung tissues of patients with NSCLC, depending on the severity of COVID-19.

PATIENTS AND METHODS

Before the start of the study, oral and written informed consent was received from the participants, approved by the Ethics Council of the National Medical Research Centre for Oncology. The study included men and women (60 people in total) with histologically or cytologically confirmed stage $T_{1-3}N_xM_0$ NSCLC, ECOG(PS) ≤ 2 working status, adequate organ function based on standard laboratory tests, including a general blood test, serum biochemistry and coagulogram. The main exclusion criteria were previous treatment of NSCLC, type II diabetes mellitus, as it could affect IGF levels, and other concomitant neoplasms over the past five years, with the exception of non-melanoma skin carcinomas. The stage was determined according to the TNM classification. The step-by-step examination included computed tomography (CT) of the chest, abdominal cavity and brain. Bone scans were performed based on symptoms. All patients were examined before the start of treatment.

The control group included 30 patients with lung cancer with asymptomatic or mild cases of COVID-19 (15 men and 15 women), the main group included 30 patients (15 men and 15 women) who had the disease in severe or moderate form. The average age of the patients was 59.11 ± 2.89 years, no significant differences between the control and main groups were noted.

According to the recommendations, a PCR smear from the nasopharynx for COVID-19 was obtained in all patients. The selection criteria included patients of both sexes, over the age of 18, and the absence of drug or alcohol dependence. In addition, patients with a known prior inflammatory condition were excluded. There were no significant differences between the groups by gender.

Quantitative assessment of IGF-I, IGF-II and IGFBP-1,2,3 content was performed by the ELISA method (Mediagnost Germany).

Statistical analysis was carried out using the Statistica 10 program. Normality was assessed using Kolmogorov-Smirnov methods, differences between groups were determined using the Student's t–test or the Mann-Whitney U-test, depending on the normality of the distribution. The value of $p < 0.05$ was considered as an indicator of statistical significance.

RESEARCH RESULTS

Initially, it was of interest to study the lung tissue not affected by the malignant process – conditionally intact tissue (resection line). The results are presented in table 1. In the control group, there were differences in the content of some IGF system factors in intact lung tissue between men and women. Thus, men had 2.6 times higher IGF-I level, 2 times higher IGFBP-1 level, and women had 1.4 times higher IGF-II level. There was no difference in the content of IGFBP-2 and IGFBP-3.

In the intact lung tissue of patients of the main group, a statistically significant increase was found relative to the indicators in the control group of IGF-I and IGF-II levels: in men – 1.6 times and 1.8 times, respectively, in women – 2.2 times and 1.8 times, respectively. At the same time, the higher content of IGF-I in men and IGF–II in women remained. Of the studied carrier proteins, the difference between the control and the main groups was found only for IGFBP-2: an increase of 1.3 times in men and 1.9 times in women.

Next, the tumor tissue was studied. It was found that in the tumor tissue of the control group of men and women, the level of IGF-I was 1.5 times and 1.9 times higher than in the corresponding conditionally intact tissue, respectively, and IGF-II – 1.5 times and 2.1 times, respectively. The level of carrier proteins was reduced: IGFBP-1 by 1.75 times in men, IGFBP-2 by 1.4 times in men and 2.2 times in women, and IGFBP-3 by 1.6 times in men and 6.4 times in women.

In the tumor tissue of the main group, an increase in the level of IGF-I in men and women relative to the corresponding intact tissue was also found by 1.6 times and 1.3 times, respectively, IGF-II – by 1.6 times and 2 times, respectively. The level of IGFBP-1 in men was 2 times lower relative to the corresponding intact tissue, in women – 6 times. The level of IGFBP-2 was 3.3 times lower only in women, and IGFBP-3 was 1.7 times lower in both men and 4.4 times lower in women. At the same time, the higher content of IGF-I in men and IGF–II in women also remained.

It turned out that in the tumor samples in men of the main group, the level of IGF-I and IGF-II was 1.7 times and 2 times higher than in the control group, respectively, against the background of 2.1 times increased IGFBP-2 and the absence of significant changes in the levels of other binding proteins, whereas in women of the main group in the samples Compared with the control tumor, the level of IGF-I and IGF-II was increased by 1.5 times and 1.7 times, respectively, but IGFBP-1 was reduced by 5 times.

The perifocal zone of the tumor is a kind of buffer area between the tumor and conditionally unaffected lung tissue. It seems that the individual absolute values of the family of insulin-like growth factors and their carrier proteins in the tissues of the perifocal zone in patients of both sexes of the control and main groups are more close to the values in the tumor tissue than in the resection line. Thus, in patients of both sexes of the control group, the level of IGF-I and IGF-II in the perifocal zone was 1.3–1.7 times higher on average, compared with conditionally intact tissue; compared with the indicators in the tumor, there were no significant differences in men, and in women IGF-II was 1.4 times lower. The content of carrier proteins in the perifocal zone in men of the control group was 1.6 times lower on average than in the resection line, in women IGFBP-2 and IGFBP-3 were 1.4 times and 6.9 times lower, respectively, but all indicators of carrier proteins did not significantly differ from the tumor and only IGFBP-2 in women were 1.5 times higher. In patients of the main group, the situation was different: in men in the n/a zone, the level of IGF-II and IGFBP-1 was higher than in the resection line, but lower IGF-II and higher IGFBP-1 than in the tumor, while the indicators of IGF-I and other carrier proteins in men of the main group did not differ from the resection line. In the women of the main group, the content of IGF-II in the n/a was lower than in the resection line and the tumor, all the carrier proteins were in greater numbers in the resection line, and IGF-I had no significant differences from the indicators in the conditionally intact tissue and in the tumor.

Of particular interest was the ratio of IGF to carrier proteins, on the one hand demonstrating the bioavailability of the studied growth factors, and on the other hand indicating the possible prevailing biological effects of IGFBP (Table 2).

When studying the ratio of insulin-like growth factors and carrier proteins in the intact tissue of all lung cancer patients, sexual differences were found. Thus, the indicators of IGF-I/IGFBP-2 and IGF-I/IGFBP-3 in women were 2.3 times and 4 times lower than in men in the control group, respectively, and in the main group, on average 2.3 times, IGF-II/IGFBP-1 and IGF-II/IGFBP-2, on the contrary, is higher: in the control group 2.7 times and 1.6 times, respectively, in the main group IGF-II/IGFBP-1 is 1.8 times higher; whereas IGF-I/IGFBP-1 and IGF-II/IGFBP-3 had no significant differences in the control group and IGF-I/IGFBP-1, IGF-II/IGFBP-2 and IGF-II/IGFBP-3 – in the main group.

At the same time, most of the calculated coefficients in intact tissue, with the exception of IGF-I/IGFBP-2 and IGF-II/IGFBP-2, were higher in patients of the main group, compared with the control group. Thus, in the intact tissue of men of the main group, the level of IGF-I/IGFBP-1, IGF-I/IGFBP-3, IGF-II/IGFBP-1 and IGF-II/IGFBP-3 was on average 1.9 times higher than in the corresponding control group. In intact tissue of women, the level of IGF-I/IGFBP-1, IGF-I/IGFBP-3, IGF-II/IGFBP-1 and IGF-II/IGFBP-3 was 1.7–3.3 times higher than in the corresponding control group.

It is obvious that there was also a dissonance between the level of IGF and carrier proteins in the tumor tissue, since the content of growth factors increased in tumor samples, and proteins, with rare exceptions (IGFBP-2 in men of the main group and IGFBP-1 in women of the control group), on the contrary, decreased. Moreover, this concerned the tumor tissue of patients of both groups. In the tumor tissue of male and female patients of the control group, all the studied coefficients significantly exceeded similar values in the corresponding intact tissues. In men, the level of IGF-I/IGFBP-1, IGF-I/IGFBP-2, IGF-I/IGFBP-3, IGF-II/IGFBP-1, IGF-II/IGFBP-2 and IGF-II/IGFBP-3 was

Table 1. Levels of insulin-like growth factors and their carrier proteins in lung tissues of cancer patients, depending on the severity of COVID-19

Groups	Sex	IGF-I ng/g t	IGF-II ng/g t	IGFBP-1 ng/g t	IGFBP-2 ng/g t	IGFBP-3 ng/g t
Resection line tissue						
Control	Males	9.6 ± 0.75 p^1 = 0.0000	6.7 ± 0.57 p^1 = 0.0000	0.35 ± 0.04 p^1 = 0.0000	22.8 ± 1.28	226.9 ± 14.8
	Females	3.7 ± 0.31	9.4 ± 0.83	0.18 ± 0.02	20.3 ± 1.78	296.7 ± 21.3
Main	Males	14.9 ± 1.18 p^1 = 0.0000 p^2 = 0.0000	11.8 ± 1.1 p^1 = 0.0000 p^2 = 0.0000	0.3 ± 0.02 p^1 = 0.0000	30.4 ± 2.3 p^1 = 0.0000 p^2 = 0.0000	204.4 ± 15.8 p^1 = 0.0000
	Females	8.3 ± 0.64 p^2 = 0.0000	16.7 ± 1.3 p^2 = 0.0000	0.24 ± 0.02	39.3 ± 2.5 p^2 = 0.0000	255.8 ± 19.4
Tumor tissue						
Control	Males	14.1 ± 1.8 p^1 = 0.0000 p^3 = 0.0000	9.8 ± 1.9 p^1 = 0.0000 p^3 = 0.0000	0.2 ± 0.03 p^3 = 0.0000	16.1 ± 1.5 p^1 = 0.0000 p^3 = 0.0000	142.2 ± 30.7 p^1 = 0.0000 p^3 = 0.0000
	Females	7.1 ± 1.3 p^3 = 0.0000	19.7 ± 1.4 p^3 = 0.0000 p^4 = 0.0000	0.2 ± 0.03	9.3 ± 2.6 p^3 = 0.0000 p^4 = 0.0000	46.2 ± 2.5 p^3 = 0.0000
Main	Males	24.4 ± 3.4 p^1 = 0.0000 p^2 = 0.0000 p^3 = 0.0000 p^4 = 0.0000	19.3 ± 2.4 p^1 = 0.0000 p^2 = 0.0000 p^3 = 0.0000	0.15 ± 0.01 p^1 = 0.0000 p^3 = 0.0000 p^4 = 0.0000	33.1 ± 2.6 p^1 = 0.0000 p^2 = 0.0000	123.4 ± 9.2 p^1 = 0.0000 p^3 = 0.0000 p^4 = 0.0000
	Females	10.7 ± 1.6 p^3 = 0.0000	33.3 ± 2.5 p^2 = 0.0000 p^3 = 0.0000 p^4 = 0.0000	0.04 ± 0.01 p^2 = 0.0000 p^3 = 0.0000 p^4 = 0.0000	11.8 ± 1.5 p^3 = 0.0000	58.7 ± 5.0 p^3 = 0.0000 p^4 = 0.0000
Perifocal zone tissue						
Control	Males	12.0 ± 0.94 p^1 = 0.0000 p^3 = 0.0000	8.6 ± 0.65 p^1 = 0.0000 p^3 = 0.0000	0.2 ± 0.03 p^3 = 0.0000	16.6 ± 1.2 p^3 = 0.0000	135.3 ± 10.0 p^1 = 0.0000 p^3 = 0.0000
	Females	6.3 ± 0.65 p^3 = 0.0000	14.6 ± 1.1 p^3 = 0.0000	0.17 ± 0.02	14.2 ± 1.2 p^3 = 0.0000	43.3 ± 3.5 p^3 = 0.0000
Main	Males	12.3 ± 1.0	16.8 ± 1.3 p^1 = 0.0000 p^2 = 0.0000 p^3 = 0.0000	0.41 ± 0.03 p^1 = 0.0000 p^2 = 0.0000 p^3 = 0.0000	34.5 ± 2.7 p^1 = 0.0000 p^2 = 0.0000	261.3 ± 21.0 p^1 = 0.0000 p^2 = 0.0000
	Females	10.3 ± 0.88 p^2 = 0.0000	11.5 ± 1.0 p^2 = 0.0000 p^3 = 0.0000	0.08 ± 0.009 p^2 = 0.0000 p^3 = 0.0000	14.2 ± 1.2 p^3 = 0.0000	77.9 ± 5.9 p^2 = 0.0000 p^3 = 0.0000

Note: statistically significant in relation to: [1] – to the indicator for women in the corresponding group; [2] – to the corresponding indicator in the control group; [3] – ro the corresponding indicator in tumor tissue; [4] – to the corresponding indicator in the perifocal zone tissue;
"g t" stands "gram of tissue".

Table 2. Ratios of insulin-like growth factors to carrier proteins in lung tissues in cancer patients, depending on the severity of COVID-19

Groups	Sex	IGF-I/ IGFBP-1	IGF-I/ IGFBP-2	IGF-I/ IGFBP-3	IGF-II/ IGFBP-1	IGF-II/ IGFBP-2	IGF-II/ IGFBP-3
Resection line tissue							
Control	Males	28.0 ± 5.4	0.42 ± 0.02 p^1 = 0.0000	0.04 ± 0.006 p^1 = 0.0000	19.6 ± 4.0 p^1 = 0.0000	0.29 ± 0.02 p^1 = 0.0000	0.03 ± 0.004
	Females	20.7 ± 1.4	0.18 ± 0.009	0.01 ± 0.0007	52.5 ± 3.7	0.46 ± 0.04	0.03 ± 0.001
Main	Males	49.9 ± 5.7 p^2 = 0.0000	0.49 ± 0.07 p^1 = 0.0000	0.07 ± 0.01 p^1 = 0.0000 p^2 = 0.0000	39.7 ± 6.1 p^1 = 0.0000 p^2 = 0.0000	0.39 ± 0.05	0.06 ± 0.009 p^2 = 0.0000
	Females	35.0 ± 5.5 p^2 = 0.0000	0.21 ± 0.03	0.03 ± 0.001 p^2 = 0.0000	69.7 ± 3.4 p^2 = 0.0000	0.43 ± 0.03	0.07 ± 0.01 p^2 = 0.0000
Tumor tissue							
Control	Main	70.6 ± 11.4 p^1 = 0.0000 p^3 = 0.0000	0.88 ± 0.16 p^3 = 0.0000	0.11 ± 0.04 p^1 = 0.0003 p^3 = 0.0000	49.4 ± 8.5 p^1 = 0.0000 p^3 = 0.0000	0.61 ± 0.1 p^1 = 0.0000 p^3 = 0.0000	0.07 ± 0.02 p^1 = 0.0000 p^3 = 0.0000
	Females	36.4 ± 9.4 p^3 = 0.0000	0.82 ± 0.28 p^3 = 0.0000 p^4 = 0.0000	0.15 ± 0.03 p^3 = 0.0000	100.0 ± 13.9 p^3 = 0.0000	2.3 ± 0.7 p^3 = 0.0000 p^4 = 0.0000	0.43 ± 0.04 p^3 = 0.0000 p^4 = 0.0000
Main	Males	165.8 ± 38.0 p^1 = 0.0006 p^2 = 0.0000 p^3 = 0.0000 p^4 = 0.0000	0.74 ± 0.07 p^3 = 0.0000 p^4 = 0.0000	0.2 ± 0.03 p^2 = 0.0000 p^3 = 0.0000 p^4 = 0.0000	128.7 ± 9.6 p^1 = 0.0000 p^2 = 0.0000 p^3 = 0.0000 p^4 = 0.0000	0.58 ± 0.1 p^1 = 0.0000 p^3 = 0.0000 p^4 = 0.0062	0.16 ± 0.02 p^1 = 0.0000 p^2 = 0.0000 p^3 = 0.0000 p^4 = 0.0000
	Females	267.5 ± 34.8 p^2 = 0.0000 p^3 = 0.0000 p^4 = 0.0000	0.91 ± 0.11 p^3 = 0.0000	0.18 ± 0.04 p^3 = 0.0000 p^4 = 0.0000	832.5 ± 94.1 p^2 = 0.0000 p^3 = 0.0000 p^4 = 0.0000	2.9 ± 0.6 p^3 = 0.0000 p^4 = 0.0000	0.57 ± 0.02 p^3 = 0.0000 p^4 = 0.0000
Perifocal zone tissue							
Control	Males	61.4 ± 11.9 p^1 = 0.0000 p^3 = 0.0000	0.73 ± 0.11 p^1 = 0.0000 p^3 = 0.0000	0.09 ± 0.004 p^1 = 0.0000 p^3 = 0.0000	43.9 ± 7.9 p^1 = 0.0000 p^3 = 0.0000	0.52 ± 0.07 p^1 = 0.0000 p^3 = 0.0000	0.06 ± 0.004 p^1 = 0.0000 p^3 = 0.0000
	Females	37.8 ± 7.1 p^3 = 0.0000	0.45 ± 0.08 p^3 = 0.0000	0.15 ± 0.02 p^3 = 0.0000	87.1 ± 13.2 p^3 = 0.0000	1.03 ± 0.08 p^3 = 0.0000	0.34 ± 0.05 p^3 = 0.0000
Main	Males	30.6 ± 4.4 p^1 = 0.0000 p^2 = 0.0000 p^3 = 0.0000	0.36 ± 0.01 p^1 = 0.0000 p^2 = 0.0000 p^3 = 0.0000	0.05 ± 0.003 p^1 = 0.0000 p^2 = 0.0000 p^3 = 0.0000	41.4 ± 1.8 p^1 = 0.0000	0.49 ± 0.08 p^1 = 0.0000	0.07 ± 0.01 p^1 = 0.0000
	Females	130.5 ± 19.3 p^2 = 0.0000 p^3 = 0.0000	0.73 ± 0.12 p^2 = 0.0000 p^3 = 0.0000	0.13 ± 0.009 p^3 = 0.0000	145.4 ± 19.7 p^2 = 0.0000 p^3 = 0.0000	0.82 ± 0.13 p^2 = 0.0000 p^3 = 0.0000	0.15 ± 0.01 p^2 = 0.0000 p^3 = 0.0000

Note: statistically significant in relation to: [1] – to the indicator for women in the corresponding group; [2] – to the corresponding indicator in the control group; [3] – to the corresponding indicator in tumor tissue; [4] – to the corresponding indicator in the perifocal zone tissue; "g t" stands "gram of tissue".

higher by more than 2 times, in women – 1.8 times, 4.6 times, 15 times, 1.9 times, 5 times and 14.3 times, respectively. At the same time, in the tumor tissue of women in the control group, almost all indicators exceeded similar values in the tissue of men.

The same pattern was observed in the tumor tissue of men and women of the main group. In men, the level of IGF-I/IGFBP-1, IGF-I/IGFBP-2, IGF-I/IGFBP-3, IGF-II/IGFBP-1, IGF-II/IGFBP-2 and IGF-II/IGFBP-3 was 1.5–3.3 times higher on average; in women, the values and the spread were wider: 7.6 times, 4.3 times, 6 times, 12 times, 6.7 times and 8.1 times, respectively. At the same time, in the tumor tissue of the women of the main group, almost all indicators (with the exception of IGF-I/IGFBP-3) exceeded similar values in the tissue of men.

In the tissue of the perifocal zone, the balance between growth factors and their carrier proteins also changed. In men and women of the control group, all the coefficients of the ratio of growth factors to carrier proteins were higher than in the resection line by an average of 1.7–2.5 times, only IGF-I/IGFBP-3 and IGF-II/IGFBP-3 in women by 15 times and 11.3 times, respectively, but in men all coefficients had no significant differences from those in the tumor, whereas in women with the exception of IGF-I/IGFBP-2 and IGF-II/IGFBP-2.

In men of the main group in the perifocal zone, only the ratio coefficients of the first insulin-like growth factor to carrier proteins were lower than in the resection line: IGF-I/IGFBP-1, IGF-I/IGFBP-2, IGF-I/IGFBP-3 on average 1.5 times, the ratio of IGF-II to proteins the carriers had no significant differences from the indicators in the resection line. Compared with the tumor in the men of the main group in the perifocal zone, all the ratio coefficients, with the exception of IGF-II/IGFBP-2, were lower: IGF-I/IGFBP-1 by 5.4 times, IGF-I/IGFBP-2 by 2.1 times, IGF-I/IGFBP-3 by 4 times, IGF-II/IGFBP-1 by 3.1 times and IGF-II/IGFBP-3 by 2.3 times. In the women of the main group, all ratio coefficients in the perifocal zone were higher than in the resection line by 1.9–4.3 times, but lower than in the tumor by 1.3–5.8 times.

It turned out that in men in the main group, only the ratio coefficients of the first insulin-like growth factor to carrier proteins were lower than in the control group by an average of 2 times, while the ratio of IGF-II to carrier proteins had no significant differences. In the perifocal zone in women of the main group, compared with the perifocal zone of the control group, IGF-I/IGFBP-1 was 3.5 times higher, IGF-I/IGFBP-2 was 1.6 times higher, and IGF-II/IGFBP-1 was 1.7 times higher, but lower than IGF-II/IGFBP-2 1.3 times and IGF-II/IGFBP-3 2.3 times, only IGF-I/IGFBP-3 had no significant differences.

That is, when considering the ratio of IGF and their carrier proteins in patients of the control group, the indicators in the tissue of the perifocal zone were closer to the values in the tumor tissue. Another trend was noted in the tissue of the perifocal zone of patients of the main group. Thus, the ratio of IFG and carrier proteins in men was closer to the values in the conditionally intact tissue, and in women – to the values in the tumor tissue.

DISCUSSION

Currently, there is no doubt that IGF-axis signaling is crucial for cellular survival, proliferation, antioxidant function and control of cell damage and death in various organs and tissues, including the lungs [17; 18]. The family of insulin-like growth factors includes IGF-I, IGF–II, their receptors – IGF-IR and IGF-IIR, and proteins binding insulin-like growth factors – IGFBP-1–6 [19]. Studies have proven the role of IGF signaling in lung development, as well as in inflammatory diseases, cancer and fibrosis [20; 21]. IGF-I and IGF-II are involved in various physiological and pathophysiological processes, including fetal growth and development, metabolic disorders, congenital disorders, inflammation, fibrosis, cancer, acute lung injury [9]. IGF-II has also been found to be overexpressed in some types of cancer, which contributes to tumor growth and survival. At the tissue level, IGF-I and IGF-II are mainly overexpressed in various types of cancer and can serve as a mitogenic stimulus in a paracrine or autocrine manner, and a violation of the regulation of the IGF axis has been demonstrated at all stages of lung carcinogenesis [22].

In his research, Shin J. with co-authors (2022) deployed that COVID-19 infection disrupts the signaling pathway of insulin-like growth factor in respiratory, metabolic and endocrine cells and tissues [23], resulting in violations of innate immune functions, such as neutrophil chemotaxis, phagocytic cell function and recruitment of inflammatory macrophages in tissues [24].

It turned out that even conditionally intact lung tissue taken on the resection line had differences in the

studied parameters of the IGF axis in patients of the control and main groups, and it was in patients who underwent COVID-19 in severe form in the studied tissue that the level of IGF-I and IGF-II was elevated, and the carrier proteins either decreased, or did not change their concentration, with the exception of IGFBP-2. This fact may indicate that in patients with lung cancer, the tumor microenvironment changes under the influence of a severely suffered COVID-19 disease. In particular, it is known that persistent unregulated inflammation at the site of injury disrupts the regeneration process and ultimately leads to the formation of tissue fibrosis and scarring [25]. And human observational studies conducted by Shin and co-authors (2022) showed that higher basal expression of insulin-like growth factor receptors may be associated with an increase in the age of lung tissue in men, as well as with comorbid diseases such as obesity and type 2 diabetes mellitus, which are well-established risk factors for severity and COVID-19 mortality [23]. It is noteworthy that higher expression of IGF receptors and lower expression of IGF/insulin signaling pathway mediators are largely associated with unfavorable critical outcomes in patients with COVID-19 and worse molecular signs of the disease, such as elevated levels of IL-1 and IL-6, cell damage and death [23].

There are six known types of IGFBP, of which IGFBP-3 is the most studied. One of the well-studied roles of IGFBP involves the delivery of IGF to target cells as its endocrine function. In addition, IGFBP-3 secretion has been reported in various tissues, which indicates its paracrine or autocrine function, in addition to endocrine. The role of IGFBP, depending on the insulin-like growth factor, includes facilitated delivery of IGF to its receptors on the cell surface and activation of the downstream signaling cascade associated with it [26].

It is noteworthy that the tumor tissue in patients with lung cancer of the main group, regardless of gender, contained significantly higher concentrations of IGF-I and IGF-II, but lower levels of IGFBP-1 binding proteins, compared with those in men and women of the control group. This may be due to the greater aggressiveness of the tumor process in patients who have undergone COVID19 in severe form, with lung tissue damage. In addition, there is evidence that lung cancer tissue is characterized by increased local production of IGF-I, IGF-II and IGF-I receptor (IGF-IR), but reduced IGFBP expression. Modulated expression of these molecules is associated with aggressive disease, local lymph node metastases, and poor clinical outcomes [27]. Several IGF-IR inhibitors are under clinical development for the treatment of solid tumors, including lung cancer [22].

CONCLUSIONS

These literature data are consistent with the results obtained by us on an increase in the production of IGF-I, IGF-II in the tumor tissue of men and women of the control group and a decrease in the expression of IGF-binding protein-3. In addition to this, we showed a change in the level of two more carrier proteins, which is of a gender nature: in the tumor tissue of men, the expression of IGFBP-1 and IGFBP-2 was also increased, and in the tumor tissue of women – IGFBP-2. Violations in the system of insulin-like factors were especially clearly reflected in the study of the ratio of IGF and carrier proteins. The increase in the ratio of IGF and carrier proteins in the tumor tissue shown by us in this study may indicate an excessive accumulation of IGF in it, which contributes to the growth and survival of neoplasm. The most pronounced disorders in the system of insulin-like growth factors were found in the tumor tissue and intact lung of patients who underwent COVID-19 in severe and moderate form. Considering that IGF-I is involved in inflammation, fibrosis, cancer, acute lung injury and ARDS, and is also a biomarker in patients with hyperoxia-induced lung damage, the results obtained can be considered as a reaction of patients' lung tissue to the infection and its intensive therapy.

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Bandovkina V. A. – preparation and editing of the manuscript, verification of critical intellectual content;
Cheryarina N. D. – statistical analysis of the results obtained, editing of the manuscript;
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South Russian Journal of Cancer 2023. Vol. 4, No. 1. P. 34-42
https://doi.org/10.37748/2686-9039-2023-4-1-4
https://elibrary.ru/hchskm
ORIGINAL ARTICLE

MAJOR AND MINOR POPULATIONS OF LYMPHOCYTES: LOCAL FEATURES IN DIFFERENT STAGES OF COLON CANCER

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ABSTRACT

Purpose of the study. Was to reveal characteristics of the immunocompetent cells in colon cancer (CC) according to the disease stage, and to identify prognostic factors of cancer development.
Materials and methods. The study included 50 patients with CC: stage I – 4 patients (8 %), II – 25 (50 %), III – 21 (42 %). All patients underwent standard surgical intervention at the initial stage, the obtained material was used for subsequent studies: a cell suspension was obtained from the tumor tissue, peritumoral zone (1–3 cm from the tumor), which was treated with an antibody panel (Becton Dickinson, USA) to identify the main subpopulations of leukocytes and lymphocytes.
Results. The tumor tissues of patients with stages I + II showed a decrease in the relative number of DP, DN, NKT and CD19+, compared to peritumoral tissues, by 33 %, 42 %, 31 % and 82 % respectively. Tumor tissues of stage III patients demonstrated elevated relative numbers of CD3+, CD4+, NK by 57 %, 34 %, 48 %, and decreased DP, DN, NKT, CD19+ by 33 %, 74 %, 31 %, 59 %, compared to peritumoral tissues. DP, DN, NKT and CD19+ in the tumor decreased by 78 %, 74 %, 39 %, 60 %, respectively, and the relative number of lymphocytes increased by 138 %, compared to the tissues of the resection line. A comparative analysis of local immunological parameters in the tumor tissues of patients with CC revealed that the relative numbers of lymphocytes and CD19+ were 58 % and 87 % higher, and DP and DN were 33 % and 27 % lower in tumor tissues of stage III patients, compared to tumor tissues of stage I + II patients.
Conclusion. Thus, the obtained features of the local population and subpopulation composition of immunocompetent cells in CC, depending on the stage of the tumor process, can be used to predict the clinical course of the disease.

Keywords:
oncology, colon cancer, local cellular immunity

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The work followed the ethical principles set forth by the Helsinki Declaration of the World Medical Association (World Medical Association Declaration of Helsinki, 1964, ed. 2013). The study was approved by the Ethics Committee of the National Medical Research Centre for Oncology (Protocol No. 32 of 10/08/2020). Informed consent was obtained from all participants of the study.

Funding: the work was performed as part of the state assignment on the "Development of prognostic and predictive algorithms based on the identification of new immunological and molecular genetic characteristics of malignant tumors and their microenvironment" topic, regist. No. 121031100251-9.
Conflict of interest: authors report no conflict of interest.

For citation:
Sagakyants A. B., Dzhenkova E. A., Mirzoyan E. A., Novikova I. A., Zlatnik E. Yu., Bondarenko E. S., Shaposhnikov A. V., Maslov A. A., Kaymakchi O. Yu., Przhedetskiy Yu. V., Shevchenko A. N. Major and minor populations of lymphocytes: local features in different stages of colon cancer. South Russian Journal of Cancer. 2023; 4(1):34-42. https://doi.org/10.37748/2686-9039-2023-4-1-4, https://elibrary.ru/hchskm

The article was submitted 11.10.2022; approved after reviewing 10.01.2023; accepted for publication 06.03.2023.

Южно-Российский онкологический журнал 2023. Т. 4, № 1. С. 34-42
https://doi.org/10.37748/2686-9039-2023-4-1-4
https://elibrary.ru/hchskm
3.1.6. Онкология, лучевая терапия
ОРИГИНАЛЬНАЯ СТАТЬЯ

ОСНОВНЫЕ И МИНОРНЫЕ ПОПУЛЯЦИИ ЛИМФОЦИТОВ: ЛОКАЛЬНЫЕ ОСОБЕННОСТИ ПРИ РАЗЛИЧНЫХ СТАДИЯХ РАКА ОБОДОЧНОЙ КИШКИ

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РЕЗЮМЕ

Цель исследования. Выявить особенности состава иммунокомпетентных клеток при раке ободочной кишки (РОК) в зависимости от стадии процесса и выделить прогностические факторы течения заболевания.
Материалы и методы. В исследование включено 50 пациентов РОК: I стадия – 4 пациента (8 %), II – 25 (50 %), III – 21 (42 %). Всем больным на начальном этапе было выполнено стандартное оперативное вмешательство. Полученный материал был использован для последующих исследований: из ткани опухоли, перитуморальной зоны (1–3 см от опухоли) была получена клеточная суспензия, которую обрабатывали при помощи панели антител (Becton Dickinson, USA) для выявления основных субпопуляций лейкоцитов и лимфоцитов.
Результаты. В тканях опухолей группы пациентов I + II стадии отмечено уменьшение относительного количества ДП, ДН, NKT и CD19+ по сравнению с перитуморальной зоной на 33 %, 42 %, 31 % и 82 % соответственно. В тканях опухоли пациентов с III стадией выявлено повышение относительного количества CD3+, CD4+, NK на 57 %, 34 %, 48 % и снижение ДП, ДН, NKT, CD19+ на 33 %, 74 %, 31 %, 59 % по сравнению с тканью перитуморальной зоны. В опухоли выявлено уменьшение ДП, ДН, NKT, CD19+ на 78 %, 74 %, 39 %, 60 %, а также увеличение относительного количества лимфоцитов по сравнению с линией резекции на 138 % соответственно. При проведении сравнительного анализа локальных иммунологических показателей в тканях опухолей больных РОК выявлено, что в тканях опухолей группы пациентов III стадии отмечено увеличение относительного количества лимфоцитов и CD19+ на 58 % и 87 % и снижение ДП и ДН на 33 % и 27 % по сравнению с тканями опухолей группы I + II стадии.
Заключение. Таким образом, полученные особенности локального популяционного и субпопуляционного состава иммунокомпетентных клеток при РОК в зависимости от стадии опухолевого процесса могут быть использованы при прогнозировании клинического течения заболевания.

Ключевые слова:
онкология, рак ободочной кишки, локальный клеточный иммунитет

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В работе соблюдались этические принципы, предъявляемые Хельсинкской декларацией Всемирной медицинской ассоциации (World Medical Association Declaration of Helsinki, 1964, ред. 2013). Исследование одобрено этическим комитетом ФГБУ «НМИЦ онкологии» Минздрава России (протокол № 32 от 08.10.2020 г.). Информированное согласие получено от всех участников исследования.

Финансирование: работа выполнена в рамках выполнения государственного задания по теме «Разработка прогностических и предиктивных алгоритмов на основе выявления новых иммунологических и молекулярно-генетических характеристик злокачественных опухолей и их микроокружения», рег. № 121031100251–9.
Конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

Для цитирования:
Сагакянц А. Б., Дженкова Е. А., Мирзоян Э. А., Новикова И. А., Златник Е. Ю., Бондаренко Е. С., Шапошников А. В., Маслов А. А., Каймакчи О. Ю., Пржедецкий Ю. В., Шевченко А. Н. Основные и минорные популяции лимфоцитов: локальные особенности при различных стадиях рака ободочной кишки. Южно-Российский онкологический журнал. 2023; 4(1):34-42.
https://doi.org/10.37748/2686-9039-2023-4-1-4, https://elibrary.ru/hchskm

Статья поступила в редакцию 11.10.2022; одобрена после рецензирования 10.01.2023; принята к публикации 06.03.2023.

RELEVANCE

Colorectal cancer (CRC) occupies one of the first places in the structure of morbidity from cancer in the Russian Federation [1; 2]. About 60 % of cases of CRC occur in colon cancer (CC), which occupies the 4th place in the structure of female cancer incidence, the 5th place – male cancer incidence [3].

To date, there are a large number of modern methods of diagnosing the oncoprocess. However, despite this, the level of neglect of colon tumors remains quite high. As a rule, at the time of treatment of patients, the cancer process has advanced stages [4; 5].

More and more research in oncology is associated with the study of the role of the links of the immune system in the occurrence, course, and progression of the cancer process. Its dual role has been proven: on the one hand, the presence of antitumor effects, on the other – tumor-activating. This fact determines the relevance of studying the role of individual components of both innate and adaptive immunity in colocancerogenesis.

The immune system of the gastrointestinal tract plays an important role in protecting the body from the action of infectious agents and toxins, their inactivation and elimination is carried out. It has been proven that both local and systemic inflammatory reactions play an important role in the progression of the tumor process, thereby affecting the outcome of the disease [6; 7]. Colorectal tumors are infiltrated by immune and inflammatory cells, the most significant of which are T-lymphocytes. It is also known that the tissue of tumors of the colon and rectum contains a small number of T-lymphocytes with CD4+ and CD8+ receptors [8].

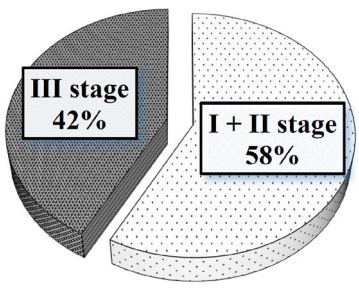

Fig. 1. Distribution of patients according to their disease stage.

Infiltration by macrophages of the tumor and perifocal zone is used as a prognostic factor: low infiltration density is associated with high invasive properties of tumors [9; 10]. There are a number of works in the literature devoted to the study of tumor-infiltrating immunocompetent cells and the assessment of their prognostic significance in CRC, which are very contradictory [11–13]. There is no doubt about the high importance of studying the role of tumor infiltration of lymphocytes, which determine the biological properties of the tumor and the features of the clinical course of the disease. At the same time, there is a small amount of information reflecting the features of lymphocytic infiltration in the tumor tissue, perifocal zone, resection line, depending on the stage of the disease, which determines the purpose of the study.

Purpose of the study was to identify the features of the population and subpopulation composition of immunocompetent cells locally in colon cancer, depending on the stage of the process and to identify prognostic factors of the course of the disease.

MATERIALS AND METHODS

The study included 50 patients with CC who were treated at the National Medical Research Centre for Oncology: 26 of them were women (52 %), whose average age was 67 ± 0.4 years and 24 men (48 %), the average age was 66 ± 0.3 years.

According to the results of postoperative histological analysis, the following distribution was revealed by stages of CC: I – 4 patients (8 %), II – 25 (50 %), III – 21 (42 %), subsequently patients of groups I and II were combined (Fig. 1).

The spread of the tumor within the intestinal wall (T1–3) was noted in 38 (76 %) patients, and a tumor that sprouted into other organs and/or visceral peritoneum T4 – in 12 (24 %). Regional lymph nodes (l.n.) (N+) were affected in 27 patients (54 %). At the 1st stage of treatment, all patients underwent surgical intervention with the collection of material for subsequent studies. A cell suspension was obtained from tumor tissues, the peritumoral zone, and the resection line, processed using an antibody panel (Becton Dickinson, USA) in order to identify the main populations and subpopulations of leukocytes and lymphocytes using a BD FACSCanto flow cytometer (Becton Dickinson, USA). The results were expressed in the relative number of the main populations and

subpopulations of lymphocytes: CD45+ – total number of lymphocytes (Lymph), CD3+ – T-lymphocytes, CD3+CD4+ – T-lymphocytes helper, CD3+CD8+ – cytotoxic T-lymphocytes, CD3+CD4+CD8+ – double positive lymphocytes (DP), CD3+CD4-CD8- – double negative lymphocytes (DN), CD3-CD56+CD16+ – NK lymphocytes, CD3+CD56+CD16+ – NKT lymphocytes, CD19+ – B lymphocytes relative to the total number of lymphocytes.

Statistical processing of the obtained results was carried out using the STATISTICA 13.3 package (StatSoft Inc., USA), which involved calculating the main statistical characteristics of the samples, determining the nature of the distribution of the determined indicators using the Shapiro-Wilk criterion. Due to the fact that the obtained results did not obey the law of normal distribution, the reliability of the differences between the samples was evaluated using the nonparametric Mann-Whitney criterion. The results were considered statistically significant at $p < 0.05$.

RESEARCH RESULTS AND DISCUSSION

We conducted a comparative analysis of the relative number of the main populations and subpopulations of lymphocytes, depending on the stage, the depth of the lesion of the intestinal wall tumor (T), the presence or absence of lesion of regional lymph nodes (N).

When analyzing the data, depending on the stage of the tumor process, it was revealed that in the tumor tissues of the group of patients of stage I–II, there was a decrease in the relative number of DP and DN cells, NKT and CD19+ lymphocytes compared with similar indicators in the peritumoral zone by 33 %, 42 %, 31 % and 82 %, respectively ($p < 0.05$). Compared with conditionally healthy tissue, tumor fragments showed an increase in the relative number of T-lymphocytes, CD3+ and NK cells by 41 %, 35 %, 50 %, while the content of DP-, DN-, NKT-, CD19+ lymphocytes was reduced by 33 %, 42 %, 31 %, 82 % ($p < 0.05$).

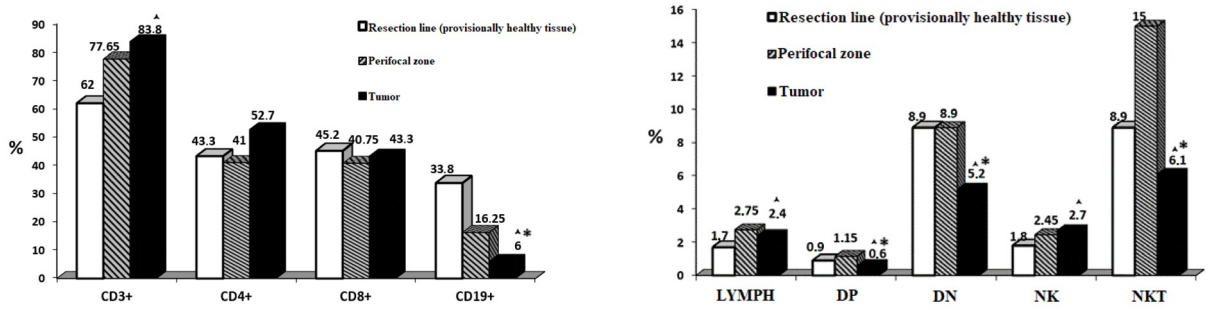

Fig. 2. Subpopulation composition in the tissues of patients with stage I – II colon cancer.
Note: Δ – statistically significant differences from the indicators of the resection line (conditionally healthy tissue); * – statistically significant differences from the indicators of the peritumoral zone.

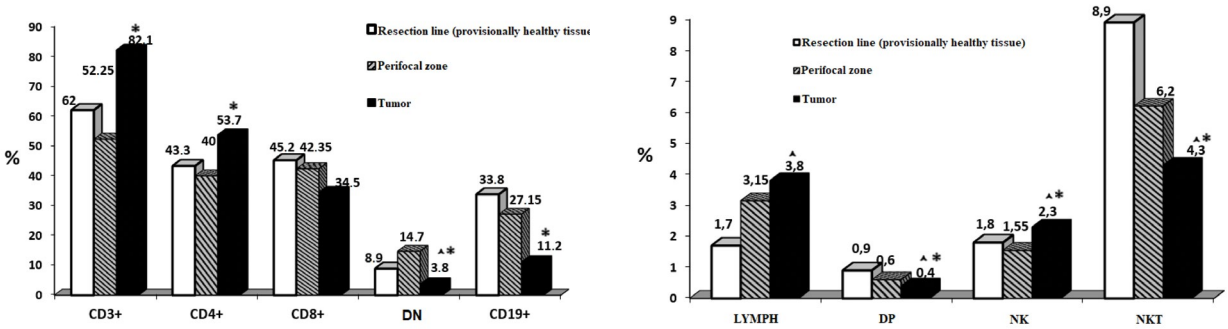

Fig. 3. The subpopulation composition in the tissues of the group of patients with stage III colon cancer.
Note: Δ – statistically significant differences from the indicators of the resection line (conditionally healthy tissue); * – statistically significant differences from the indicators of the peritumoral zone.

In the peritumoral zone, a low content of CD19+ (by 52 %) was noted, as well as an increased content of lymphocytes, CD8+, DP, NK, NKT on 62 %, 25 %, 27 %, 36 % and 69 %, respectively, compared with the indicators in the resection line ($p < 0.05$) (fig. 2).

Analysis of the results of the study of tissues of patients with stage III CC showed that in the tumor tissue there is an increase in the relative number of CD3+, CD4+, NK lymphocytes by 57 %, 34 %, 48 % and a decrease in DP, DN, NKT, CD19+ cells by 33 %, 74 %, 31 %, 59 % by compared with the tissue of the peritumoral zone ($p < 0.05$). In the tumor, there is a decrease in DP, DN, NKT, CD19+ by 78 %, 74 %, 39 %, 60 %, against this background, an increase in the relative number of lymphocytes compared to the resection line was revealed by 138 %, respectively ($p < 0.05$) (Fig. 3).

A comparative analysis of local immunological parameters in the tumor tissues of CC patients revealed that in the tumor tissues of the group of stage III patients there was an increase in the relative number of lymphocytes and CD19+ cells by 58 % and 87 % and a decrease in DP and DN of lymphocytes by 33 % and 27 % compared with the tissues of tumors of stage I and II ($p < 0.05$) (fig. 4).

Based on the data obtained, it was revealed that the tumor is characterized by the accumulation of T-lymphocytes, in particular, T-helper-inductor and B-cells (CD19+), which is especially pronounced in stage III of the disease in patients with CC and a low content of DP and DN lymphocytes, as well as NKT cells.

The existing data in the literature indicate that the tissue of tumors of the colon and rectum is infiltrated by a small number of T-lymphocytes. In 1987, J. R. Jass et al. It has been shown that pronounced lymphocytic infiltration of the perifocal zone is a prognostic factor for longer overall survival of CRC patients [11–13]. It has been proven that the pronounced accumulation of CD8+ T cells in tumor tissue correlates with longer survival of patients [14; 15].

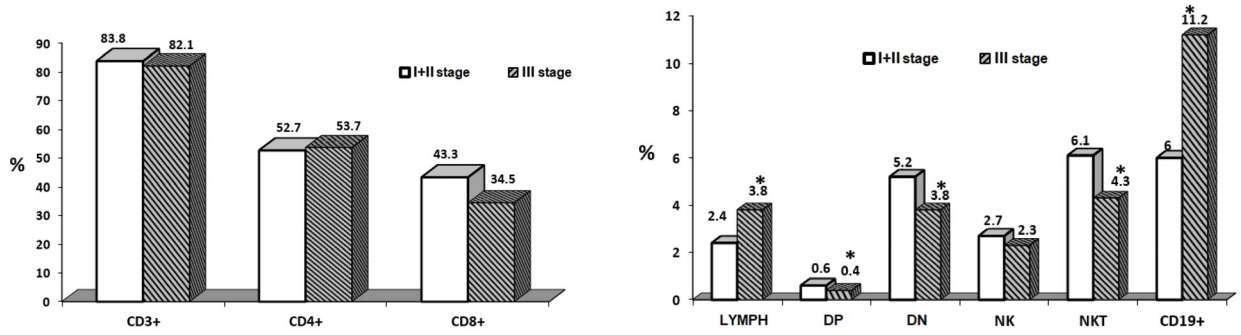

Fig. 4. The subpopulation composition in the tumor tissues of a group of colon cancer patients, depending on the stage.
Note: * – statistically significant differences ($p < 0.05$).

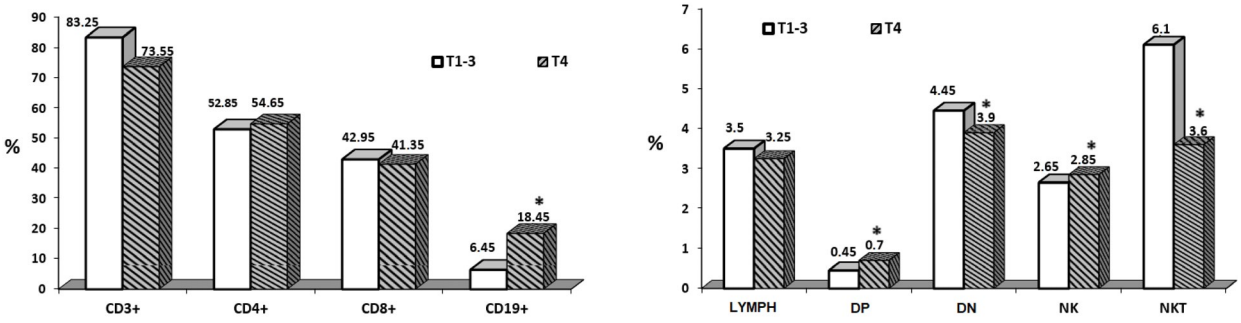

Fig. 5. The subpopulation composition in the tumor tissues of a group of colon cancer patients, depending on the criterion T.
Note: * – statistically significant differences ($p < 0.05$).

Analysis of the subpopulation composition of lymphocytes in tumor tissue, depending on the criterion of the level of invasion of the intestinal wall tumor (T), showed that in the tumor tissues of the T4 patient group, an increase in the relative number of DP cells and CD19+ lymphocytes was noted compared to the tissues of the T1-3 group by 56 % and 186 %, respectively ($p < 0.05$), and also, a decrease in the relative number of NKT lymphocytes by 41 % ($p < 0.05$) (fig. 5).

When comparing the results obtained, depending on the lymph node lesion, it was noted that in the tumor tissues of the group of patients with node lesion (N+), an increase in the relative number of lymphocytes and CD19+ was noted by 96 % and 97 %, as well as a decrease in the relative number of NKT lymphocytes by 32 % compared with the tumor tissues of the group of patients N0 ($p < 0.05$) (Fig. 6).

Our results somewhat contradict the data of Tachibana T. et al., according to which tumor infiltration by NKT cells positively correlated with a smaller number of lymph node metastases [16].

By a group of authors A. C. Diederichsen et al. It has been demonstrated that a low CD4+/CD8+ ratio, i.e. the prevalence of cytotoxic T-lymphocytes in the tumor against the background of a decrease in T-cells with helper-inductor function, is a prognostic factor for long-term survival of CRC patients [17]. The data obtained in our study, indicating certain features of the tissue composition of immunocompetent cells in the tumor and its microenvironment, in some cases agree with the data of other authors and may, in our opinion, be the basis for the development of criteria for the prognosis of the development of CRC metastases [18].

DN lymphocytes – Pinocchio cells (Pinocchio cells) are intermediate elements of differentiating T-lymphocytes. This type of cells resembles cells of innate immunity in functional activity, they form early barrier formations aimed at maintaining immune homeostasis [19].

When studying the features of the immunological organization of soft tissue sarcomas, it was shown that in the tissue of recurrent sarcomas there is a high level of DN T-lymphocytes, which belong to a subpopulation of T cells with TCRγΔ and may have the properties of T-regs [20]. An increase in their level in the blood in some malignant tumors presupposes some negative changes in the immune status [21].

However, there is also an opposite opinion, according to which both DN and DP lymphocytes, being cells of innate immunity, have the opposite effect. In our study, the opposite trend was found – in the case of disease progression, its later stages or the presence of metastases, it was accompanied by an increase in the content of these cells, which, however, does not allow us to draw a final conclusion about the functional significance of this fact.

Currently, there is no clear opinion about the role of B-lymphocytes in the development of tumors and sensitivity to therapy. It has been shown that the number of circulating antibodies increases in the blood of cancer patients, and in the tumor tissue of tumor-infiltrating B-lymphocytes, which perform a protective function [22]. According to another opinion, B cells increase the aggressiveness of the tumor, thereby worsening the prognosis [23]. Under certain conditions, B cells can perform the function of antigen-presenting cells: express stimulating CD80, CD86 and ICOS molecules and activate CD4+, CD8+ T cells [24].

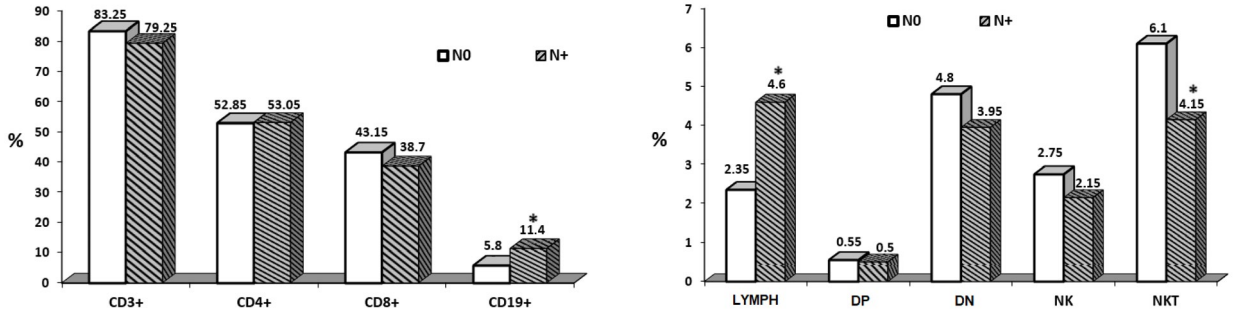

Fig. 6. The subpopulation composition in the tumor tissues of the CC patient group, depending on criterion N.
Note: * – statistically significant differences ($p < 0.05$).

However, activated B-lymphocytes and plasmocytes synthesize antibodies that block the antigens of tumor cells, disrupting their recognition [25]. It has been shown that one of the varieties of B-lymphocytes are cells with high regulatory potential, which is expressed in the secretion of IL-10, IL-35, IL-6, transforming growth factor-β (TGF-β), which causes immunosuppression of antitumor immunity [26]

Despite certain differences in the results of studying the immunological microenvironment of various malignant tumors obtained by various research groups, understanding the mechanisms involved in the interaction between tumor cells and the microenvironment opens up a great prospect for changing the treatment strategy that will help fight tumors more effectively [27].

CONCLUSION

1. The tumor tissue in CC is characterized by the accumulation of T-helper-inductor lymphocytes and B cells (CD19+) and depletion of DP and DN lymphocytes, as well as a decrease in the number of NKT cells, which is more pronounced in stage III of the disease in patients with CC.

2. At the III stage of the disease, in CC, there is a decrease in the activity of local innate immunity, which is manifested in a decrease in the content of NKT cells in the primary tumor, as well as the tension of the humoral link of immunity, due to the high content of tumor CD19+ lymphocytes, the same trend is observed with the defeat of regional lymph nodes (N+).

3. It is possible that an increased number of CD19+ cells is a factor predisposing to the occurrence of lymphogenic metastasis, and is also associated with a more advanced stage of CC.

Thus, the data obtained on the features of the local population and subpopulation composition of immunocompetent cells in colon cancer, depending on the stage, T and N, can be used in the prognosis of the clinical course of the disease.

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South Russian Journal of Cancer 2023. Vol. 4, No. 1. P. 43-51
https://doi.org/10.37748/2686-9039-2023-4-1-5
https://elibrary.ru/hrhtif
ORIGINAL ARTICLE

DYNAMIC ASSESSMENT OF PRESSURIZED INTRAPERITONEAL AEROSOL CHEMOTHERAPY IMPACT ON PERITONEAL CARCINOMATOSIS IN OVARIAN CANCER (PRELIMINARY RESULTS)

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ABSTRACT

Purpose of the study. Dynamic assessment of the direct impact of pressurized intraperitoneal aerosol chemotherapy (PIPAC) on peritoneal carcinomatosis in ovarian cancer.

Patients and methods. The study involved 164 people with visually detectable and morphologically verified ovarian cancer with peritoneal carcinomatosis of the peritoneum (IIIb-IIIc stages of ovarian cancer). All patients underwent combined treatment of ovarian cancer, which included primary cytoreduction and 6 courses of chemotherapy according to the TC scheme. In the main group, the standard treatment was supplemented with 3 PIPAC procedures. Statistical processing was carried out by analyzing the exact criterion of the Wilcoxon-Mann-Whitney sums, the distribution of patients in groups by age and peritoneal lesion was estimated. It was found that the distribution of the analyzed parameters was random. The distribution in the groups by stages of the disease was homogeneous, which is justified by the use of the Barnard criterion. The dynamics of the parameters of the study was evaluated by the methods of basic statistics. Used software packages: MedCals, Statistica.

Results. The results obtained demonstrate a distinct positive dynamics in the group of patients receiving PIPAC in addition to standard treatment of newly diagnosed ovarian cancer: a significant decrease in the peritoneal cancer index, therapeutic pathomorphosis in peritoneal samples during treatment, reduction of ascites.

Conclusion. The team of authors managed to establish that PIPAC simultaneously with standard combined treatment for primary ovarian cancer with peritoneal carcinomatosis makes it possible to achieve a dynamic regression effect of peritoneal carcinomatosis of the peritoneum, morphological regression of carcinomatosis and complete resorption of ascites in the vast majority of treated patients. The revealed therapeutic effect was prolonged and persistent with an objective assessment 6 months after the end of treatment.

Keywords:
ovarian cancer, ascites, peritoneal carcinomatosis, drug pathomorphosis, pressurized intraperitoneal aerosol chemotherapy, PIPAC

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The work followed the ethical principles set forth by the Helsinki Declaration of the World Medical Association (World Medical Association Declaration of Helsinki, 1964, ed. 2013). The study was approved by the Committee on Biomedical Ethics at the National Medical Research Centre for Oncology (extract from the protocol of the meeting No. 660 dated 04/09/2021).
Informed consent was obtained from all participants of the study.

Funding: this work was not funded.
Conflict of interest: authors report no conflict of interest.

For citation:
Dzasokhov A. S., Kostin A. A., Astashov V. L., Andreev M. A., Turiev A. V., Uskov A. D. Dynamic assessment of pressurized intraperitoneal aerosol chemotherapy impact on peritoneal carcinomatosis in ovarian cancer (preliminary results). South Russian Journal of Cancer. 2023; 4(1): 43-51. https://doi.org/10.37748/2686-9039-2023-4-1-5, https://elibrary.ru/hrhtif

The article was submitted 15.12.2022; approved after reviewing 31.01.2023; accepted for publication 06.03.2023.

Южно-Российский онкологический журнал 2023. Т. 4, № 1. С. 43-51
https://doi.org/10.37748/2686-9039-2023-4-1-5
https://elibrary.ru/hrhtif
3.1.6. Онкология, лучевая терапия
ОРИГИНАЛЬНАЯ СТАТЬЯ

ДИНАМИЧЕСКАЯ ОЦЕНКА ВОЗДЕЙСТВИЯ ВНУТРИБРЮШНОЙ АЭРОЗОЛЬНОЙ ХИМИОТЕРАПИИ ПОД ДАВЛЕНИЕМ НА КАНЦЕРОМАТОЗ БРЮШИНЫ ПРИ РАКЕ ЯИЧНИКОВ (НЕПОСРЕДСТВЕННЫЕ РЕЗУЛЬТАТЫ)

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РЕЗЮМЕ

Цель исследования. Динамическая оценка непосредственного воздействия внутрибрюшной аэрозольной химиотерапии под давлением (ВАХД) на перитонеальный канцероматоз при раке яичников.

Пациенты и методы. В исследовании приняли участие 164 человека с визуально определяемым и морфологически верифицированным раком яичников с перитонеальным канцероматозом брюшины (III*b*-IIIc стадии рака яичников). Всем пациенткам проводилось комбинированное лечение рака яичников, включавшее первичную циторедукцию и 6 курсов системной полихимиотерапии (ПХТ) по схеме ТС. В основной группе стандартное лечение было дополнено 3-мя сеансами ВАХД. Статистическая обработка проведена посредством анализа точного критерия сумм Уилкоксона-Манна-Уитни произведена оценка распределения пациенток в группах по возрасту и поражению брюшины. Установлено, что распределение по анализируемым параметрам было случайным. Распределение в группах по стадиям заболевания было гомогенным, что обосновано использованием критерия Барнарда. Динамика параметров исследования оценена методами базовой статистики. Используемые пакеты программ: MedCalc, Statistika.

Результаты. Полученные результаты демонстрируют отчетливую положительную динамику в группе пациенток, получавших ВАХД в дополнение к стандартному лечению впервые выявленного рака яичников: достоверное уменьшение индекса перитонеального канцероматоза, терапевтический патоморфоз в образцах брюшины в процессе лечения, редукция асцита.

Заключение. Авторскому коллективу удалось установить, что проведение ВАХД совместно со стандартным комбинированным лечением по поводу впервые выявленного рака яичников с перитонеальным канцероматозом позволяет достичь нарастающего в динамике эффекта регрессии перитонеального канцероматоза брюшины, морфологической регрессии канцероматоза и полной резорбции асцита у подавляющего большинства пролеченных пациенток. Выявленный терапевтический эффект был пролонгированным и стойким при объективной оценке через 6 мес. по окончании лечения.

Ключевые слова:
рак яичников, асцит, перитонеальный канцероматоз, лекарственный патоморфоз, внутрибрюшная аэрозольная химиотерапия под давлением, ВАХД

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В работе соблюдались этические принципы, предъявляемые Хельсинкской декларацией Всемирной медицинской ассоциации (World Medical Association Declaration of Helsinki, 1964, ред. 2013). Исследование одобрено Комитетом по биомедицинской этике при ФГБУ «НМИЦ онкологии» Минздрава России (выписка из протокола заседания № 660 от 09.04.2021 г.).
Информированное согласие получено от всех участников исследования.

Финансирование: финансирование данной работы не проводилось.
Конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

Для цитирования:
Дзасохов А. С., Костин А. А., Асташов В. Л., Андреева М. А., Туриев А. В., Усков А. Д. Динамическая оценка воздействия внутрибрюшной аэрозольной химиотерапии под давлением на канцероматоз брюшины при раке яичников (непосредственные результаты). Южно-Российский онкологический журнал. 2023; 4(1): 43-51. https://doi.org/10.37748/2686-9039-2023-4-1-5, https://elibrary.ru/hrhtif

Статья поступила в редакцию 15.12.2022; одобрена после рецензирования 31.01.2023; принята к публикации 06.03.2023.

INTRODUCTION

Unsatisfactory results of treatment of peritoneal carcinomatosis in ovarian cancer are one of the most actual problems of modern oncogynecology. Ovarian cancer is characterized by the latent nature of the disease in the early stages, the absence of pathognomonic symptoms, diagnostic difficulties in detecting early forms of the disease, recurrent nature and relatively low effectiveness of anti-relapse treatment [1]. Peritoneal carcinomatosis is one of the main obstacles to achieving high efficiency of primary and anti-recurrent treatment of ovarian cancer. The incidence of metastatic lesions of the peritoneum in newly diagnosed ovarian cancer is 65–70 % of cases according to worldwide data.

With the progression of ovarian cancer, the defeat of the peritoneum by metastases is noted in 65 % of cases according to various studies [2]. In the vast majority of cases, complete cytoreduction with widespread peritoneal carcinomatosis is technically impossible, and systemic drug treatment does not give a stable clinical effect, which makes peritoneal carcinomatosis an unfavorable prognostic sign in ovarian cancer [3].

Systemic intravenous cytostatic therapy of metastatic lesions of the peritoneum still does not have a high efficiency and a persistent clinical antitumor effect, due to the low bioavailability of cytostatics in metastases on the peritoneum. For many years, attempts have been made to increase the bioavailability of antitumor drugs to carcinomatous foci on the peritoneum. One of such options is locoregional application of cytostatics, a special case of which is intraperitoneal administration of chemotherapy drugs in the form of a normothermal solution of cytostatics [4].

Through intra-abdominal chemotherapy, it is possible to create a high concentration of chemotherapy drugs in tumor foci without resorptive effect and associated systemic toxic effects [5].

In 2000, a group of researchers led by M. Reymond proposed a new variant of intraperitoneal chemotherapy – intraperitoneal aerosol chemotherapy under pressure or PIPAC (Pressurized IntraPeritoneal Aerosol Chemotherapy) – which is the injection of a solution of cytostatics into the closed abdominal cavity in the form of a fine aerosol in the conditions of normothermal carboxyperitoneum [6]. The method ensures uniform distribution of the aerosol over the entire metastatic surface of the peritoneum, which determines its advantage over other types of peritoneal lavage, and increased intra-abdominal pressure increases the depth of penetration of drugs into the peritoneal tissue. With each PIPAC procedure, diagnostic laparoscopy and multifocal biopsy of the peritoneum are performed, which makes it possible to objectively assess the condition of the peritoneum in dynamics through repeated procedures.

Currently, the results of 16 foreign studies of the effectiveness of PIPAC in ovarian cancer with peritoneal carcinomatosis have been published. Pronounced therapeutic pathomorphosis and a decrease in the peritoneal cancer index (PCI) were noted in 69 % of cases [7–15].

At the same time, there are no references in the available literature to the simultaneous use of cytoreductive surgery and PIPAC in ovarian cancer, except for their own first experience of clinical use of the PIPAC method in combination with surgical cytoreduction in primary ovarian cancer with peritoneal carcinomatosis [4].

The authors created a "Method for the treatment of peritoneal carcinomatosis in ovarian cancer", which was the basis for the development of the world's first protocol of a prospective open randomized controlled phase II clinical trial "Intraperitoneal aerosol chemotherapy under pressure (PIPAC) in the treatment of primary ovarian cancer with peritoneal carcinomatosis", approved by the Independent Committee on Biomedical Ethics at the National Medical Research Centre for Oncology [16].

Purpose of the study: to evaluate the direct effect of intra-abdominal aerosol chemotherapy under pressure on metastatic altered peritoneum, carried out in addition to the standard combined treatment of ovarian cancer.

PATIENTS AND METHODS

The study included 164 patients with primary ovarian cancer with visually detectable and morphologically verified peritoneal carcinomatosis. Prior to inclusion in the study, informed consent was obtained from all patients to participate in the study and to conduct PIPAC on the condition of complete anonymity. The work followed the ethical principles

set forth by the Helsinki Declaration of the World Medical Association (World Medical Association Declaration of Helsinki, 1964, ed. 2013). The study was approved by the Committee on Biomedical Ethics at the National Medical Research Centre for Oncology (extract from the protocol of the meeting No. 660 dated 04/09/2021).

Research stages and ongoing activities

Prior to inclusion in the study, patients were examined according to the recommendations of the AOR for patients suffering from ovarian cancer. The examination period did not exceed 7 days.

Upon completion of the examination, surgical intervention was performed in the following volume: extirpation of the uterus with appendages, omentectomy and multifocal biopsy from the 4 most altered areas of the peritoneum. The volume of cytoreduction in all cases was suboptimal.

Randomization was performed directly in the operating room after urgent morphological verification of metastatic peritoneal lesion by generating a random value of 0 or 1 on the site https://www.random.org/. Where the value 0 corresponded to the patient's getting into the control group, and 1 – into the main one.

In the control group, standard suturing of the anterior abdominal wall was performed. In the main group, after the completion of the organ-bearing stage of the operation, the patient underwent a PIPAC procedure.

In the postoperative period, a set of standard postoperative diagnostic and therapeutic measures was carried out in accordance with the clinical recommendations of the AOR, as well as taking into account the patient's condition and the specific clinical situation.

On the 8th day after cytoreductive surgery, patients of both groups underwent the 1st course of systemic polychemotherapy according to the TC scheme: paclitaxel 175 mg/m^2, carboplatin AUC 5–7. The first course of PCT was performed on the 2nd week of the study as part of a single hospitalization with a simultaneous organ-bearing stage and a PIPAC stage, the duration of systemic chemotherapeutic treatment was 1 day. The next (second course) intravenous chemo was performed after 21 days, and then 4 more courses (6 in total) were conducted with an interval of 21 days between them.

At the 3rd and 5th hospitalization, patients from the main group underwent the second and third PIPAC procedures, consistently and gradually performing diagnostic laparoscopy and pressurized intraperitoneal aerosol chemotherapy with an assessment of the peritoneal cancer index and multifocal biopsy of the peritoneum in the volume indicated earlier.

As part of the 3rd and 5th hospitalization, the activation of patients was performed the day after the PIPAC (2nd and 3rd procedures), and the day after activation, systemic intravenous PCT was performed according to the TC scheme.

6 months after the completion of the course of treatment, the patients from the main group, after a standard preoperative examination, underwent diagnostic laparoscopy with an assessment of the index of peritoneal carcinomatosis and a multipoint biopsy examination of the peritoneum.

At the same time, patients from the control group underwent a follow-up examination that corresponded to the recommendations of the AOR in terms of dispensary follow-up of patients who had undergone combined treatment for ovarian cancer.

Evaluation of the peritoneal carcinomatosis index

The scale of peritoneal metastasis lesion is estimated by calculating the index of peritoneal carcinomatosis in points for each PIPAC procedure. To do this, the parietal peritoneum and several sections of the visceral are conditionally divided into 13 zones: 0 – central, 1 – right dome of the diaphragm, 2 – epigastrium, 3 – left dome of the diaphragm, 4 – left lateral canal, 5 – left iliac region, 6 – pelvis, 7 – right iliac region, 8 – right lateral canal; additionally, 4 zones of the visceral peritoneum are evaluated: 9 – the proximal part of the jejunum, 10 – the distal part of the jejunum, 11 – the proximal part of the ileum, 12 – the distal part of the ileum. In the absence of a lesion in the selected zone, a score of 0 points is given, 1 point – the presence of formations up to 5 mm in size, 2 points – the presence of formations from 6 to 25 mm in size, 3 points – the presence of formations larger than 25 mm or drain formations.

The results of the calculation are entered into a standard form (Fig. 1). After that, all the scores are added, the result is an index of peritoneal carcinomatosis.

Histological assessment of tumor pathomorphosis

To assess the therapeutic effect directly in peritoneal metastases at the morphological level, we used the classification of G. A. Lavnikova, based on the assessment of the structure of both the tumor tissue as a whole and its individual cells. As a material for the study, 4 biopsies of the peritoneum were used, obtained during each PIPAC procedure and during control diagnostic laparoscopy 6 months after the end of treatment.

Within the framework of this classification, 4 degrees of pathomorphosis are distinguished:

- Grade I – more than 50 % of the tumor parenchyma is preserved;
- Grade II – 20–50 % of tumor parenchyma is preserved;
- Grade III – up to 20 % of the tumor parenchyma has been preserved as separate foci;
- Grade IV – complete absence of tumor parenchyma.

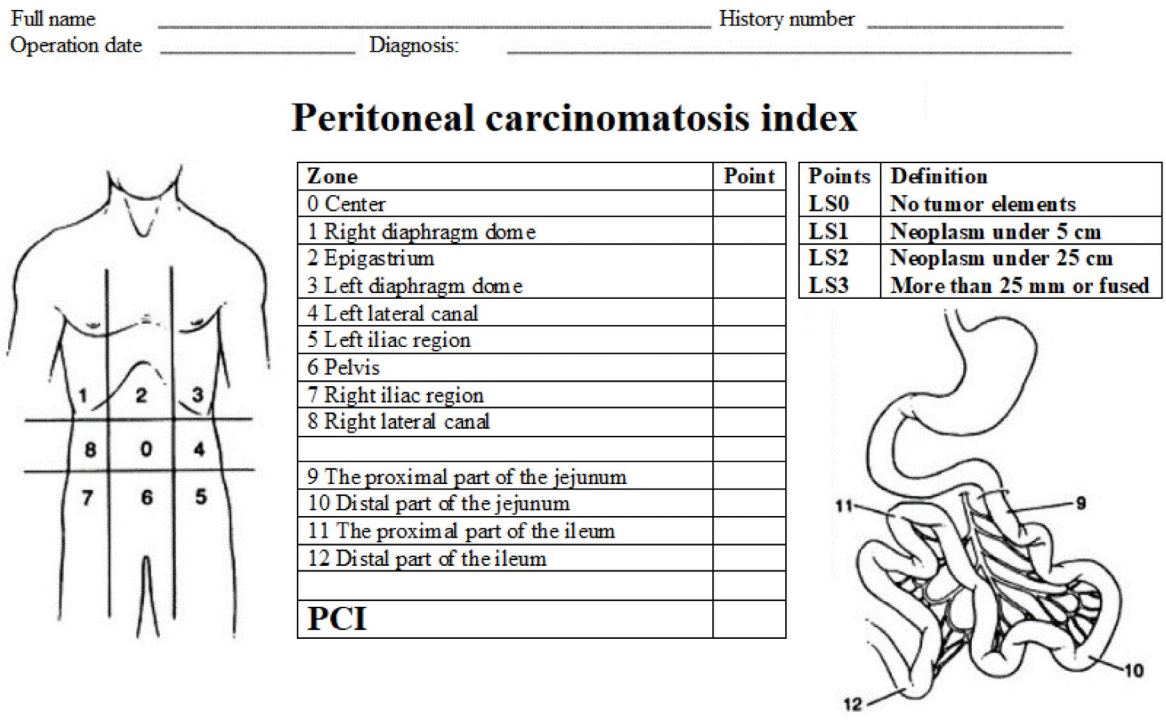
Clinical and demographic composition of the groups

The final sample included 164 patients, 79 of them in the main group, 85 in the control group. The average age of patients in the main group was 56.8 years, in the control group – 56.2 years. Analysis using the exact Wilcoxon-Mann-Whitney sum criterion showed a significance level of 0.779, which indicates a random distribution of patients between groups.

All the patients involved in the study were diagnosed with stage III serous ovarian cancer. In the main group, stage IIIb was established in 17 patients, and stage IIIc in 62. In the control group at 13 and 72, respectively. When evaluating the Barnard criterion, a significance level of $p = 0.364$ was obtained, hence the distribution among groups is homogeneous.

Also, peritoneal carcinomatosis was detected and verified in all patients at the time of initiation of treatment. After randomization, the lesion volume reflected in the PCI index varied in the range from 7 to 39 and from 5 to 39 points in the control and main groups, respectively. A more detailed distribution by the degree of peritoneal lesion is presented in Table 1.

The average PCI index in the main group was 23.1, and in the control group – 23.7 points. When compared using the exact Wilcoxon-Mann-Whitney sum criterion, the significance level was 0.642. Consequently, the distribution of patients between groups according to the degree of peritoneal lesion is random.


Full name _____ History number _____
Operation date _____ Diagnosis: _____

Peritoneal carcinomatosis index

Zone	Point
0 Center	
1 Right diaphragm dome	
2 Epigastrium 3 Left diaphragm dome	
4 Left lateral canal	
5 Left iliac region	
6 Pelvis	
7 Right iliac region	
8 Right lateral canal	
9 The proximal part of the jejunum	
10 Distal part of the jejunum	
11 The proximal part of the ileum	
12 Distal part of the ileum	
PCI	

Points	Definition
LS0	No tumor elements
LS1	Neoplasm under 5 cm
LS2	Neoplasm under 25 cm
LS3	More than 25 mm or fused

Fig. 1. Standard form for assessing the peritoneal carcinomatosis index.

The duration of hospitalization after treatment conducted at stage 1 of the study in the control and main groups averaged 7.2 days (range from 5 to 13 days) and 7.6 days (range from 6 to 12 days), respectively. There were no statistically significant differences between the groups for this indicator.

In the future, patients from both groups underwent 6 courses of systemic PCT according to the TC scheme. For patients from the control group, this therapy was the only one. In the main group, treatment was carried out in the mode of bidirectional chemotherapy with the addition of PIPAC sessions between courses of intravenous chemotherapy. Each of the patients had at least 1 PIPAC session, two sessions were conducted in 72 patients, three sessions each in 69 patients. Thus, a total of 220 PIPAC procedures were conducted in 79 patients, with an average of 2.8 sessions for each patient. The duration of each session ranged from 62 to 87 minutes, an average of 74 minutes. The duration of hospitalization after PIPAC varied from 2 to 5 days, on average 3 days. Also, 404 courses of systemic PCT were conducted in the main group, that is, an average of 5.6 courses per patient. In the control group, a total of 384 PCT sessions were conducted in 67 patients, which is 5.7 courses per person. The study of the direct effect of PIPAC on the metastatically altered peritoneum was carried out in the main group by assessing the dynamics of the PCI index, the morphological picture of drug pathomorphosis and the volume of ascitic fluid during diagnostic laparoscopy at the 2nd and 3rd PIPAC sessions.

Immediate results

As indicated earlier in the main group, the distribution of patients by the degree of peritoneal lesion was as follows: from 1 to 10 points on the PCI scale in 6 patients (7.6 %); from 11 to 20 points – in 26 (32.9 %); from 21 to 30 points – in 29 (36.7 %) and 18 (22.8 %) of patients revealed the most massive lesion of the peritoneum 31–39 points. In other words, at the time of detection of the disease, total peritoneal metastasis was present in 92.4 % of patients. When re-evaluated during the 2nd PIPAC procedure, a significant decrease in PCI was noted in almost all patients. Thus, tumor elements were no longer detected in 31 patients (PCI 0 points), which is 43 % of cases; in one patient (1.3 %), PCI was 17 points. In the remaining 40 cases (50.6 %), the values of this indicator were in the range from 1 to 10 points. Thus, already at the 2nd session of the PIPAC, it was noted that complete regression of total carcinomatosis occurred in 43 % of cases, and in 50.6 % of cases total carcinomatosis transformed into limited, which corresponds to partial regression.

According to the results of histological examination of the biopsy material taken during the second session of the PIPAC, it was found that grade IV pathomorphosis (complete absence of viable tumor cells) was detected in 40 patients, which is 55.5 % of cases. This discrepancy with clinically determined pathomorphosis in favor of morphological pathomorphosis is due to the fact that metastases on the peritoneum at the stages of drug pathomorphosis initially underwent replacement with fibrous tissue, which looked like metastases during video endoscopic revision, and morphological examination recorded complete drug pathomorphosis with total replacement of metastasis with fibrous elements.

The III degree of pathomorphosis was detected

Table 1. PCI in the main and control groups

Peritoneal Carcinomatosis Index	Main group	Control group
1 to 10 points	6	4
11 to 20 points	26	30
21 to 30 points	29	35
31 to 39 points	18	16

in 17 cases (23.6 %), the II degree – in 13 cases (18.1 %), and the I degree in only 2 patients (2.8 %). In other words, complete morphological regression of peritoneal carcinomatosis took place in more than half of the patients (55.5 %), and partial regression in 41.7 %. The cumulative effective morphological response was 97.2 %.

When assessing the PCI index during the 3rd session of PIPAC (69 patients), visible lesions of the peritoneum were absent in 58 cases (84.0 %), in 11 (16.0 %) the index value did not exceed 6 points. Histologically, grade IV tumor pathomorphosis was detected in 50 cases (72.5 %), grade III – in 10 (14.5 %) cases, grade II – in 5 (7.2 %) cases and in 4 (5.8 %) patients, grade I pathomorphosis was detected. That is, complete clinical regression of peritoneal carcinomatosis at the 3rd session of PIPAC was 84.0 % versus 43 % at the 2nd session, and complete morphological regression was noted in 72.5 % at the 3rd session of PIPAC versus 55.5 % at the second session.

At the control diagnostic laparoscopy 6 months after the end of treatment, there were no signs of peritoneal lesion in all 47 patients (100 %). At the same time, 36 (76.6 %) of them had drug–induced tumor pathomorphosis of the IV grade, 9 (19.2 %) – of the III grade, and only two of the I and II grade (2.1 % each).

A similar dynamic was observed with respect to the volume of ascitic fluid in the abdominal cavity. At the beginning of the study in the main group, ascites was absent in only one patient (1.2 %), a volume of up to 1 liter was detected in 38 cases (48.1 %), from 1 to 2 liters – in 14 cases (17.7 %), 7 patients (8.9 %) had from 2 to 3 liters of free fluid in abdominal cavity, another 6 (7.6 %) – from 3 to 4 liters, and massive ascites with a volume of more than 4 liters were detected in 13 cases (16.5 %). During the second PIPAC session, ascites were no longer detected in the vast majority of cases (59 out of 72, i.e. 81.9 %), in the remaining 13 cases (18.1 %) its volume did not exceed 200 ml. During 3 sessions of PIPAC, only 3 patients out of 69 (4.4 %) had an insignificant amount of exudate (volume no more than 200 ml). Accordingly, ascites resorbed completely in 95.6 % of patients by the 3rd PIPAC session. During the control laparoscopy, 6 months after the end of therapy, only 1 (2.1 %) of 47 patients had an insignificant amount of exudate with a volume of no more than 100 ml. At the same time, there were no clinical manifestations of ascitic syndrome in all 47 cases (100 %).

CONCLUSION

Preliminary results of primary ovarian cancer with peritoneal carcinomatosis, supplemented with pressurized intraperitoneal aerosol chemotherapy. During the second PIPAC prosedure, 31 patients (43 %) revealed complete clinical regression of peritoneal carcinomatosis, and according to the results of histological examination of biopsy material, therapeutic pathomorphosis of the IV degree (complete morphological regression) was registered in 40 patients (55.5 %). During the 3rd PIPAC procedure, further development of the therapeutic effect was noted: complete regression of metastases on the peritoneum was registered in 58 patients (85.3 %), and morphologically determined pathomorphosis of the IV degree in 50 people (73.5 %). Later, during diagnostic laparoscopy, 6 months after the completion of treatment, complete clinical regression was detected in all patients (100 % of cases), and in 36 people (76.6 %), drug pathomorphosis of the IV degree was established, which corresponds to complete morphological regression of peritoneal carcinomatosis and the long-term effect of the treatment.

A similar positive trend was noted with respect to the frequency and severity of ascites. So at the beginning of the study, it was detected in all patients of the main group, except one, which was 98.8 %. At the same time, at the time of the second PIPAC procedure, complete resorption of ascites was noted in 59 patients (81.9 %). This trend was observed throughout the entire track of the study and persisted after the end of therapy (at the time of the control diagnostic laparoscopy, ascites syndrome was not registered in any case). This effect is clinically significant because it leads to a significant improvement in the general condition and, as a result, significantly improves the tolerability of the treatment and the quality of life of patients.

Our observations allow us to draw several conclusions.

1) pressurized intraperitoneal aerosol chemotherapy simultaneous with standard combined treatment for primary ovarian cancer with peritoneal carcinomatosis makes it possible to achieve complete clinical regression (PCI = 0) of peritoneal carcinomatosis in 85.3 % of patients by the 3rd session of PIPAC,

complete morphological regression of carcinomatosis (IV degree drug pathomorphosis according to Lavnikova) in 73.5 % patients, and complete resorption of ascites in 81.9 % of patients.

2) With laparoscopic control 6 months after the end of treatment, complete clinical regression was noted in 100 % of cases, complete morphological regression in 76.6 % of cases, ascites syndrome was absent in 100 % of patients. The revealed trend, in our opinion, indicates a prolonged and persistent therapeutic effect of PIPAC on peritoneal carcinomatosis accompanying primary ovarian cancer.

3) It seems promising to conduct a comparative assessment of the main and control groups of the study in terms of long-term treatment results by comparing the duration of the relapse-free period and overall survival.

4) The results obtained allow us to consider the possibility of expanding the indications for the use of PIPAC as a method of palliative and symptomatic care in patients at the terminal stage of the disease, accompanied by an intensive accumulation of ascites.

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South Russian Journal of Cancer 2023. Vol. 4, No. 1. P. 52-64
https://doi.org/10.37748/2686-9039-2023-4-1-6
https://elibrary.ru/iicmmc
REVIEW

MODERN APPROACHES TO GLIOBLASTOMA THERAPY

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ABSTRACT

Glioblastoma (GBM) is the most malignant and the most common primary tumor of the central nervous system. During the last several years GBM has been classified and managed according to the World Health Organization (WHO) criteria which subdivide it into primary and secondary GBM. As it is suggested, GBM originates from glial cells and has a diffuse growth pattern, but its etiology and pathophysiology are poorly investigated up to date. Its rapid progression and anatomical location in the brain often limits the effectiveness of therapeutic interventions. Despite all scientific and technological advances, GBM remains an incurable disease with a median survival of approximately 18 months. Standard treatment options involving maximal safe resection of the tumor followed with radiotherapy and chemotherapy do not provide satisfactory results.

Better understanding of the molecular pathology of GBM and its associated signaling pathways has opened up possibilities for new treatments for newly diagnosed and relapsing tumors. A multitargeted therapeutic approach using compounds capable of inhibiting more than one specific molecular target is a promising alternative to conventional therapies.

Currently, specialists study such innovative treatment options as small molecule inhibitors aimed at signaling pathway disruptions, immunotherapy, including checkpoint inhibitors, oncolytic vaccines, CAR T-cell therapy, and drug delivery systems. In terms of an innovative approach, the elaboration of targeted drug delivery systems is of particular interest, since this strategy looks the most promising due to its ability to increase the bioavailability and effectiveness of both standard and newly tested agents. This review discusses results of preclinical and clinical studies of innovative therapeutic approaches, their advantages and disadvantages. An interdisciplinary approach is expected to be able to combine the results of cutting-edge research in this area and to provide novel promising therapeutic strategies for patients with GBM.

Keywords:
glioblastoma, nanoparticles, immunotherapy, small-molecule inhibitors

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Funding: this work was not funded.
Conflict of interest: authors report no conflict of interest.

For citation:
Kuznetsova N. S., Gurova S. V., Goncharova A. S., Zaikina E. V., Gusareva M. A., Zinkovich M. S. Modern approaches to glioblastoma therapy. South Russian Journal of Cancer. 2023; 4(1): 52-64. https://doi.org/10.37748/2686-9039-2023-4-1-6, https://elibrary.ru/iicmmc

The article was submitted 08.06.2022; approved after reviewing 17.01.2023; accepted for publication 06.03.2023.

Южно-Российский онкологический журнал 2023. Т. 4, № 1. С. 52-64
https://doi.org/10.37748/2686-9039-2023-4-1-6
https://elibrary.ru/iicmmc
3.1.6. Онкология, лучевая терапия.
ОБЗОР

СОВРЕМЕННЫЕ ПОДХОДЫ К ТЕРАПИИ ГЛИОБЛАСТОМЫ

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РЕЗЮМЕ

Глиобластома (ГБМ) является наиболее злокачественной и часто встречающейся первичной опухолью центральной нервной системы. В течение последних лет ГБМ классифицировали и лечили в соответствии с критериями Всемирной организации здравоохранения (ВОЗ), которая подразделяет ее на первичную и вторичную. Считается, что ГБМ происходит из глиальных клеток, имеет диффузный характер роста, однако ее этиология и патофизиология не вполне изучены на сегодняшний день. Быстрое прогрессирование опухоли, её анатомическая локализация в головном мозге часто ограничивают эффективность терапевтических вмешательств. Несмотря на все научно-технические достижения, ГБМ остается неизлечимым заболеванием с медианой выживаемости пациентов примерно 18 мес. Стандартные схемы лечения, включающие в себя максимальное хирургическое удаление опухоли с последующим облучением и химиотерапией, не обеспечивают удовлетворительных результатов.

Значительные успехи в понимании молекулярной патологии ГБМ и связанных с ней сигнальных путей открыли возможности для новых методов лечения впервые диагностированных и рецидивирующих опухолей. Многоцелевой терапевтический подход, направленный на использование соединений, способных ингибировать более чем одну конкретную молекулярную мишень, представляет собой многообещающую альтернативу стандартным методам лечения. В настоящее время изучаются такие инновационные варианты лечения как применение низкомолекулярных ингибиторов, нацеленных на нарушение сигнальных путей, иммунотерапия, включающая ингибиторы контрольных точек, онколитические вакцины, CAR-T-терапия, использование систем доставки лекарств. С точки зрения применения инновационного подхода особый интерес представляет разработка систем адресной доставки лекарств, так как именно эта стратегия выглядит наиболее перспективной в связи с ее способностью увеличивать биодоступность и эффективность как стандартных, так и впервые тестируемых препаратов. В данном обзоре обсуждаются результаты доклинических и клинических исследований инновационных терапевтических подходов, их преимущества и недостатки. Ожидается, что реализация междисциплинарного подхода способна объединить результаты передовых исследований в этой области, привести к созданию новых обнадеживающих терапевтических стратегий в отношении пациентов с ГБМ.

Ключевые слова:
глиобластома, наночастицы, иммунотерапия, низкомолекулярные ингибиторы

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Финансирование: финансирование данной работы не проводилось.
Конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

Для цитирования:
Кузнецова Н. С., Гурова С. В., Гончарова А. С., Заикина Е. В., Гусарева М. А., Зинькович М. С. Современные подходы к терапии глиобластомы. Южно-Российский онкологический журнал. 2023; 4(1): 52-64. https://doi.org/10.37748/2686-9039-2023-4-1-6, https://elibrary.ru/iicmmc

Статья поступила в редакцию 08.06.2022; одобрена после рецензирования 17.01.2023; принята к публикации 06.03.2023.

INTRODUCTION

Glioblastoma (GBM) is the most malignant and common primary tumor of the central nervous system (CNS), accounting for 30 % of all CNS tumors [1]. It is believed that GBM originates from glial cells, has a diffuse growth pattern, and its etiology and pathophysiology have not yet been fully studied to date [2]. In recent years, GBM has been classified and treated in accordance with the criteria of the World Health Organization (WHO), which divides it into primary and secondary [3]. According to WHO, primary GBM occurs de novo, aggressive in nature, is characteristic mainly of the elderly (median age 62 years), while secondary develops through malignant progression from less aggressive tumors, such as diffuse astrocytoma (grade II) or anaplastic astrocytoma (grade III) and manifests itself in younger patients (median 45 years) [3]. Although GBM can occur at any age, it should be noted that the incidence increases with age, with the average age of diagnosis being about 65 years, the median overall survival is about 15–18 months, and the average time interval before relapse is about 7 months, with a 5-year survival rate of less than 10 % [4].

To date, the standard of treatment for patients with GBM involves maximum surgical resection followed by radiation and chemotherapy, temozolomide (TMZ) is used as a first-line drug [4]. Due to the high degree of invasiveness, radical resection of the primary tumor mass does not lead to a complete cure, since infiltrating tumor cells invariably remain in the surrounding tissues. In this regard, further stages of treatment in the form of radiation (LT) and chemotherapy (CT) are required to prevent the progression and/or recurrence of the disease [4; 5] LT is one of the ways to combat malignant neoplasms/ cells based on the use of ionizing radiation. Cell death is caused by two causes: cellular stress and DNA damage, represented as single-stranded and double-stranded breaks [5].

The chemotherapeutic stage is based on the use of TMZ, which belongs to the class of alkylating agents with the ability to overcome the blood-brain barrier (BBB). After absorption, TMZ undergoes spontaneous hydrolysis and turns into an active metabolite of 5-(3-methyltriazene-1-yl) imidazole-4-carbosamide, which is further hydrolyzed to the methyldiazonium cation and 5-aminoimidazole-4-carboxamide [6].

The mechanism of action of the drug is realized by transferring an electrophilic alkyl group to a nucleophilic DNA atom, methylation of the nitrogenous bases of DNA adenine (at position N3) and guanine (position N7) occurs. At the same time, various types of damage formed in DNA activate specific repair pathways that allow to eliminate damage and can contribute to resistance to radio and chemotherapy. In this regard, the efforts of researchers are aimed at developing various approaches to the treatment of GBM, aimed at new molecular targets that could be used as therapeutic alternatives. However, most of them fail during clinical trials [6–11]. These failures may be associated with compensatory mechanisms due to the activation of the DNA repair system, high systemic toxicity, insufficient stability of drugs and other factors.

Nevertheless, new approaches to the creation of optimized treatment methods related to the understanding of the complex biology of GBM are able to increase the survival rate of patients with this disease [7].

In this regard, the purpose of the review was to consider some options for new therapeutic strategies currently being developed, such as inhibition of pathological signaling pathways, immuterapeutic drugs, drug delivery systems, as well as to discuss their advantages and disadvantages.

1. Therapeutic targets associated with the p53 signaling pathway.

TP53 is one of the most frequently deregulated genes in terms of cancer. It encodes the protein p53, which is associated with invasion, migration, proliferation, prevention of apoptosis and the properties of GBM stem cells.

Normally, p53 exhibits suppressor activity by altering the expression of genes involved in cell cycle arrest, apoptosis, stem cell differentiation, and cellular aging. It is usually activated in response to DNA damage, genotoxicity, oncogen activation, aberrant growth signal transmission and hypoxia [8]. Under normal conditions, its activity is low and is controlled by MDM2 and MDM4 proteins through ubiquitination and subsequent degradation.

MDM2 and MDM4 act as oncogenic inhibitors of p53 suppressive activity against tumors. MDM2 negatively regulates p53, causing its degradation in the

proteasome. Thus, inhibition of MDM2/p53 interaction for reactivation of p53 function is a promising strategy for cancer treatment, including GBM [9]. MDM2 transcription is induced by p53, creating a negative feedback loop. MDM4, unlike MDM2, which is responsible for cleavage of p53, inhibits this protein by binding it to the transcription activation domain.

Amplification of MDM2 and MDM4 can inactivate p53, which leads to the loss of various functions of tumor suppressors: growth arrest, apoptosis, and aging [11; 12]. MDM2 and MDM4 genes have been shown to be amplified and/or overexpressed in several different types of cancer [10].

P53 and α5ß1 integrins also play an important role in cellular processes, being part of the convergence pathway that controls the apoptosis of malignant neoplasms, which encourages researchers to look for effective molecules that can regulate both targets simultaneously [11].

For example: idasanutlin (RG7388,) is an MDM2 inhibitor, has greater efficacy and selectivity [12]. It has been recognized as an attractive therapeutic strategy for reactivating p53 by inhibiting MDM2 and MDM4, negative suppressors of p53. However, acquired resistance and toxicity continue to limit the development of this MDM2 inhibitor as a clinical antitumor agent [13].

Nutlins belong to the cis-imidazoline group of molecules that were detected by screening a chemical library of molecules to study anti-cancer efficacy. Some studies on animal models have shown that nutlin treatment, in particular nutlin3, led to an increase in p53 concentration, increased apoptosis and decreased oncogenicity in cells [14].

Later, nutlin analogues RG7388, MI77301, CGM097, MK8242 and AMG232 were developed and tested in clinical trials. Among them, AMG232 (KRT-232) has been shown to be the most effective and selective oral MDM2/p53 inhibitor with favorable toxicological properties *in vitro* and *in vivo* [15]. AMG232 showed relative selectivity towards wt-p53 stem cells and was very effective in inhibiting the growth of three-dimensional tumor spheroids [16]. It is assumed that the molecule will have a low clearance rate and a long half-life in humans.

2. RTK inhibition.

Signaling cascades of receptor tyrosine kinases (RTK) coordinate intracellular signaling in response to growth factors, chemokines, and other extracellular stimuli to control biological processes [17]. In healthy cells, receptor activity is strictly controlled, and RTK signaling regulates cellular processes such as apoptosis, growth, survival, and translation. RTK activation is triggered by the binding of extracellular ligands, which leads to the oligomerization of receptors and autophosphorylation of tyrosine residues in cytoplasmic domains, which leads to further signal transmission, the result of which is a change in the expression of a number of proteins important for cell life [17; 18].

RTKs include more than 50 different human receptors, including platelet growth factor receptors (PDGFR), vascular endothelial growth factor receptors (VEGFR) and epidermal growth factor receptors (EGFR/HER/ERBB) [18]. It has been demonstrated that RTK mutations associated with the occurrence and progression of multiple malignancies, including GBM.

A large number of studies have shown that malignant neoplasms, including GBM, are characterized by active angiogenesis due to the secretion of regulatory growth factors, such as vascular endothelial growth factor (VEGF), platelet growth factor (PDGF) [19].

The platelet growth factor (PDGF) family is necessary for a wide range of physiological processes, such as migration and proliferation of pericytes, which contribute to the formation and proper functioning of blood vessels. The deregulated activity of PDGFR contributes to the occurrence of various pathological conditions, and, consequently, members of the PDGF/PDGFR family are important therapeutic targets [20].

There are three main approaches to inhibiting the PDGF/PDGFR pathway: 1) sequestration of the ligand with neutralizing antibodies, soluble extracellular parts of the receptors; 2) disruption of the interaction between the ligand and the receptor by blocking the receptor with receptor-specific antibodies or low molecular weight inhibitors; 3) using low molecular weight inhibitors to block the kinase activity of PDGFR [21].

Imatinib is one such drug that has an inhibitory effect on PDGFR. Although imatinib has activity against other malignancies, it has not shown significant activity against GBM during clinical trials. Tumor growth and overall survival remained unchanged

regardless of whether the drug was used in mono- or combination therapy [22; 23].

Tandutinib is another PDGFR inhibitor that has shown little therapeutic effect in clinical trials for recurrent GBM. AG1433 is another PDGFR inhibiting molecule that has proven its activity in preclinical studies on several HGG cell lines (gliomas of high malignancy) *in vitro*. In 2019, it was tested on 11 and 15 HGG cell lines with and without radiation therapy. It was found that the AG1433 molecule is effective, but the combination with irradiation does not increase its activity [23].

Vascular endothelial growth factor (VEGF) plays a crucial role not only in stimulating the growth of tumor vessels, but also in the formation of an immunosuppressive state. VEGF can inhibit the function of T cells, enhance the involvement of regulatory T cells (Tregs) and suppressor cells of myeloid origin (MDSC), and hinder the differentiation and activation of dendritic cells (DC) [23]. The VEGF family includes VEGF A, VEGF B, VEGF C, VEGF D and placental growth factor (PlGF). These ligands with different affinities bind to three endothelial receptor tyrosine kinases: VEGFR1, VEGFR2 and VEGFR3 [24].

VEGF promotes tumor angiogenesis by stimulating, proliferating and surviving endothelial cells, as well as increasing vascular permeability and recruiting vascular progenitor cells from the bone marrow. Unlike the formation of mature vessels under normal conditions, intra-tumor vessels are complex, disorganized, irregular and leaky, which leads to hypoxia and ineffective delivery of antitumor agents into the tumor microenvironment [24]. The combination of these factors makes it possible to consider an angiogenesis inhibitor as one of the options for antitumor therapy.

However, the absence of an antitumor effect when using a VEGF inhibitor, observed in some models of orthotopic GBM xenografts in rodents, may be due to a decrease in permeability and vasogenic cerebral edema. Several adaptive resistance mechanisms can neutralize the potential initial benefit provided by antiangiogenic therapy. Under conditions of inhibition of VEGF signaling, the tumor and its microenvironment release alternative proangiogenic growth factors to stimulate VEGF-independent angiogenesis, which is further enhanced by recruiting proangiogenic myeloid cells [24; 25].

One of the options for antiangiogenic therapy is bevacizumab, which is an antibody to VEGF. Although bevacizumab has become a standard part of the treatment of GBM relapses, numerous studies have shown that it nevertheless does not increase survival [25–27].

It is assumed that the simultaneous administration of low-molecular-weight VEGF and PDGF inhibitors may have a positive effect on the results of chemoradiotherapy. Sorafenib is a multipurpose RTK inhibitor that is active in VEGF (VEGFR-2 and –3) and PDGF (PDGF β and Kit). In a preclinical assessment on cells, U87 administered in monotherapy mode showed a significant improvement in survival, but there was no positive dynamics in clinical studies. Vatalanib (PTK787) is another of the low molecular weight inhibitors of VEGFR, PDGFR and c-Kit., which has demonstrated safety and tolerability during clinical trials for the treatment of GBM [26]. Vandetanib (ZD6474), a low molecular weight tyrosine kinase inhibitor of VEGFR, EGFR and RET 23, in combination with other chemotherapeutic agents in clinical trials in patients with GBM showed good tolerability, but the survival rate did not change significantly. An unsatisfactory result may be associated with a number of problems, such as heterogeneity, inability to overcome BBB [26].

The epidermal growth factor receptor (EGFR) plays a central role in cell division, migration, adhesion, differentiation, and apoptosis. When bound to ligands, EGFR is activated by homodimerization or heterodimerization on the cell surface, which leads to phosphorylation of its intracellular tyrosine kinase domain. Studies have shown that EGFR amplification and mutation are the most common genetic changes occurring in more than 50 % of GBM cases [26; 27].

Many EGFR inhibitors such as erlotinib, gefitinib and lapatinib have been widely evaluated in the clinic for the treatment of GBM. Gefitinib in neoadjuvant mode showed that its concentration in the tumor was 20 times higher than in plasma, but this discovery was not associated with inhibition of the downstream pathway. Thus, the drug effectively acts on the EGFR receptor, but does not affect the downstream targets of this pathway. The same conclusion can be made to erlotinib and lapatinib [27]. These studies show that first-generation EGFR does not effectively inhibit EGFR signaling in GBM, and the

above observation may be the reason for the failure of these drugs.

Another of the selective EGFR inhibitors is AZD3759, which effectively penetrates the BBB, has a free concentration in the blood, cerebrospinal fluid and brain tissues.

The main problems of modern EGFR targeting strategies are the lack of BBB permeability, the molecular heterogeneity of GBM and the need to increase the specificity of low molecular weight EGFR mutation inhibitors [27].

2.1. Therapeutic targets, related to the I3K/Akt/mTOR pathway.

Several studies has shown that, with GBM signal transmission is realized through PI3K/AKT/mTOR.

PI3K/AKT/mTOR, the central component of which is phosphatidylinositol-3-kinase (PI3K), as well as AKT and mTOR kinases, is considered one of the universal signaling pathways characteristic of most human cells. It is responsible for avoiding apoptosis, growth, cell proliferation, and metabolism. The PI3K/Akt/mTOR signaling cascade is considered as a promising target of modern combination therapy. A number of inhibitors targeting key components of this pathway are undergoing clinical trials.

2.1.1. PI3K inhibitors.

PI3K is involved in proliferation, differentiation, migration, metabolism and survival and is divided into three classes depending on their substrate specificity and homological sequence. A growing amount of preclinical and clinical data suggests that PI3K inhibitors offer promising treatment options for oncological diseases, including GBM [28].

One of the PI3K inhibitors buparlisib is promising for the treatment of GBM due to its ability to penetrate the BBB. In xenograft models, buparlisib demonstrated antitumor activity regardless of EGFR status. In addition, the synergistic activity of buparlisib in combination with TMZ was manifested in xenografts of mice. However, clinical results have shown insufficient inhibition of general signaling by tolerated doses in patients with relapse. The reason for the lack of efficacy is that the PI3K pathway cannot be completely blocked in tumor tissues. Recent studies have shown that buparlisib in combination with the PARP inhibitor rukaparib shows improved antitumor efficacy compared to monotherapy with these molecules [29].

It has also been shown that PQR309 (bimiralisib) is an effective PI3K/mTOR inhibitor with good BBB penetration. This molecule has a strong inhibitory effect on PI3K, rather than on mTOR. It has been confirmed that bimiralisib has antitumor activity against GBM *in vitro* and *in vivo*. In addition, the combination of this molecule with an AKT inhibitor shows strong activity against GBM in the LN-229 63 cell line xenograft model in BALB/c Nude mice [30].

Another PI3K and mTOR inhibitor with good pharmacokinetic parameters is GNE-493. However, its poor penetration into the brain limits its use as a treatment for GBM. This molecule was used as a starting compound to obtain its analogues with improved permeability, by reducing the number of hydrogen bond donors. One of such analogues is GNE-317. It was developed taking into account the aforementioned shortcomings, and is an effective brain-penetrating PI3K inhibitor [30; 31].

The PI3K/mTOR inhibitor voxtalisib showed good activity on GBM xenografts, both in monotherapy and in combination with conventional therapeutic agents [31].

2.1.2. AKT/mTOR inhibitors.

In addition to PI3K, such components of this signaling as AKT and mTOR also contribute to the development and progression of GBM. It has been shown that an increase in the level of activated phosphorylated AKT, as well as hyperactivation of mTOR, contribute to uncontrolled growth of GBM cells and a decrease in survival, and therefore they can be considered as possible therapeutic targets [32–34].

In particular, GDC-0068 (ipatasertib) is a highly selective ATP-competitive inhibitor of pan-AKT, which leads to increased antiproliferative activity in cell lines with PI3K/AKT activation. Preclinical data have shown that ipatasertib can enhance the antitumor activity of classical chemotherapeutic drugs [35].

Among the mTOR inhibitors sirolimus, temsirolimus and everolimus are approved by the FDA. Sirolimus, a well-studied drug with antifungal, immunosuppressive and antitumor effects, is a macrolide antibiotic. Sirolimus is known for its ability to inhibit the mTOR signaling pathway and has been extensively studied for its therapeutic potential [36].

Palomin 529 (P529) is a dual mTORC1/2 inhibitor that can increase the effectiveness of radiation therapy by delaying the DNA repair mechanism [37]. P529 penetrates well into the brain, which provides support for further evaluation of its use in the treatment of GBM. AZD2014 is also a dual inhibitor of mTORC1/2, which enhances radiosensitivity both *in vitro* and in orthotopic conditions *in vivo*. It is assumed that a dual mTORC1/2 inhibitor may be a suitable radiosensitizer for the treatment of GBM [38].

Rapalink-1 is a third-generation mTOR inhibitor, which consists of sirolimus and MLN0128. It showed good inhibitory activity in mice with intracranial xenografts U87MG, was well tolerated and significantly improved survival.

Currently, there are a large number of targeted drugs targeting the PI3K/Akt/mTOR pathway that are undergoing preclinical or clinical trials. However, targeted GBM therapy has not yet demonstrated significant clinical survival benefits. Currently there are several possible reasons for the limited effect: 1) BBB, therefore targeted drugs cannot reach effective concentrations; 2) heterogeneity of GBM [39].

3. Immunotherapy.

For a long time, based on experimental data, the central nervous system was considered as an "immunoprivileged" system due to a small number of antigen-presenting cells (APC) and limited penetration of lymphocytes through the BBB. Currently, some studies have refuted this postulate and demonstrated the penetration of activated T-lymphocytes through the BBB, thereby showing that the central nervous system interacts with the immune system [40]. With a variety of pathological processes, there is a change in the permeability of the BBB due to anti-inflammatory cytokines. As a result, a large number of lymphoid and myeloid immune cells penetrate into the tissues of the central nervous system.

However, in comparison with other solid tumors, GBM is characterized by low infiltration of NK and T cells, nevertheless, various immunotherapy strategies for malignant brain tumors are currently being actively developed. The basic principle is that the host immune system can destroy the tumor provided the effector function is enhanced, this leads to the elimination of cancer cells by improving the recognition of tumor agents [41]. Immunotherapy is based on such strategies as immunomodulatory cytokine therapy, anti-cancer vaccines, checkpoint inhibitors, CAR-T therapy.

3.1. Cytokine therapy.

Cytokine therapy uses mediators of immune activation and proliferation, such as interleukins, interferons and granulocyte-macrophage colony stimulating factor, to create a broad antitumor response. Interleukins activate lymphocytes to initiate innate and adaptive immune responses. Interferons induce immune cells and inhibit angiogenesis in cancer immunotherapy [42].

However, the administration of cytokine therapy to patients with GBM is ineffective due to the short half-life and limited ability to overcome BBB. To solve these problems, high doses of cytokines should be administered, which in turn can lead to cytokine storms, autoimmune reactions and systemic side effects [43].

3.2. Immune control checkpoints inhibition.

Immune checkpoint inhibitors (ICIs) are molecules that reduce the activity of regulatory pathways that limit the activation of T cells. These inhibitors are aimed at interacting with cellular proteins that prevent the cytotoxic effect of T-lymphocytes [44]. The most studied molecules for cancer immunotherapy using ICI inhibitors are CTLA-4 receptors (cytotoxic T-lymphocyte-associated protein 4), PD-1 (Programmed cell death 1) and its PD-L1 ligand (Programmed death-ligand 1).

CTLA-4 and PD-1 are expressed on the surface of T cells. Tumor cells, evading the immune ones, express PD-L1. However, despite the positive results obtained during preclinical trials, some clinical studies using ICI inhibitors (anti-PD-1 and anti-CTLA-4, separately and in combination) in GBM showed no improvement in patient survival [45–47]. These and other studies have revealed the reasons for the low effectiveness of these inhibitors: BBB, low infiltration by tumor T cells and multilevel immunosuppression by elements of the tumor microenvironment [47].

3.3. Vaccines.

Vaccines are known as a means to stimulate immune effector cells and enhance their infiltration into tumors. They are divided on the basis of nucleic

acids, neoantigens, peptides and cells. Therapeutic vaccines contribute to the determination of antigens expressed by tumor cells for further detection and destruction of the cancer focus by the immune system.

Nucleic acid-based vaccines are injected as a segment of genes, DNA or RNA encoding tumor antigens and causing an immune response. Vaccines containing RNA have certain advantages over those containing DNA, this is due to the direct translation of antigenic proteins and higher safety. However, one should not forget that "pure" RNAs are susceptible to nucleases and can be destroyed before APC transfection [48].

Neoantigenic vaccines are new epitopes resulting from mutations in the genome of tumor cells. They have high specificity, antigenicity and safety [48]. At the stage of the first clinical trials is a personalized combined vaccine GAPVAC-101, containing neoantigen and unmutated antigen targeted against GBM.

Cellular vaccines are mainly created using dendritic cells (DC), which are responsible for activating adaptive immunity and stimulating B and T lymphocytes. In this type of immunotherapy, DC is isolated from the blood of patients to stimulate antigen-presenting properties *in vitro*, and then injected back into the patient to activate effector cells [49]. The advantages of therapy with this type of vaccine are the induction of an antitumor T-cell response, an increase in tumor immunogenicity due to the strengthening of antigen-presenting functions of DC and the ability to link innate immunity with adoptive immunity. This is important, in particular for low-immunological tumors, such as GBM [50].

3.4. Chimeric antigen receptor T cells (CAR).

Adaptive T-cell immunotherapy is an antigen-specific approach based on the transformation of the patient's own immune cells. T-cells obtained from patients with tumor diseases undergo modification outside the human body. As a result of modification, the T-lymphocyte acquires a tumor-specific chimeric antigen receptor (CAR) to provide more effective target recognition [51].

One of the barriers affecting the effectiveness of CAR-T-cell therapy in solid tumors, such as GBM, is the high heterogeneity and diverse expression of tumor antigens. The creation of CAR T cells targeting multiple antigens by expressing multiple CAR on T cells is considered as an approach to overcoming this limitation [37].

4. Alternative drug delivery systems.

The search for alternative effective treatment methods is associated not only with the emergence of new therapeutic agents, but also with the development of drug delivery systems. Systemic drug delivery is seen as a promising and universal prospect that can overcome the failure of systemic drug administration. In this area of research, there are a number of materials that can be used to increase the absorption of chemotherapeutic drugs by cells. In some works, the results of work in the field of application of nanostructures of various sizes, physico-chemical properties and forms for the treatment of oncological diseases were demonstrated. They may include lipid and/or polymer materials that are capable of generating structures such as liposomes, micelles, exosomes, polymer and inorganic nanoparticles, polymer conjugates. In this regard, their properties depend on the components used, which determine their further function [52].

Each nanostructure should be carefully studied and designed to achieve maximum therapeutic effect with minimal possible side effects on the body. Most of them can be modified so that they respond to various internal or external stimuli, which is an advantage for controlling the release of encapsulated therapeutic substances. The design of drug delivery systems must be specific in order to successfully target the affected area without affecting the surrounding tissues [53].

Nanoparticles (NPS) are transport systems ranging in size from 1 to 100 nm. Their use can provide such advantages as prevention of premature degradation of drugs in the bloodstream, improved penetration into cells, targeted delivery of immune drugs and enhanced absorption [54]. Also, LPS are used to overcome BBB, which is known to be one of the main reasons complicating the delivery of therapeutic molecules into the brain, thereby limiting their effectiveness. To overcome this limitation, modern therapeutic agents are loaded inside polymer or lipid nanostructures that have the ability to penetrate through the BBB.

Lipid nanocarriers are divided into categories depending on the physicochemical properties and methods of creation. The main lipid – based carriers include: 1) niosomes, which are lamellar self-assembling structures consisting of nonionic surfactants and cholesterol; 2) transferosomes, similar to niosomes and liposomes, consisting of a lipid bilayer created from a lipid matrix stabilized by various surfactants; 3) liposomes, which are spherical vesicles created by a lipid bilayer of phospholipids; 4) solid lipid nanoparticles consisting of a solid lipid core and 5) nanostructured lipid carriers whose core contains a liquid lipid phase inside a solid lipid phase [55–58].

Solid lipid nanoparticles are one of the newly developed groups of lipid-based nanocarriers. They have the ability to efficiently deliver both lipophilic and hydrophilic drugs, as well as other therapeutic molecules, to numerous affected tissues. They reduce the toxicity of the therapeutic molecule they carry, while protecting them from clearance by the reticuloendothelial system. Their inherent ability to dissolve poorly in water leads to a controlled and delayed release of drugs, long-term stability allows them to be used for a long period of time. Against the background of many advantages, solid lipid nanoparticles have a number of disadvantages: displacement of the encapsulated therapeutic agent, tendency to gelation and low encapsulation efficiency. The low encapsulation efficiency is due to the internal structure of the lipid nucleus, which does not create empty spaces during crystallization, which makes it difficult to retain the potentially encapsulated substance inside the solid phase [56–59].

Polymer nanoparticles are stable structures that provide controlled and delayed release of the drug and can be modified in such a way as to respond to external or internal stimuli. In the literature, most nanoparticle delivery systems that have been used to treat brain diseases consist of synthetic polymers such as polyethylene glycol, polylactide, chitosan, poly(L-lactide-co-glycolide) (PLGA), polyacrylic acid (PAA), polylactide (PLA), polyvinyl alcohol (PVA). Their chemical composition affects stability, biodegradability, biocompatibility, bio-distribution, cellular and subcellular fate. They can be modified to package and deliver therapeutic agents to the desired site of action or to respond to certain physiological and external stimuli [57; 58]. One of the conditions for the development of polymer nanoparticles for medical applications is their biodegradability, which should depend on the therapeutic application, target sites (organs, tissues, cellular or subcellular organelles) and the route of administration.

This system has a negative impact on humans: low solubility and decomposition in acidic by-products is a limitation for their use in brain diseases. In addition, the use of organic solvents to produce most of these nanoparticles is another disadvantage that can cause problems of increased toxicity [58].

Metal nanoparticles (MNPs) are a nanomaterial for targeted therapy and visualization of malignant brain tumors. Conjugation of peptides or antibodies with the surface of MNCs allows direct targeting of the surface of tumor cells and potentially disrupting active signaling pathways. Most MNCs are being developed as contrast agents for magnetic resonance imaging (MRI) and computed tomography (CT) probes [59]. However, most of these studies are only preclinical.

Among MNPs, only iron oxide nanoparticles (IONP) are approved by the FDA for preclinical and diagnostic studies. Their unique properties, such as low toxicity, biocompatibility, superparamagnetic properties, excellent solubility in water and catalytic behavior, make them promising candidates for biomedical applications [59].

Medicines created thanks to the development of nanotechnology have been widely used in the biomedical field in the last decade. These compounds can be inorganic or organic, of various shapes and sizes. The combination of different materials gives these nanostructures their universal properties and makes them so attractive in nanomedicine.

CONCLUSION

Up to the date, an obvious need to develop new effective methods of treating GBM still remains. The solution of this difficult biomedical problem is greatly facilitated by the pronounced progress of interdisciplinary research and the promising results obtained during them. One of the priorities in this area is the development of low-molecular-weight inhibitors of signaling pathways associated with the development of this disease. Also, the poten-

tial possibility of using immunotherapeutic strategies aimed at strengthening the functions of the immune system in the aspect of recognizing tumor cells and their subsequent destruction deserves close attention. From the point of view of applying an innovative approach, the development of drug delivery systems is of particular interest, which can increase the bioavailability and effectiveness of both already approved antitumor drugs and new promising compounds. It is expected that ideas that can combine the most outstanding results of individual research areas can lead to the creation of new promising therapeutic approaches for patients with GBM.

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Goncharova A. S. – literature data processing;
Zaikina E. V. – обработка текста;
Gusareva M. A. – literature data processing;
Zinkovich M. S. – literature data retrieval.

South Russian Journal of Cancer 2023. Vol. 4, No. 1. P. 65-78
https://doi.org/10.37748/2686-9039-2023-4-1-7
https://elibrary.ru/izbfnt
REVIEW

MOLECULAR FEATURES OF MALIGNANT GASTRIC TUMORS

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ABSTRACT

Gastric cancer is one of the most widespread cancers and makes a significant contribution to the global mortality rate from malignant neoplasms. The late onset of clinical symptoms is the main reason why the disease is often diagnosed at an advanced stage, and this limits the available therapeutic approaches. Despite the fact, that extensive studies have been carried out to identify the mechanisms and markers of the development and progression of the disease, their results are currently not fully included in clinical practice. As a consequence, only marginal improvement in long-term survival has been achieved and patient prognosis remains poor. Understanding the molecular genetic features of gastric malignant tumors can provide insight into their pathogenesis, help identify new biomarkers for prognosis and diagnosis, and identify new therapeutic targets. In recent decades, advances in high throughput sequencing technologies have improved understanding of the molecular genetic aspects of gastric cancer. This review considers molecular level changes, including information on tumor suppressor genes, oncogenes, cell cycle and apoptosis regulators, cell adhesion molecules, loss of heterozygosity, micro-satellite instability and epigenetic aberrations (change in methylation level and modification of histones). The review is also devoted to the molecular aspects of pathogenesis – changes in the signaling pathways involved in the gastric cancer development; the classification of sporadic and hereditary gastric cancer at the molecular genetic level is considered. The characteristics and classification of GC presented in this review at the genetic and epigenetic levels confirms that this disease is heterogeneous. These data can be used both to develop and test potential markers and new targeted therapeutic approaches.

Keywords:
gastric cancer, heredity, sporadic forms, tumor suppressor genes, oncogenes, epigenetics, microsatellite instability

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Funding: this work was not funded.
Conflict of interest: authors report no conflict of interest.

For citation:
Gevorkyan Yu. A., Dashkov A. V., Soldatkina N. V., Kolesnikov V. E., Timoshkina N. N., Kutilin D. S., Bondarenko O. K. Molecular features of malignant gastric tumors. South Russian Journal of Cancer. 2023; 4(1): 65-78. https://doi.org/10.37748/2686-9039-2023-4-1-7, https://elibrary.ru/izbfnt

The article was submitted 06.09.2022; approved after reviewing 31.01.2023; accepted for publication 06.03.2023.

Южно-Российский онкологический журнал 2023. Т. 4, № 1. С. 65-78
https://doi.org/10.37748/2686-9039-2023-4-1-7
https://elibrary.ru/izbfnt
3.1.6. Онкология, лучевая терапия
ОБЗОР

МОЛЕКУЛЯРНЫЕ ОСОБЕННОСТИ ЗЛОКАЧЕСТВЕННЫХ ОПУХОЛЕЙ ЖЕЛУДКА

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РЕЗЮМЕ

Рак желудка является одним из широко распространенных онкологических заболеваний и вносит существенный вклад в показатель глобальной смертности от злокачественных новообразований. Позднее появление клинических симптомов является основной причиной того, что заболевание часто диагностируется на запущенной стадии, а это ограничивает доступные терапевтические подходы. Несмотря на то, что были проведены обширные исследования для выявления механизмов и маркеров развития и прогрессирования заболевания, их результаты в настоящее время полностью не вошли в клиническую практику. Как следствие этого, достигнуто лишь незначительное улучшение долгосрочной выживаемости, и прогноз у пациентов остается неблагоприятным. Понимание молекулярно-генетических особенностей злокачественных опухолей желудка может дать представление об их патогенезе, помочь в идентификации новых биомаркеров для прогнозирования и диагностики, а также выявить новые терапевтические мишени. В последние десятилетия достижения в области технологий высокопроизводительного секвенирования улучшили понимание молекулярно-генетических аспектов рака желудка. В этом обзоре рассмотрены изменения на молекулярном уровне, включающие информацию о генах-супрессорах опухолей, онкогенах, регуляторах клеточного цикла и апоптоза, молекулах клеточной адгезии, потери гетерозиготности, микросателлитной нестабильности и эпигенетических аберрациях (изменение уровня метилирования и модификации гистонов). Обзор также посвящен молекулярным аспектам патогенеза – изменениям в сигнальных путях, вовлеченных в развитие рака желудка; рассматривается классификация спорадического и наследственного рака желудка на молекулярно-генетическом уровне. Представленная в данном обзоре характеристика и классификация РЖ на генетическом и эпигенетическом уровне подтверждает, что это заболевание является гетерогенным. Эти данные можно использовать как для разработки, так и для тестирования потенциальных маркеров и новых таргетных терапевтических подходов.

Ключевые слова:
рак желудка, наследственность, спорадические формы, гены-супрессоры опухолей, онкогены, эпигенетика, микросателлитная нестабильность

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Финансирование: финансирование данной работы не проводилось.
Конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

Для цитирования:
Геворкян Ю. А., Дашков А. В., Солдаткина Н. В., Колесников В. Е., Тимошкина Н. Н., Кутилин Д. С., Бондаренко О. К. Молекулярные особенности злокачественных опухолей желудка. Южно-Российский онкологический журнал. 2023; 4(1): 65-78.
https://doi.org/10.37748/2686-9039-2023-4-1-7, https://elibrary.ru/izbfnt

Статья поступила в редакцию 06.09.2022; одобрена после рецензирования 31.01.2023; принята к публикации 06.03.2023.

INTRODUCTION

Worldwide, gastric cancer (GC) remains one of the leading causes of cancer death. The late appearance of clinical symptoms is the main reason that the disease is often diagnosed at an advanced stage, and this limits the available therapeutic approaches [1]. Despite the fact that extensive studies have been conducted to identify signaling pathways and genes involved in the development and progression of the disease, their results have not fully entered clinical practice at the present time. As a consequence, only a slight improvement in long-term survival has been achieved and the prognosis in patients with GC remains unfavorable. Adenocarcinoma is the main histological type of GC, which accounts for 90–95 % of all malignant neoplasms of the gastric. Morbidity is closely related to environmental factors reflecting the peculiarities of the geographical distribution of this disease [2].

GC is the result of a complex interaction of environmental factors and multiple genes. Obvious risk factors for GC are Helicobacter pylori infection and Epstein-Barr virus (EBV), smoking, consumption of foods with a high salt content or N-nitroso compounds, family history and molecular factors [2; 3]. The latter include multiple genetic and epigenetic changes in oncogenes, tumor suppressor genes (TSG), cell cycle regulators and DNA repair genes [4].

Thus, a systematic look at the molecular basis of GC is necessary for the development of new strategies for the prevention and treatment of this disease. Therefore, the purpose of this review was to analyze and systematize information about currently known epigenetic and genetic changes in GC of various subtypes.

1. Classification of gastric cancer based on molecular profile studies.

According to the Lawrence classification, gastric adenocarcinoma is divided into intestinal, diffuse, mixed and non-deterministic [5]. They differ not only in morphology, but also in epidemiology, the nature of progression, genetics and clinical picture. Histopathologically, the intestinal type is characterized by malignant epithelial cells that exhibit cohesiveness and glandular differentiation infiltrating surrounding tissues [6]. On the contrary, the diffuse subtype is characterized by tumor cells that exhibit poor differentiation and lack of cohesion. It is believed that the intestinal type of GC is associated mainly with the influence of environmental (exogenous) factors, whereas the diffuse type is due to genetic hereditary and non-hereditary (endogenous) factors. These histological classifications are not sufficient to reflect the molecular characteristics of GC or to develop personalized treatment strategies. Several molecular classification systems have been proposed, and individual molecular subtypes have been identified [7–9].

To date, the Cancer Genome Atlas (TCGA) has characterized 295 cases of gastric adenocarcinoma using high-throughput sequencing technologies, including gene copy number analyses, DNA methylation, matrix RNA and microRNA sequencing, proteome and microsatellite instability (MSI) analysis, as well as genome-wide sequencing data [7]. Based on this, four subtypes of GC were described in 2014 (Table 1):

(1) EBV-positive (8.8 %),
(2) microsatellite unstable (MSI, 21.7 %),
(3) genomically stable (19.7 %),
(4) chromosomally unstable (CIN, 49.8 %) [7].

These subtypes of GC showed various epigenetic changes and mutations in different genes. Thus, EBV+ tumors had mutations in *PIK3CA* and *ARIDIA*, DNA hypermethylation and significant amplification of *JAK2*, *PD-L1* and *PD-L2*. Most EBV-positive tumors occurred in male patients in the bottom or body of the gastric. All EBV-positive RS demonstrated hypermethylation of the *CDKN2A* promoter and the absence of hypermethylation of the *MLH* promoter characteristic of the RS phenotype associated with MSI (CIMP) [7; 10].

Tumors of the MSI-H subtype, as a rule, occur in female patients, are diagnosed at late stages and are characterized by an increased frequency of mutations, including mutations of genes encoding target oncogenic signaling proteins [11].

The genomically stable subtype (GS) lacked numerous molecular changes and correlated well with the diffuse histological variant of Loren, but contained mutations in *CDH1* and *RHOA* or CLDN18-ARCHGAP fusion. It is known that the active form of RHOA associated with GTP activates STAT-3 to stimulate oncogenesis. According to the Lauren classification, GC is divided into intestinal and diffuse types, which have different clinical, pathological and prognostic

features. They differ not only in morphology, but also in epidemiology, the nature of progression, genetics and clinical picture. It has recently been observed that the location of the tumor is also important, since there is a difference between proximal and distal non-diffuse GC in terms of the expression level of different sets of genes [12; 13]. Despite significant progress in the diagnosis and treatment of GC, the survival rate is still low, only about 20 % of patients with GC can achieve 5-year survival. At the same time, surgical treatment is the only therapeutic method that provides the greatest probability of cure.

Finally, tumors of the CIN subtype were often found in the gastrointestinal junction/cardia, correlated well with the intestinal histological variant of Loren, showed pronounced aneuploidy and contained focal amplifications of receptor tyrosine kinases, in addition to TP53 mutations and RTK-RAS activation [7].

Table 1. Molecular classification of gastric adenocarcinoma based on cancer genome atlas with characteristic features of each subtype

Classification of the Cancer genome Atlas	Defining characteristics
EBV+	Mutations in *PIK3CA, ARID1A, TP53* genes
	CDKN2A inhibition
	PD-L1/L2 gene over-expression
	Hypermethylation of CpG residues
	Prevalence in males
	Over-expression of the signals by neural cells
MSI	*TP53*, *KRAS*, *PIK3A*, *ARID1A* mutations
	Hypermethylation of CpG residues
	MLH1 inhibition
	Prevalence in elderly people
	Prevalence in female
GS	*CDH1*, *RHOA* gene mutations
	Cell adhesion genes excess expression
	CLDN18-ARHGAP fusion
	Diagnosed prevalently in younger patients
	Diffuse histology
CIN	RTK-*RAS* gene activation
	Aneuploidy
	Mutations in *TP53*
	More often in the gastro-esophageal junction and cardia
	intestinal histology

In 2015, the Asian Cancer Research Group (ACRG) proposed a new classification system related to various genomic changes, disease progression and prognosis [10]. Four molecular subtypes were identified based on genome-wide sequencing, profiling of gene expression and the number of their copies, as well as targeted gene sequencing:

(1) Microsatellite unstable (MSI),

(2) with signs of epithelial-mesenchymal transition (MSS/EMT),

(3) Microsatellite stable with TP53 mutation (MSS/TP53+),

(4) microsatellite stable with wild-type TP53 (MSS/TP53) [10].

MSI tumors are hypermuted, intestinal type, usually antral, and are diagnosed at clinical stage I/II. MSI tumors had the best prognosis; their recurrence rate after surgical removal of primary GC was the lowest among all four subtypes (22 %). MSS/TP53+ tumors were associated with EBV infection and also had a good prognosis. MSS/EMT tumors appeared at a younger age, were mainly diagnosed at clinical stage III/IV and had a diffuse histological type according to Loren. The MSS/EMT subtype had the worst prognosis and the highest recurrence rate (63 %), with relapses localized mainly in the abdominal cavity [10]. In one of the studies, the RS samples were divided into two clusters according to the frequency of mutations in the genes – with a normal frequency (cluster 1) and with a high frequency of mutations (cluster 2). Cluster 1 was further divided into two subgroups, C1 and C2. The first subgroup (C1) had mutations in the TP53, XIRP2 and APC genes and was associated with a significantly better outcome than C2. And C2 was associated with mutations in the genes ARID1A, CDH1, PIK3CA, ERBB2 and RHOA (Table 2) [10].

Table 2. Molecular classification of gastric adenocarcinoma based on the Asian Cancer Research Group with characteristic features of each subtype

Classification of the Asian Cancer Research Group	Defining characteristics
MSI	Primary histology of intestinal type
	Predominantly in the antrum
	A large number of mutations in genes
	High rate of relapses and metastases confined to the liver
	Worse overall survival, higher stage at diagnosis
MSS /EMT	Worse overall survival, higher stage at diagnosis
	Young age
	Primarily diffuse histology
	Highest relapse rate, peritoneal spread
	Lowest mutation load
MSS / *TP53 +*	Second best overall survival
	The highest percentage among EBV1 related tumors
MSS / *TP53 -*	Higher rate of recurrence and metastases confined to the liver

2. Molecular profile of sporadic malignant tumors of the stomach.

The molecular characterization of GC continues to evolve. Many molecular classifications have been proposed and various molecular subtypes have been identified [9]. An important role in this was played by the study of the gene copy index.

It is known that the genes of various receptor tyrosine kinases (RTK), such as the human epidermal growth factor receptor (*EGF*), *EGFR1*, mesenchymal epithelial transition factor (MET) and GF2 fibroblast receptor (FGFR2) are amplified in GC [10; 11; 14; 15]. According to GI-screen (a nationwide cancer genome screening project), changes in gene copy are often detected: *ERBB2* (11.3 %), *CCNEI* (11.1 %), *KRAS* (3.7 %), *FGFR2* (3.3 %), *ZNF217* (3.3 %), MYC (2.7 %), *CCND1* (2.3 %) and *CDK6* (2.1 %) [16].

A change in the copy Number Variation (CNV) is a type of genetic polymorphism, the result of which may be a decrease or increase in the number of copies of a certain gene (which is often observed in various oncopathologies), and, consequently, a reduced or increased expression of the gene product – protein or non-coding RNA [17].

A lot of works by Russian authors have been devoted to the study of changes in the copyicity of genes in gastric cancer. In 2014–2015, the National Medical Research Centre for Oncology received data indicating the important role of changes in the copyicity of the genes *BAX*, *CASP3*, *CASP8*, *OCT4*, *C–MYC*, *SOX2*, *BCL2*, *NANOG*, *CASP9*, *NFKB1*, *HV2*, *ACTB*, *MKI67*, *IL-10*, *GSTP1* and *P53* in the malignancy of gastric tissues. It was found that the change in the copyicity of these genes is specific for cancer of a certain histological type, and also depends on the stage of differentiation of tumor cells and metastasis [18–23]. The obtained data formed the basis of the "Method of differential diagnosis of gastric cancer of various histological types" (Patent for invention No. 2613139. Date of state registration 03/15/2017), "Method for predicting the development of metastases in patients with gastric cancer" (Patent No. 2016122160 dated 06/03/2016), "Method for predicting the development of metastases to regional lymph nodes in patients with gastric adenocarcinoma" (Patent No. (19) RU(11)2661600(13) C1 dated 07/17/2018) and "Test systems for predicting the development of metastases in patients with gastric cancer" based on the determination of the number of copies of HV2 mtDNA (Patent No. 2683571 dated 03/29/2019).

Currently, the understanding of the molecular aspects of GC is improving thanks to studies using next-generation sequencing (NGS), which provide a high-performance method for the systematic detection of genetic changes in GC. By doing NGS Li-Chang et al. mutations of several driver genes were shown, including; *TP53*, *PIK3CA*, *CTNNB1*, *CDH1*, *SMAD4* and *KRAS* [24]. It was found that some of the tumor suppressor genes (TSG), such as APC, CDH1, CDH4, THBS1 and UCHL1, are inactivated by hypermethylation [25]. It has been shown that 59 % of RS have a mutation in chromatin remodeling genes such as *ARID1A*, *PBRM1* and *SETD2*. New mutated driver genes *MUC6*, *CTNN2A* and *GLI3* were found as a result of genome-wide sequencing [26; 27]. It was also found that genes involved in cell adhesion and chromosome organization demonstrate frequent mutations in patients with gastric adenocarcinoma, which confirms the presence of 30 driver mutations in primary tissues and lymph node tissues. Primary tumors show more mutations than metastatic tumors, but surprisingly, the researchers did not find any metastatic specific mutations. Several loci on chromosome 17q12 have been identified that are often amplified in GC: PPPIRIB-STARD3-T-CAP-PNMT, PERLD1-ERBB2-MAC14832-GRB7 [28]. In addition, two genes, CDKN2A and *CDKN2B*, located on chromosome 9p21, showed a decrease in the number of copies (CN = 0.8 ~ 1.32). These two genes encode proteins that perform a very important function – they inhibit cyclin-dependent kinases CDK4 and CDK6, and control cell proliferation, preventing entry into the S phase of the cell cycle, so their inactivation can lead to uncontrolled cell growth [28].

2.1 Genetic changes in gastric cancer.

Gene mutations in GC are divided into three categories:

1) Over-frequent drivers, demonstrate a high recurrence rate (> 5–10 %) in several tumors.

2) Rare drivers, mutate in the range of 1–10 %, but still contribute to the pathogenesis of the disease.

3) Mutations of the passenger/witness type arise as a consequence of the main mutational processes, but do not functionally contribute to oncogenesis [29].

Currently, the importance of mutations in the *RTK*/*RAS*/*MAPK* signaling pathway, frequent mutations in the *ERBB3* gene and *NRG1*/*ERBB4* ligand genes in GC has been established. With the help of NGS, the importance of changes in the *ARID1A* and *RHOA* genes in GC was revealed. *ARID1A*, as is known, encodes components of the chromatin remodeling complex and participates in the regulation of cell proliferation and the cell cycle, is mutated in 10–15 % of rye. *ARID1A* mutations are usually inactivating. The consequences of mutation in both *ARID1A* and RHOA are different. *ARID1A* mutations are distributed across the gene, whereas RHOA mutations are localized in the hot spot of the N-terminal region (Ty42, Arg5 and Gly17). It is assumed that *ARID1A* modulates the downstream transmission of Rho signals.

Mutations in *RHOA* can confer resistance to anoikis (a form of programmed cell death that occurs after the separation of cells from a solid substrate). From a clinical point of view, the detection of *RHOA* mutations provides a concrete pathway for the development of new targeted therapeutic approaches for diffuse type of GC, traditionally associated with an extremely poor prognosis [29].

Next, we will consider in detail changes in tumor suppressor genes, oncogenes, genes regulating the cell cycle, apoptosis and cell adhesion in GC.

1) Tumor suppressor genes (TSG). TSG (tumor suppressor genes) usually perform a protective role in preventing malignant cell transformation by repairing DNA, inhibiting cell proliferation, and initiating programmed cell death (apoptosis). TSGs are involved in the regulation of a number of cellular functions, including cell adhesion, intercellular interaction, cytoplasmic signal transmission and nuclear transcription [30]. Over the past decades, there has been a rapid increase in the number of TSG members who have been identified in connection with a wide range of hereditary and non-hereditary human oncological diseases. A better understanding of the TSG expression pattern in GC may allow the identification of specific biomarkers that can be used for early diagnosis and the development of targeted treatment. Overexpression of the P53 gene and decreased expression of the *PTEN*, *CDH1* (E-cadherin), *SMAD4*, *MGMT*, and *CD82* genes are largely associated with poor prognosis in malignant gastric tumors [30].

2) Oncogenes. Oncogenes are genes whose normal activity promotes cell proliferation. Oncogenes can be divided into five classes: secreted GF; cell surface receptors; components of intracellular signal transmission systems; DNA-binding nuclear proteins; components of a network of cyclins, CDK and kinase inhibitors that regulate the course of the cell cycle [31].

Oncogenes have the ability to turn normal cells into malignant ones. These genes make patients more predisposed or susceptible to cancer by altering or disrupting several mechanisms [31]:

(1) the production of nuclear transcription factors (TF) that control cell growth (e.g., *MYC*),

(2) signaling within cells (e.g., *RAS*),

(3) interactions of GFs and their receptors (e.g. *HER*/*NEU*).

Mutations transform proto-oncogenes into oncogenes through several processes such as amplification, translocation, and point mutation. Oncogenes are activated in many ways: by amplification, by point mutation and the formation of chimeric gene products. Consider the changes in some oncogenes.

The RAS gene is the first identified human oncogene, which is associated with the development of 20 % of all human malignancies. This gene encodes a protein that binds guanine nucleotides and performs various functions in the transmission of a mitogenic signal. And the activity of the protein itself is controlled by the GTP or GDP binding states (active – GTP-bound and inactive – GDP-bound).

The C-myc gene is another oncogene located on chromosome 8 encoding a nuclear phosphoprotein that acts as a transcription factor whose main function is to regulate the transcription of target genes by induction and suppression of expression [32]. It is also involved in the modulation of proliferation, differentiation and angiogenesis, as well as DNA repair and apoptosis [32]. Overexpression of *C-myc* is found in more than 40 % of gastric tumors and is associated with poor patient survival. It was found that in benign gastric lesions, including chronic atrophic gastritis, gastric ulcer and *H. pylori* infection, high expression of the *C-myc* gene is also observed [32].

The PRR11 gene was identified in 2013 as a new important regulator of the progression and oncogenesis of GC. Switching off *PRR11* in several gastric cell lines inhibited the rate of proliferation, migration of cancer cells, formation of cell colonies and

tumor growth *in vivo* experiments [33]. The results showed that mRNA and *PRR11* protein are activated in the tissues of the GC compared to the normal gastric mucosa. The expression of the PPR11 gene is associated with aggressive cancer phenotypes, including tumors with an increased degree of invasion, increased tumor differentiation and late-stage disease [33].

3) Regulators of the cell cycle. Cyclins are proteins that control the passage of key control points in the cell cycle by binding and activating specific cyclin–dependent kinases (CDKs). The transition from the G1-S phase is regulated by the activity of cyclin D, cyclin E, cyclin A and their catalytic partners, such as CDK 2, 4 and 6. The G2/M transition is regulated by cyclin-associated B-type kinase. Cyclin-CDK complexes stimulate cell cycle progression, and CDKI (CDK inhibitors) cause cell cycle arrest by suppressing CDK activity [34]. Moreover, unregulated expression of these molecules associated with the cell cycle leads to uncontrolled proliferation and malignant transformation of the cell [34]. Cell cycle control is regulated by D-type cyclins, which are most often mutated in tumor cells. There is increasing evidence that gastric carcinogenesis is associated with abnormalities in the expression of cyclins and other genes associated with the cell cycle [34].

4) Apoptosis regulatory genes. Initially, apoptosis was described by its morphological characteristics, including cell shrinking, membrane swelling, chromatin condensation and nuclear fragmentation [35]. The realization that apoptosis is a gene-driven program has had profound implications for understanding the biology of development and tissue homeostasis, it implies that the number of cells can be regulated by factors affecting cell survival, as well as those that control proliferation and differentiation. Moreover, the genetic basis of apoptosis implies that cell death, like any other program of metabolism or development, can be disrupted by mutation. In fact, it is now believed that defects in the pathways of apoptosis contribute to a number of human diseases, from neurodegenerative disorders to malignant neoplasms [35]. What triggers apoptosis during tumor development? Various factors are important. Extracellular factors include depletion of growth factors, hypoxia, radiation, and loss of cell-matrix interaction. Internal imbalance can also cause apoptosis, including DNA damage, telomere disruption, and inadequate proliferative signals caused by oncogenic mutations.

Cloning and characterization of the *Bcl-2* oncogene have established the importance of apoptosis in tumor development. *Bcl-2* was first identified at the chromosomal break point t (14; 18) in the human leukemia cell line [36]. To date, at least 15 *Bcl-2* family member proteins have been identified in mammalian cells, including proteins that promote apoptosis and those that prevent it [36]. In the gastric mucosa of patients with GC, compared with subjects with superficial gastritis, there is a decrease in the expression of the GKN1 protein and its mRNA [37]. GKN1 maintains the integrity of the gastric mucosa, protects it from the action of gastric juice and enzymes, as well as from mechanical damage, bacteria or foreign antigens [38]. It has been shown that GKN1 inhibits the growth of tumor cells and reduces the number of cell colonies, stopping the G2/M cell cycle instead of inducing apoptosis [39].

5) Genes are regulators of cell adhesion. Classical cadherins are transmembrane adhesion molecules containing five calcium-dependent domains that provide homotypic interactions, and cytoplasmic contact that binds to a number of effectors for transmitting physical and biochemical signals to the cell.

The names of cadherins were originally based on the type of cells in which their expression was first described, but now the generally accepted nomenclature defines classical cadherins as *CDH1* (E-cadherin), *CDH2* (N-cadherin), *CDH3* (P-cadherin), *CDH4* (R-cadherin) and *CDH15* (M-cadherin) [40]. The key role of E-cadherin during normal epithelial function is the function of a tumor suppressor. Mutations inactivating E-cadherin during RJ deletion inside the reading frame caused by the omission of exons 7 or 9, or random mutations of the reading frame shift.

The expression of E-cadherin is mainly limited to epithelial cells, whereas cells of neural or mesenchymal origin usually express N-cadherin. Epithelial cells differ phenotypically from mesenchymal cells; from an oncological point of view, the latter are more mobile and migrate. "Cadherin switching" (epithelial-mesenchymal transition, EMT) in cancer is defined as the absence of E-cadherin expression and N-cadherin expression [41], which induces or increases the metastatic ability of the tumor cell.

During EMT, type I cadherin (epithelial cadherin, E-cadherin encoded by the *CDH1* gene on human

chromosome 16q22.1), which supports key intracellular binding structures such as desmosomes and claudins, switches to neural cadherin (N-cadherin encoded by the *CDH2* gene), which is predominantly expressed among mesenchymal cells [42]. Reduction of E-cadherin with an immunoglobulin-like domain on the cell surface (capable of uniting neighboring cells) and an intracellular region (binds α- and β-catenin to the actin cytoskeleton) plays a crucial role in EMT, changing the components of intercellular adhesion and regulating various signaling pathways [43].

In GC, the expression of E-cadherin is suppressed by increased expression of aquaporin 3 (AQP3), thereby activating EMT. The *PI3K*/*AKT*/*SNAIL* signaling pathway is also involved in the induction of EMT in GC [44]. Caveolin-1 is modulated by HSP90 and functions as an important EMT regulator in GC. Insulin-like IGF-I induces EMT by increasing levels of Zeb2, which depends on the PI3K/Akt signaling pathway in GC cells [45].

GC is one of the typical malignant neoplasms associated with oxidative stress [46]. Hypoxia is also a significant inducer of EMT in gastric cancer. Under hypoxic conditions, the expression of E-cadherin decreases, and the expression of N-cadherin, vimentin, Snail, Sox2, Oct4, and Bmi1 increases, indicating that the hypoxic microenvironment induces EMT, accompanied by cytoskeletal remodeling [47]. Recent data indicate that EMT is a key factor in the progression of GC and plays a fundamental role in the early stages of invasion, metastasis and recurrence of GC [47].

2.1.1. Loss of heterozygosity (LOH).

This is a genetic phenomenon often observed with tumor suppressor genes in cancer. Since the human karyotype is diploid, mutation of one allele of the tumor suppressor gene is not enough to cause cancer. In heterozygous individuals, the wild-type allele provides a functional phenotype. However, when a "second strike" occurs, for example, due to improper chromosome segregation, this individual (or cell) may lose its "heterozygosity", which leads to a complete tumor phenotype. Karaman et al. [48] found a significant correlation between the prevalence of 17p (*TP53*) LOH and precancerous gastric lesion, indicating that the loss of TP53 may be an early event of gastric carcinogenesis [48].

Recent studies have shown that, although *PTEN* mutations in GC are rare, LOH of this gene is more common. Byun et al. (2003) found a decrease in the expression of PTEN and LOH to 47 % in 5 GC cell lines and 36 % of GC tissue samples [49]. The LOH level was significantly higher in the late stages than in the early stages of GC; it was also significantly higher in low-differentiated than in high- and medium-differentiated GC. This suggests that complete functional inactivation of *PTEN* does not necessarily cause gastric carcinogenesis, the loss of one allele is sufficient [49].

Malignant gastric tumors are characterized by high LOH frequencies in chromosomal regions 1p, 2q, 3p, 4p, 5q, 6p, 7p, 7q, 8p, 9p, 11q, 12q, 13q, 14q, 17p, 18q, 21q and 22q [50]. LOH at these sites leads to the loss of fragments/whole genes (tumor suppressor genes, cell cycle regulators and DNA repair).

2.1.2. Microsatellite instability.

In hereditary (most cases) and sporadic GC, another type of genomic instability, MSI (microsatellite instability), was also detected [51]. In patients with gastric cancer with the MSI phenotype, there is a high frequency of DNA replication errors leading to insertions/deletions of nucleotides in microsatellite repeats in tumor tissues [51]. These errors are detected and corrected by the MMR (repair of unpaired bases) protein complex. The development of the MSI phenotype in gastric cancer is usually associated with the inactivation or loss of MMR genes (for example, *MLH1* or *MSH2*), which leads to additional genetic anomalies (for example, inactivation of tumor suppressor genes and LOH) [51; 52].

MMR disruption can occur:

(1) as a result of mutational inactivation of one or two MMR genes

(2) as a result of epigenetic inactivation of MMR (CIMP) genes [51].

The MSI-type of GC is mainly associated with epigenetic disorders in MMR genes [52; 53], which leads to multiple mutations in other loci regulating cell growth (*TGF-β.RII*, *IGFIIR*, *RIZ*, *TCF4* and *DP2*), apoptosis (*BAX*, *BCL10*, *FAS*, *CASPASE5* and *APAF1*) and DNA repair (*hMSH6*, *hMSH3*, *MED1*, *RAD50*, *BLM*, *ATR* and *MRE11*) [54]. These changes further contribute to genetic instability and enhance the development of a malignant phenotype [54]. The genomes of gastric tumor cells with MSI are characterized by the presence of multiple mutations at many loci [55]. A high incidence of MSI in GC (MSI-H GC) is more

likely to occur with antral localization, with intestinal type, with expansive type and with seropositivity to *H. pylori* and correlates with a lower prevalence of lymph node metastasis [55]. MSI is a promising tool for identifying patients with genetic instability and patients with precancerous lesions [54; 52].

2.2. Epigenetic disorders.

Epigenetic disorders include changes in the transcriptional activity of genes, the regulation of which is not associated with a violation of the native DNA sequence [52; 56]. DNA methylation and histone modifications are usually studied as epigenetic events. Currently, the term epigenetics has been expanded to include inherited and transient/reversible changes in gene expression that are not accompanied by a change in the DNA sequence. A comprehensive understanding of various biological activities, such as DNA methylation, chromatin structure, transcriptional activity and histone modification, contributed to the development of epigenetics. The two main epigenetic modifications are DNA methylation and chromatin remodeling. DNA methylation is a chemical change in nucleosides that most often occurs in the cytosine portion of CpG dinucleotides. Chromatin remodeling occurs through histone modifications (mainly at the N-terminal tails), which ultimately affect the interaction of DNA with the chromatin-modifying protein. Both DNA methylation and histone modifications are associated with suppression of critical TSG and activation of oncogenes involved in cancer development [56].

2.2.1. Hypermethylation.

DNA methylation is a reversible chemical modification of cytosine in the CpG islands of the promoter sequence, catalyzed by a family of DNA methyltransferases. DNA methylation does not change the genetic information, but changes the "reading" from DNA and can lead to gene inactivation [56]. In general, methylation of CpG islands results in gene silencing. Methylated CpG islands also recruit histone deacetylases (HDACs) and other factors involved in transcriptional repression [56]. TSG inactivation via hypermethylation of CpG islands in promoter regions is an important event in carcinogenesis [56]. Hypermethylation of the p16 INK4a promoter was found in gastric carcinoma. Hypermethylation of *CDKN2A* may contribute to the malignant transformation of premalignant gastric lesions. *DAPK* hypermethylation is observed in intestinal, diffuse, and mixed types of gastric cancer and correlates with the presence of lymph node metastases, late stage, and poor survival [57]. Epigenetic silencing of the *XAF1* gene by aberrant promoter methylation has been reported in gastric cancer [57]. Caspase-1, a member of the cysteine protease family, exhibits a loss of expression in 19.3 % of gastric carcinomas [57], with the expression level being reversed when the cell line is treated with 5-aza-2'-deoxycytidine and/or trichostatin.

Hypomethylation of certain genes also contributes to gastric carcinogenesis. Initially, global genome hypomethylation was thought to be an exceptional event in the development of cancer [57]. Loss of methylation in cancer is mainly due to hypomethylation of repetitive DNA sequences. During the development of a neoplasm, the degree of hypomethylation of genomic DNA increases as the lesion passes from a benign disease to a metastatic one [57]. DNA demethylation can promote mitotic recombination, leading to deletions, translocations, and chromosomal instability [56]. Demethylation of MAGE, synuclein-γ (SNCG), and cyclin D2 has been described in gastric carcinoma [57].

In parallel with global hypomethylation, hypermethylation of CpG islands also has a silencing effect on miRNAs. MicroRNAs are short, 18–22 nucleotides, non-coding RNAs that regulate many cellular functions, including cell proliferation, apoptosis, and differentiation, by suppressing specific target genes through translational repression or mRNA degradation [58].

2.2.2. Histone modification.

In a normal cell, a precise balance maintains the nucleosomal DNA in either active/acetylated or inactive/deacetylated form. This adequate balance is controlled by acetylating enzymes (histone acetyltransferases) and deacetylating enzymes (HDACs). The modification involves methylation of the arginine and lysine residues of the histones. This methylation is catalyzed by histone methyltransferase and this process is involved in the regulation of a wide range of gene activity and chromatin structures. In general, lysine methylation at H3K9, H3K27, and H4K20 is associated with the suppression of gene transcription, while methylation at H3K4, H3K36, and H3K79 is associated with gene activation [59].

3. Features of the molecular profile of hereditary gastric cancer.

While the vast majority of gastric cancer cases are sporadic, familial aggregation occurs in about 10 % of cases, and of these, only 1–3 % are clearly hereditary. Hereditary gastric cancer includes syndromes such as hereditary diffuse gastric cancer, gastric adenocarcinoma and proximal gastric polyposis (GAPPS) and familial intestinal gastric cancer (FIGC). Gastric cancer has also been identified as part of other hereditary cancer syndromes such as hereditary nonpolyposis colorectal cancer, Li-Fraumeni syndrome, familial adenomatous polyposis, and Peutz-Jeghers syndrome [60].

Hereditary diffuse gastric cancer (HDGC) is one of the most genetically characterized forms of hereditary gastric cancer. HDGC is mainly associated with heterozygous *CDH1* (E-cadherin) mutations, including frameshift, nonsense and missense mutations, and large rearrangements [60]. A pathogenic mutation in *CDH1* increases the risk of developing diffuse gastric cancer at the age of 80 years to 70 % [60]. The histopathology of HDGC is comparable to sporadic diffuse gastric cancer, although the presence of typical precancerous lesions, *in situ* or *pagetoid* signet cells, is specific for CDH1-associated HDGC.

CONCLUSION

GC is a collection of various genetic and epigenetic changes, and its molecular landscape is extremely complex. Improvement in our understanding of the genetics of gastric cancer has accelerated significantly over the past decades, allowing us to redefine the definition of disease at the molecular level. These results may lead to the identification of high-risk groups and ultimately to improved treatment outcomes. The TGCA and ACRG classifications have opened the door to a complete understanding of the complex molecular landscape of gastric cancer. Studies of the genomic and epigenomic profile provide a better understanding of the molecular basis of gastric cancer. In this review, the characterization and classification of gastric cancer at the genetic and epigenetic levels confirms that this disease is highly heterogeneous. Clinicians should use the information gained from these studies to both develop and test potential markers and new targeted therapeutic approaches.

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Bondarenko O. K. – data analysis and interpretation.

South Russian Journal of Cancer 2023. Vol. 4, No. 1. P. 79-93
https://doi.org/10.37748/2686-9039-2023-4-1-8
https://elibrary.ru/jffeih
REVIEW

OPTIMAL MANAGEMENT OF LONG-TERM AIR LEAKAGE AFTER LUNG RESECTIONS FOR CANCER

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ABSTRACT

Lung resection is the main diagnostic and therapeutic surgical intervention in terms of lung cancer management. Air leak through pleural drains often occurs after lung resections due to damage to the pulmonary parenchyma. Therefore, proper drainage of the pleural cavity is very important for the successful outcome of the operation. The installation of a single pleural drainage after anatomical resection, the refusal to use vacuum aspiration and the earliest possible removal of drains contribute to the rapid activation of patients in the postoperative period. Prolonged air leakage (PAL) after lung resection, on average, develops in 15 % of lung cancer patients, remaining one of the most common complications adversely affecting the rehabilitation of patients and leading to delayed discharge from the hospital. The incidence of empyema with prolonged air leakage is 10.4 % with air discharge for more than 7 days compared to 1 % with air leaks less than or equal to 7 days. PAL requires prolonged drainage of the pleural cavity, which increases postoperative pain, causing shallow breathing, difficulty coughing leads to an increased risk of pneumonia, decreased mobility is accompanied by a high risk of thromboembolic complications. In addition, the treatment of complications is associated with the need to perform additional invasive interventions such as chemical or mechanical pleurodesis. Prolonged air leakage is associated with an increase in hospital mortality. Patients with an air leak have a 3.4 times greater risk of death than patients without it. Active tactics in relation to PAL include preoperative prediction of a high risk of complications, intraoperative measures to prevent air leak from the lung parenchyma and postoperative treatment to reduce the duration of PAL. The urgency of the problem is due to the fact that prolonged air leakage in patients with lung cancer after organ-preserving operations is associated with an increased risk of infectious complications due to the need for prolonged drainage of the pleural cavity. In this review, the main attention is paid to two components of postoperative management of PAL: diagnosis with an accurate assessment of the intensity of air leak and treatment of alveolar-pleural fistulas.

Keywords:
lung resection, prolonged air leakage, chest tube management, digital drainage systems, autologous blood patch pleurodesis, outpatient management

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Funding: this work was not funded.
Conflict of interest: authors report no conflict of interest.

For citation:
Iozefi K. D., Kharagezov D. A., Lazutin Yu. N., Stateshny O. N., Milakin A. G., Leyman I. A., Ayrapetova T. G., Vitkovskaya V. N., Gappoeva M. A., Mirzoyan E. A., Khomidov M. A., Shevchenko A. N., Dimitriadi S. N. Optimal management of long-term air leakage after lung resections for cancer. South Russian Journal of Cancer. 2023; 4(1): 79-93. https://doi.org/10.37748/2686-9039-2023-4-1-8, https://elibrary.ru/jffeih

The article was submitted 14.09.2022; approved after reviewing 25.01.2023; accepted for publication 06.03.2023.

Южно-Российский онкологический журнал 2023. Т. 4, № 1. С. 79-93
https://doi.org/10.37748/2686-9039-2023-4-1-8
https://elibrary.ru/jffeih
3.1.6. Онкология, лучевая терапия
ОБЗОР

ОПТИМАЛЬНОЕ ЛЕЧЕНИЕ ДЛИТЕЛЬНОЙ УТЕЧКИ ВОЗДУХА ПОСЛЕ РЕЗЕКЦИЙ ЛЕГКОГО ПО ПОВОДУ РАКА

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РЕЗЮМЕ

Резекция легкого – основное диагностическое и лечебное хирургическое вмешательство при раке легкого. Сброс воздуха по плевральным дренажам нередко возникает после операций на легких из-за повреждения легочной паренхимы. Следовательно, правильное дренирование плевральной полости имеет весьма важное значение для успешного исхода операции. Установка единственного плеврального дренажа после анатомической резекции, отказ от применения вакуум-аспирации и максимально раннее удаление дренажей способствуют быстрой активизации больных в послеоперационном периоде. Длительная утечка воздуха (ДУВ) после резекции легкого в среднем, развивается у 15 % больных раком легкого, оставаясь одним из наиболее распространенных осложнений, неблагоприятно влияющим на реабилитацию больных и приводящим к задержке выписки из больницы. Частота развития эмпиемы при ДУВ составляет 10,4 % при сбросе воздуха более 7 дней по сравнению с 1 % при утечках воздуха менее или равных 7 дням. ДУВ требует длительного дренирования плевральной полости, что усиливает послеоперационную боль, вызывая поверхностное дыхание, затрудненное откашливание приводит к повышенному риску развития пневмонии, снижение подвижности сопровождается высоким риском тромбоэмболических осложнений. Кроме того, лечение осложнения связано с необходимостью выполнения дополнительных инвазивных вмешательств таких как химический или механический плевродез. Длительная утечка воздуха связана с увеличением госпитальной летальности. Пациенты с утечкой воздуха имеют в 3,4 раза больший риск смерти, чем больные без нее. Активная тактика применительно к ДУВ включает в себя предоперационное прогнозирование высокого риска осложнения, интраоперационные мероприятия для предотвращения сброса воздуха из паренхимы легкого и послеоперационное лечение для сокращения продолжительности ДУВ. Актуальность проблемы обусловлена тем, что длительная утечка воздуха у больных раком лёгкого после органосохранных операций связана с повышением риска развития инфекционных осложнений в связи с необходимостью длительного дренирования плевральной полости. В данном обзоре основное внимание уделено двум составляющим послеоперационного ведения ДУВ: диагностике с точной оценкой интенсивности сброса воздуха и лечению альвеолярно-плевральных свищей.

Ключевые слова:
резекция легких, длительная утечка воздуха, ведение плевральных дренажей, цифровые дренажные системы, плевродез аутологичной кровью, амбулаторное ведение

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Финансирование: финансирование данной работы не проводилось.
Конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

Для цитирования:
Иозефи К. Д., Харагезов Д. А., Лазутин Ю. Н., Статешный О. Н., Милакин А. Г., Лейман И. А., Айрапетова Т. Г., Витковская В. Н., Гаппоева М. А., Мирзоян Э. А., Хомидов М. А., Шевченко А. Н., Димитриади С. Н. Оптимальное лечение длительной утечки воздуха после резекций легкого по поводу рака. Южно-Российский онкологический журнал. 2023; 4(1): 79-93. https://doi.org/10.37748/2686-9039-2023-4-1-8, https://elibrary.ru/jffeih

Статья поступила в редакцию 14.09.2022; одобрена после рецензирования 25.01.2023; принята к публикации 06.03.2023.

INTRODUCTION

Lung resection remains the main diagnostic or therapeutic intervention in thoracic surgery. In addition to a comprehensive preoperative examination, careful surgical intervention, proper postoperative care is absolutely necessary to achieve a favorable result of surgical treatment of lung cancer (LC). Air discharge through pleural drains often occurs after lung operations due to damage to the pulmonary parenchyma. The frequency of air discharge after lung resection ranges from 25 % to 50 % on the 1st day after surgery and up to 20 % on the 2nd day [1; 2]. Therefore, proper drainage of the pleural cavity is very important for the successful outcome of the operation. The installation of a single pleural drainage (PD) after anatomical resection, the refusal to use vacuum aspiration and the earliest possible removal of drainage against the background of sufficient anesthesia contribute to the rapid activation and rehabilitation of patients in the postoperative period. Air discharge in most cases stops spontaneously, but when it continues for 5–7 days after surgery, such a prolonged air leak (PAL) is considered a complication [3]. PAL due to the communication of the alveoli of the pulmonary parenchyma distal to the segmental bronchus with the pleural cavity [3] after lung resection, on average, develops in 15 % of patients with RL, remaining one of the most common complications adversely affecting the rehabilitation of patients and leading to delayed discharge from the hospital [4].

Improved Postoperative Rehabilitation (ERAS) programs are designed in such a way as to counteract possible complications with a scientifically based approach to their prevention and treatment. Active tactics in relation to PAL include preoperative prediction of a high risk of complications, intraoperative measures to prevent air discharge from the lung parenchyma and postoperative treatment to reduce the duration of PAL. In this review, the main attention is paid to two components of postoperative management of PAL: diagnosis with an accurate assessment of the intensity of air discharge and treatment of alveolar-pleural fistulas.

Number of pleural cavity drains

After anatomical resection of the lungs, an apical drainage tube for air removal and a basal drainage tube for fluid removal are traditionally installed. The need for a traditional approach has recently been challenged in the literature. To date, 4 randomized clinical studies (RCSs) have been conducted [5–8], one non-randomized study [9] and two meta-analyses [10; 11] that examined the results of postoperative management of pleural drainage after anatomical lung resections. They report on the duration of standing PD, the duration of hospital stay (DHS), the severity of postoperative pain and complications. No study provides data on the advantage of two PD compared to one pleural drainage. A shorter duration of standing PD and DHSwas found in one RCS [6] and in both meta-analyses [10; 11]. One meta-analysis [10] and 3 clinical studies [5; 7; 9] indicate a decrease in postoperative pain. Study of postoperative complications in 3 RCSs [5–7] and in both meta-analyses [12; 13] revealed no differences depending on the amount of PD, as well as in the need for repeated drainage of the pleural cavity.

It turns out that one drainage of the pleural cavity is quite enough, and the combined data indicate that one PD reduces the duration of standing PD and DHS. In fact, in patients with PAL and clinical manifestations of pneumothorax that are not controlled by a single PD, it may be necessary to install a second drainage. It is important that according to the literature data, there was no decrease in the need for repeated drainage of the pleural cavity when installing two drains [5–7; 10; 11]. ERAS protocols indicate the successful use of a single PD to control air discharge after lung resection [14]. Therefore, despite the traditional use of two drains, one signal drainage of the pleural cavity is quite sufficient to control air discharge and manage patients with advanced PAL.

Assessment of air discharge intensity

Traditional analog systems allow only a subjective static assessment of air discharge by PD. Digital devices provide more objective data on the intensity of air discharge by measuring and continuously recording the values of the air flow in the form of volume per unit of time, i.e. ml/min. The role of chest radiography has also been recently revised. In addition, patients are often discharged with portable drainage devices and, therefore, need to be assessed on an outpatient basis [12]. It is important to note that the methods discussed below are limited only to the discharge of air from the alveolar-pleural, but not the bronchopleural fistula, which always requires

a different treatment tactic.

The initial pleural drainage systems (IPDS) were three-balloon drainage devices. The most common analog drainage devices used today are the well-known: Pleur-evac® (Teleflex Incorporated, USA) and Atrium® (Maquet Getinge Group, Germany) [12]. The devices consist of a liquid collection chamber and a water gate chamber for measuring the intensity of air discharge. Air discharge is measured by the formation of air bubbles in the water gate chamber. The assessment of air discharge is made by registering a number on a numbered column into which air bubbles fall when the patient coughs or exhales; the higher the number reached, the greater the intensity of air discharge. Attempts to quantify the intensity of air discharge have been made in several classifications. However, the Robert David Cerfolio Classification System, represented by 4 classes of air discharge, remains the most frequently cited: 1st degree when coughing, 2nd degree when exhaling, 3rd degree when inhaling and 4th degree with constant discharge (bubbling) during inhalation and exhalation [13]. Observation of air bubbles in the water gate chamber is a very subjective method, creating uncertainty about the presence or absence of a small air discharge, which makes provocative overlap of the PD permissible [15]. Provocative overlaps of PD delay the discharge of patients with no PAL or carry the risk of developing pneumothorax and subcutaneous emphysema in patients with PAL.

Since 2007, digital SDPS have become popular, which contribute to reducing variability in the assessment of the intensity of air discharge when making clinical decisions and timely diagnosis of PAL. They allow you to accurately measure intrapleural pressure and maintain its stable negative parameters using an electronic sensor and a digital console. In addition, digital IPDSs are more portable compared to water-gate IPDSs, which facilitates physical activity of patients [12].

It was expected that the protocols developed on the basis of the use of digital IPDS would lead to simpler postoperative management of pleural drainage. Objective measurement of the intensity of air discharge will allow medical personnel to determine in time when the air leak has stopped, which should facilitate the earliest possible removal of PD and discharge from the hospital. On the other hand, it was assumed that the use of digital IPDS would ensure the active identification of patients with the development of PAL. Identification of PAL will help to determine in a timely manner the optimal management tactics for such patients and those who can be discharged from the hospital with portable drainage devices.

Currently, Thopaz® (Medela Healthcare, Baar, Switzerland) and Atmos® (Medizin Technik, Germany) are available to measure the intensity of air discharge, which allow continuous measurement of air flow and record it as a graph for 12–48 hours [15]. The potential advantages of more objective measurements provided by digital SDPS are considered to be: the possibility of the earliest possible removal of PD, fewer attempts of provocative squeezes and early prediction or early diagnosis of PAL [12]. Numerous RCSs have been carried out comparing the effectiveness of digital and analog IPDS with the primary endpoint in the form of the duration of hospital stay and the duration of standing PD (Table 1).

The advantage of digital SDPS in terms of reducing the duration of standing PD and reducing DHShas been demonstrated in 5 studies [16–20]. One study showed a shorter duration of standing PD without a significant difference in DHS [21]. The absence of significant differences in the duration of standing PD and in DHSwas registered in four studies [1; 22; 23] (Table 1). Two randomized studies showed that digital devices led to fewer provocative pinching PD [1; 22].

Possible explanations for such different results are the lack of consensus on the intensity of air discharge before the removal of PD and different amounts of PD. In fact, the intensity of the air flow used as a threshold value before removing drains from the pleural cavity ranges from 0 to 40 ml/min during various time intervals from 8 to 12 hours [20–24]. In addition to air discharge, the amount of liquid separated by PD is another criterion that is usually taken into account before removing drains. There is also no consensus regarding the amount of liquid allowed for the removal of PD with fluctuations in volume from 200 to 450 ml in 24 hours [21–24]. In addition, PD is not necessarily removed immediately after the cessation of air discharge, but usually during the day after the morning round. Thus, as soon as the final criteria are established, continuous monitoring of air discharge by digital drainage devices will finally make it possible to really benefit from the timely removal of PD.

The first meta-analysis concerning the use of various SDPS after lung resection was undertaken by S. Coughlin et al. in 2012. It analyzes 4 RCSs conducted during the period from 2001 to 2007 [16–19]. There were no significant differences in terms of the duration of air discharge, the frequency of blowing, the duration of standing PD and the duration of hospital stay when comparing the use of IPDS with vacuum aspiration or with a water gate [25]. In 2018, J. Zhou and colleagues conducted a meta-analysis of 10 RCSs involving 1601 patients on the same issues and in the same comparison groups. As a result, based on the results of their meta-analysis, the role of a water-gate or vacuum-aspiration IPDS still remained unclear. Nevertheless, the need for selective application of vacuum aspiration was justified by the presence of residual or increasing pneumothorax [26]. Recently, the use of digital IPDS after lung resections has become more and more popular. J. Zhou et al. in 2018 and N. Wang et al. in 2019. They spoke in favor of the clinical use of digital IPDS in patients who underwent lung resection to reduce the time of air discharge, the duration of standing PD, the duration of hospital stay compared with aspiration IPDS [26].

The last systematic review revealed 21 comparative RCSs of the effectiveness of digital and analog IPDS with the participation of 3399 patients, men

Table 1. Results of 10 RCSs comparing the effectiveness of digital and analog pleural drainage systems

Author/year	N/M (%)/ Ave. Age	Approach	Surgery type	Complications (%)	DHS
Cerfolio R. J., Bryant A. 2008 [20]	100/51 %/ 62.0	VATS: 0 % Thoracotomy: 100 %	LE: 55 % SE: 16 % AR: 29 %	No data	3.3 vs. 4.0 Days (p = 0.055)
Filosso P. L. et al. 2010 [22]	31/67.7 %/ 69.6 ± 3.4	VATS: 0 % Thoracotomy: 100 %	LE: 100 %	No data	8 vs. 7 Days (p = 0.0385)
Brunelli A. et al. 2010 [21]	166/72.9 %/ 66.7 ± 10.9	VATS: 0 % Thoracotomy: 100 %	LE: 100 %	15,06 %	6.4 vs. 6.3 Days (p < 0.05)
Bertolaccini L. et al. 2011 [24]	100/59 %/ 65.5 ± 13.6	No data	LE: 48 % SE: 6 % AR: 46 %	2 %	6.5 vs.7.1 Days (p = 0.09)
Pompili C. et al. 2014 [23]	390/52.3 %/ 66.2	VATS: 80.84 % Thoracotomy: 19.16 %	LE: 85.3 % SE: 14.7 %	No data	4.6 vs. 5.6 Days (p < 0.0001)
Lijkendijk M. et al. 2015 [27]	105/37.1 %/ 68.3	VATS: 39.04 % Thoracotomy: 60.96 %	LE: 100 %	No data	4 vs. 5 Says (p = 0.65)
Gilbert S. et al. 2015 [1]	176/36.3 %/ 68.0	VATS: 72.09 % Thoracotomy: 27.91 %	LE: 76.74 % SE: 23.26 %	13,64 %	4.0 vs. 4.0 Days (p = 0.09)
Lococo F. et al. 2017 [29]	95/51.5 %/ 63.6 ±13.0	No data	LE: 52.63 % AR: 47.37 %	2,11 %	5.8 vs. 6.2 Days (p = 0.5)
Plourde M. et al. 2018 [28]	215/43.2 %/ 67.5 ± 9.3	VATS: 83.72 % Thoracotomy: 16.28 %	LE: 93.49 % SE: 4.19 % AR: 2.32 %	5,12 %	4 vs. 5 Days (p = 0.47)

Note: N – number of patients; M (%) – male sex in %; VATS – video–assisted thoracoscopic surgery; LE – lobectomy; SE – segmntectomy; AR – atypical resection; DHS- duration of hospital stay.

make up 58.9 %, the average age of the subjects is 63.2 years, which were included in the meta-analysis [27]. The meta-analysis aimed to compare the clinical efficacy of digital and aspiration SDPS with a drainage device with a water gate in terms of their effect on the duration of standing PD, the frequency of PAL after lung resection and DHS. Data on surgical access were obtained in 2326 patients: 1439 (61.87 %) patients underwent thoracotomy and 887 (38.13 %) underwent video–assisted thoracoscopic surgery (VATS). The type of surgical intervention was established in 2744 patients: 2089 (76.13 %) underwent lobectomy or bilobectomy, 189 (6.89 %) – segmentectomy and 466 (16.98 %) – atypical resection or lung biopsy. Complications after lung resections, such as PAL, bleeding, atelectasis and pneumonia, are not uncommon, they account for about 6–23 %, 0.1–0.3 %, 1–20 % and 3–25 %, respectively. 9 RCSs selected for meta-analysis reported different rates of complications after lung resection in the range from 2 % to 61.54 % [12; 17; 19; 21–26].

13 studies [1; 16–20; 22–24; 28–31] with the participation of 1870 patients were analyzed to study the primary control point for which DHSwas selected. The use of digital IPDS or IPDS with a water gate was significantly associated with a shorter hospital stay than with the use of IPDS with vacuum aspiration; MD ranges between –1.40 (95 % CI: –2.20– –0.60) for digital IPDS and –1.05 (95 % CI: –1.91– –0.18) for IPDS with a water gate [27]. Regarding the duration of standing PD, 10 studies involving 2124 patients were analyzed [1; 17; 18; 20; 23; 24; 28–31]. Digital IPDS significantly reduced the duration of standing PD (MD: –0.68; 95 % CI: –1.32– –0.04), while the value of the IPDS with a water gate in reducing the duration of standing PD remained unconvincing. 14 studies have been studied on the problem of the occurrence of PAL, including data from 2,709 patients [17–21; 25; 28–31]. Despite the fact that digital and water–gate IPDS had a positive effect on the prevention of PAL, both methods did not achieve statistical significance (digital: OR = –0.76; 95 % CI: 0.42–1.39; water-gate: OR = 0.95; 95 % CI: 0.56–1.62) [27].

Meta-analysis showed that the use of both digital SDPS and a water gate is significantly associated with a shorter DHSthan when connecting PD to aspiration SDPS. Digital IPDS provided a reduction in the duration of standing PD by 0.68 days (MD: –0.68, 95 % CI: from –1.32 to –0.04), and a water gate by 0.45 days (MD: –0.45, 95 % CI: from –1.11 to 0.20) compared to the IPDS with vacuum aspiration. Digital SDPS led to a reduction in DHSby 1.4 days (MD: –1.40, 95 % CI: –2.20 to –0.60), while the use of a water gate is associated with a reduction in DHSby 1.05 days (MD: –1.05, 95 % CI: –1.91 to –0.18) compared with aspiration SDPS [27]. It is logical that earlier removal of PD leads to a shorter stay in the hospital, which is the main result confirmed by meta-analysis.

The difference in results between the hospital stay and the duration of standing PD is explained: firstly, by the heterogeneity of the analyzed studies presented by different clinics and surgeons with their own experience; secondly, by the fact that the studies were conducted at different times for almost 20 years and, consequently, the results could be influenced by innovations in the field of anesthesiology and thoracic surgery.

As for PAL after lung resections, the use of digital IPDS had a positive, although not statistically reliable, effect on their frequency (OR = 0.76; CI: 0.42–1.39; p = 0.78). IPDS with a water gate also has a lower odds ratio OR (OR = 0.95; 95 % CI: 0.56–1.62) in the prevention of PAL in comparison with a vacuum-aspirated IPDS [27]. The results obtained are consistent with the recommendations for accelerated rehabilitation after lung surgery published in 2019 [32]. Routine use of vacuum aspiration for PD management after lung resection in the postoperative period is no longer recommended.

Thus, despite the absolute importance of drainage of the pleural cavity, PD causes pain, worsens lung function and prevents patients from performing physical exercises regardless of the surgical approaches used [33]. The inconveniences created by prolonged standing PD delay the postoperative rehabilitation of patients. Therefore, early removal of PD is essentially the ultimate goal of optimizing postoperative management after lung resection, allowing to reduce DHSand the costs associated with treatment [33].

In the postoperative period, chest radiography is usually prescribed, which, despite the minimal side effect, causes discomfort in patients, especially in the first days after surgery [1; 34]. In addition, it is now known that asymptomatic pneumothorax is safe and ERAS protocols recommend standard PD management [12; 35].

A retrospective review of 1,550 radiographs and related prospectively collected clinical data in 176 patients showed that the results of the RGC did not change the management tactics of patients who did not have clinical symptoms such as shortness of breath, chest pain, tachycardia or decreased oxygen saturation [29]. Similarly, in a meta-analysis involving 3,649 patients, the appointment of RGC only for clinical indications reduced the number of radiographs per patient by 3.15 without increasing mortality, stay in the intensive care unit or DHS [36].

The RCS results discussed in detail above include parameters important for ERAS protocols, such as: the frequency of PAL, the duration of standing PD, DHSand the presence of residual pneumothorax after removal of drains from the pleural cavity. Obviously, it makes no sense to repeat, noting that two protocols aimed at standardizing the management of patients after lung resection established a PD management regime with their connection to active vacuum aspiration until the 1st day of the postoperative period, followed by a transition to a water shutter in the absence of contraindications [12; 35]. It seems at the moment that the tactics of PD management adopted in a particular center are probably more important than the ongoing debate about the benefits and harms of using active vacuum aspiration.

Despite the fact that modern literature generally focuses on the conservative treatment of PAL, including outpatient management for persistent air discharge and observation of pneumothorax detected in RGC [35], patients who do not tolerate PAL, as well as with the threat of postoperative pneumonia or pleural empyema, invasive measures are shown to eliminate the complication. Among other things, it is necessary to continue studying new and old methods of active resolution of PAL.

Pleurodesis

Pleurodesis is performed without surgery at the patient's bedside using a chemical substance or autologous blood. The use of both methods is reported in small cohort and RCSs. Literature sources indicate that autologous blood pleurodesis (PAC) appears to be a promising way to resolve PAL.

Many drugs, such as talc, silver nitrate, doxycycline, tetracycline, bleomycin and interferon, are injected into the pleural cavity in order to cause inflammation leading to the adhesive process. For the formation of pleural accretions, chemicals require a good apposition of the visceral and parietal pleura. An inflammatory reaction often causes pain, fever, shortness of breath and even acute respiratory distress syndrome (ARDS). The literature supporting the use of chemical pleurodesis in the postoperative period is limited [38]. However, a retrospective review of 41 patients after lung resection who received chemical pleurodesis using talc, doxycycline and a combination of these drugs revealed successful termination of PAL in 40 (97.6 %) patients. The average duration of PAL after administration of the sclerosing agent was 2.8 days. Pleural empyema developed in 1 (2.5 %) patient [39]. An interesting clinical study of the effectiveness of three methods of treatment of PAL after lung resection was published by S Jabłonski, in 2018. Chemical pleurodesis with an aqueous solution of iodine in 30 patients and intrapleural administration of 200 mg of doxycycline in 34 patients was compared with a control group of 35 patients who were administered only lidocaine solution. The shortest standing time of PD and DHSwas observed in the pleurodesis group with an aqueous solution of iodine ($p < 0.001$), which was associated with strongly noticeable chest pain ($p < 0.0001$) [39]. Despite the seemingly encouraging results, surgeons are reluctant to use chemical pleurodesis after lung resection, since talc, being essentially a foreign body, causes a rough adhesive process that makes repeated surgical intervention extremely difficult. Other methods of chemical pleurodesis with the introduction of other drugs are accompanied by severe pain and are not always effective.

On the contrary, PAK as a method of pleurodesis has been studied more thoroughly and is more often discussed in the timely literature. PAK was proposed 35 years ago for the treatment of patients with spontaneous pneumothorax. The first report on the use of PAK in patients with PAL after lobectomy was published 30 years ago. It refers to 2 patients who were successfully treated with PAK as a "last resort" of conservative therapy for PAL [40]. Several theories have tried to explain the mechanism of action; one hypothesis suggests that blood initiates an inflammatory reaction of the pleura, leading to an adhesive process, while another hypothesis supports the idea that the alveolar-pleural fistula is directly clogged with blood [41].

Since then, several studies have been conducted on this issue. The usefulness of ABP for the treat-

ment of PAL has been the subject of two systematic reviews and meta-analysis [42]. The first review published by K. Manley and colleagues in 2012 included patients with PAL, which occurred both as a result of spontaneous pneumothorax and after lung resection. The second review, devoted to the study of the role of ABP, included 10 studies involving 198 patients who developed PAL after thoracic surgery [42].

Usually, 50 to 120 ml of blood is taken from the peripheral vein of the patient and injected into the pleural cavity through drainage. The timing of the procedure, depending on the day of the postoperative period in which the procedure was performed, is shown in Table 2. The amount of blood used for pleurodesis varied from 45 to 250 ml. In one study, patients were randomized for ABP with blood volumes of 50 or 100 ml and it was concluded that patients of the second group had a significantly shorter drainage time of the pleural cavity [43]. In 9 studies, blood was injected directly through pleural drainage, and in one study, an additional catheter was installed through pleural drainage to ensure more targeted blood injection [44]. Patients with a "residual space" confirmed by the results of chest radiography were included in two studies [45; 46], and in the third study, most patients had a "residual space" [47]. In 4 studies, it is reported that in some cases more than one blood injection was required [44, 46–48], so in one observation, four injections were reported in one patient table 2 [44]. ALK TKI in subsequent lines of targeted therapy of previously treated ALK-positive NSCLC.

In 3 studies, PAL was present for almost two weeks before the decision was made to proceed with PAK [44; 47]. In addition, in a number of studies before PAK, such measures as pleurodesis with the introduction of tetracycline or other methods of chemical pleurodesis were unsuccessfully used. Therefore, it is realistic to assume that the PAK has successfully eliminated PAL in patients with prolonged or very prolonged air discharge.

As for the treatment of PD after the PAK procedure, it is more often described lifting the drainage tube above the patient's level with the cessation of vacuum aspiration, in one study it was reported that vacuum aspiration continued when the drainage was raised, and in another one – the drainage was squeezed for 30 minutes, and then connected to a water gate. It is worth noting that in the last study, all patients had a second PD, which remained connected to the water gate without aspiration. Usually, ABP were performed without any additional blood treatment, in one study blood was mixed with Picibanil [45], and in another study a pneumoperitoneum was applied the day before ABP [46].

In 2 studies, complications after ABP were not reported [43; 48], in 2, one case of empyema was registered [44; 49]. In addition, a total of 17 patients had fever after PAK, but only two had a positive microbiological examination (Table 2). It is important to observe complete sterility, since blood is a known nutrient medium for bacteria [42].

As a result, meta-analysis showed that the success rate of ABP for resolving postoperative PAL within 48 hours was 83.7 % (95 % CI: 75.7–90.3) for all included patients and 85.7 % (95 % CI: 74.4–94.0) in patients who underwent lung resection. The total frequency of empyema after the procedure was 1.5 %, and the frequency of fever was 8.6 %. To identify a potential correlation between the amount of blood used for pleurodesis and the success of the ABP, the Pearson coefficient was calculated; no correlation could be detected (r = 0.049, p = 0.893) [42].

A relatively small study by J. J. Rivas de Andres et al. demonstrated the same level of success as the meta-analysis. At the same time, according to the results of the last RCS from Mayo Clinic, the resolution rate of PAL after PAK was 65 %, which contributed to a tendency to decrease the duration of standing PD from 16 to 11 days (HR = 1.5–2; p = 0.14,), DHS (p = 0.13) and a significant decrease in the number of repeated hospitalizations (HR = 0.16; p = 0.02), and repeated operations for PAL or empyema (HR = 0.11; p = 0.05) [48].

In general, the literature supports the opinion that PAK is an effective means of eliminating PAL in patients after lung resections. Taking into account the available evidence of efficacy and the low complication rate, ABP should be considered for the elimination of PAL within the framework of ERAS protocols [13]. In addition, it is interesting to conduct studies to compare ABP with the management of patients on portable drainage devices, taking into account the duration of standing PD and DHSas the main endpoints.

Endobronchial valves (EV)

EV are currently being implemented in the form of endobronchial valves (Zephyr®, PulmonX Inc.)

and intrabronchial valves (IBV/SVS system®, Spiration Inc.) [50]. EVimplantation is described in detail and is carried out in three stages: 1. Identification of segmental or sub-segmental bronchus leading to PAL by means of successive balloon inflations with monitoring of the termination of air discharge through the drainage of the pleural cavity; 2. selection of a suitable valve size according to the caliber provided by the manufacturer; 3 valve installation [51].

In the modern literature, special attention is not paid to the treatment of postoperative PAL using the installation of EC. Publications on their use for the treatment of PAL are limited to a series of cases that include postoperative PAL, along with other causes such as spontaneous, traumatic and iatrogenic pneumothorax [51]. An international study involving 40 patients who had EVinstalled to eliminate PAL included 8 patients with postoperative PAL. After the installation of EV in 19 (47.5 %) of 40 patients, PAL was completely eliminated, in 18 (45 %) patients the intensity of air discharge decreased, in 2 (5 %) there was no response. The median and average duration of pleural drainage after the procedure were 7.5 days and 21 days, respectively. The median and average DHSvalues after valve installation were 19 days and 11 days, respectively [52].

In another study, 9 patients with an average duration of PAL of more than 4 weeks were treated with the help of EC. Successful valve installation was performed in 7 (77.8 %) patients; 3.5 valves were used on average. The average duration of PAL after valve installation was 1 day and four patients were discharged within 2–3 days after valve installation [53]. In another study, 21 (10 after lung resection) patients with PAL underwent 24 procedures to install EC. Drainage of the pleural cavity lasted on average for 15 days, and the average DHS was 5 days after the valve was installed [54].

Obviously, the use of EVfor the treatment of postoperative PAL is limited to a small number of cases. Endobronchial valves were mainly used as a last resort or in patients with the inability to use other methods of treatment. Perhaps their earlier use can improve the results. To compare EV with the standard treatment of PAL, a multicenter prospective RCS (Valves Against Standard Therapy) is currently being conducted, which is not limited to postoperative PAL [55]. In addition to the risk of increased exacerbations of COPD, the development of pneumonia and hemoptysis, the installation of EVin postoperative PAL may be accompanied by the development of atelectasis. Therefore, until more data is obtained, EV should remain the last resort to eliminate postoperative PAL.

Repeated operation

There are no studies comparing repeat surgery with other PAL treatments. Many intraoperative methods of preventing PAL have been described, including: strengthening of mechanical suture lines, the use of surgical sealants, the creation of pleural awnings and

Table 2. Clinical studies of pleurodesis with autologous blood

Author/year	Patients number	Time of con-ducting the procedure	Volume of the infused fluid (ml)	Complictions
Yokomise H., et al.1998 [45]	10	8.7 ± 4.7	50	Fever 5
Droghetti A., et al. 2006 [47]	21	11 on average	50–150	Fever 1
Andreetti C., et al. 2007 [43]	25	6 on average	50 or 100	Absent
Oliveira FH., et al. 2010 [44]	27	10.6 on average	90	Empyema 1, Fever 1
Korasidis S., et al. 2010 [46]	39	No data	100	Fever 6
Dye K., et al. 2020 [49]	19	7 on average	45–120	Empyema 1
Hasan IS., et al. 2021 [48]	34	6 days	90	Absent

the imposition of pneumoperitoneum [12]. However, all of them have not been studied in conditions of repeated use.

However, repeated intervention is rarely required [56]. Probably, the operation is most indicated when intensive air discharge is unexpectedly detected during the first 24 hours after lung resection. Early repeated surgery helps to eliminate the failure of bronchial sutures, identify and suture damage to the lung parenchyma or strengthen the lines of the mechanical suture and apply the above-mentioned methods to prevent PAL. ERAS protocols for lung resection do not provide for repeated operations and are mainly focused on more conservative treatment of PAL [14]. Repeated surgery, as a rule, is not indicated for many patients and is performed in the early postoperative period with intensive PAL or in cases of delayed occurrence of massive air discharge.

Outpatient management

PAL, will develop independently of the best practice of thoracic surgery. Until the invasive methods of treatment of PAL are thoroughly studied to maximize the impact, ERAS protocols provide for outpatient management of PAL. Three positions should be clearly defined in the protocols: 1. when to connect a sick patient to a portable drainage device; 2. how and when to conduct outpatient monitoring; 3. what are the criteria for removing drainage from the pleural cavity.

R. J. Cerfolio et al. connected the Heimlich valve to PD in 55 patients with air discharge, of which 22 stopped during the day, but 33 patients were diagnosed with PAL. In 6 cases, the Heimlich valve had no effect, requiring the PD to be reconnected to the water valve or to the vacuum aspirator; in all patients, air discharge was of the 4th degree according to the Robert David Cerfolio Classification System. In the end, all 33 patients were discharged home with the Heimlich valve and treated on an outpatient basis. In a larger study involving 193 patients with PAL, R. J. Cerfolio et al. It was shown that 190 of them were cured without serious complications, and all 3 patients with complications had impaired immunity [57].

A retrospective review of prospectively collected data from 65 patients discharged with portable drainage devices found a decrease in DHSby an average of 3.65 days compared to the STS (Society of Thoracic Surgeons) database as a control [58]. Another retrospective analysis of the data of 73 patients discharged from the clinic over a 10-year period again showed a decrease in DHS (average 3.88 days) compared with the control group in the same institution (average 5.68 days). There was no increase in the number of complications in patients discharged with a portable drainage device, and only two patients required repeated hospitalizations [59].

In another study, PD was connected to the Heimlich valve on the 4th day of the postoperative period, and patients were discharged between 5 and 11 days of the postoperative period after learning how to check the air discharge, after which the drainage was removed. With PAL for more than 2 weeks, patients were hospitalized for provocative pinching of PD and resolving the issue of their removal [55]. Later R. J. Cerfolio et al. He reported connecting 193 patients to a portable drainage device on the 3rd day after surgery with discharge on the 4th day. All patients were discharged with the recommendation of oral antibiotics. Drains from the pleural cavity were removed on average 16.5 days after discharge, even in the presence of PAL or pneumothorax according to the results of the RGC [57]. A. M. Royer with col. the patients were examined within 3 days after discharge and all performed RGC. Drains from the pleural cavity were removed on average 4.7 days after discharge [58]. R. K. Schmocker and colleagues examined patients 4–5 days after discharge with the help of RGC and assessment of the presence of air discharge. Drains were removed an average of 8.3 days after discharge [59].

It is obvious that PAL negatively affects the timing of the start of adjuvant treatment [60]. Thus, there is retrospective evidence that patients can be safely discharged home with portable drainage devices. In most studies, patients were discharged on the 4th or 5th day after surgery, followed by follow-up for 3–5 days. Pleural drainage was usually removed within 4–11 days after discharge, and one study showed that all PD can be removed about 17 days after discharge, even in the presence of PAL or pneumothorax [59]. In the future, early identification of patients who can be discharged with portable drainage systems and forecasting the day of termination of air discharge will ensure timely discharge and follow-up planning, reducing the cost of medical care.

CONCLUSION

Thus, PAL after lung resection remains the most common postoperative complication in thoracic surgery. The analysis of modern literature indicates that digital drainage systems, providing objective, documented evidence of the cessation of air discharge, will be useful for the implementation of ERAS protocols aimed at the earliest possible removal of pleural drains. Clear evidence is presented that the use of active vacuum aspiration does not prevent air discharge, but possibly enhances it, therefore algorithms based on the experience of a particular institution will ensure optimal management of pleural drains, in particular, during the development of postoperative PAL. The use of routine RGC is minimized if there are no clinical indications. Pleurodesis with autologous blood appears to be the most promising minimally invasive way to eliminate PAL. At the same time, until its role is confirmed by a large-scale randomized clinical trial, there will remain a need for conservative management of PAL with early discharge of patients with portable drainage devices.

The latest advances in technology and evidence-based approaches in thoracic surgery provide a platform for eliminating contradictions in the postoperative care of patients who have undergone lung resection, creating a solid foundation for the development of algorithms to combat PAL.

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South Russian Journal of Cancer 2023. Vol. 4, No. 1. P. 94
https://doi.org/10.37748/2686-9039-2023-4-1-9
https://elibrary.ru/zjuihk

MONOGRAPH REVIEW

KIT OI, SHAPOSHNIKOV AV. GENERAL CARCINOGENESIS. EXOGENOUS TUMOROGENIC EFFECTS. ROSTOV-ON-DON: LLC "DON PUBLISHING HOUSE"; 2022, 128 P.

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The second part of a three-component work on general carcinogenesis has been published. The first part (2021) deploys modern theories, models of general carcinogenesis: mutation theory, models of genomic instability, Darwinian and non-genotoxic models, and also explains the roles of inflammation, immunological deviations and tumor microenvironment in carcinogenesis.

Particular attention has been paid to the biological characteristics of tumor cells, genetic characteristics and metabolic changes that initiate and promote tumor growth.

A general concept of carcinogenesis has been formulated, which boils down to the presence of "ring" spheres: tumorigenic environment → the organism as a whole system and its subsystems → target organ → protective tumorigenic tissue microenvironment → target cell → genetic alterations, eventually leading to malignant growth.

The second part of the manual (2022) is devoted to the mechanisms of carcinogenesis. In this monograph, the main attention is paid to the "outer ring of carcinogenesis" – a tumorigenic environment, which includes a wide range of various negative factors: from cosmic and terrestrial to food, medical and iatrogenic ones.

The book provides modern facts about the prevalence of certain agents, their influence on the body and the creation of carcinogenic background at the organ-cellular and gene-molecular levels. Attention is focused on the dependence of the cancer effects of exogenous influences on their intensity, duration, organotropism and genotoxicity. In conclusion, a general assessment of these effects as inducers of malignant growth is given.

The manual contains 20 tables reflecting all the key positions of the factual material. The monograph is illustrated with 29 color figures brilliantly prepared by the authors. The print quality is amazing.

Each chapter is accompanied by references, predominantly for the last 5–7 years.

In our opinion, Chapter 3.7 “Medical influences as inducers of carcinogenesis” is of particular interest.

There are 3 groups of carcinogenic medical effects: A – causing the development of primary, previously non-existing malignant neoplasms (MNs) at the preventive, diagnostic, therapeutic and rehabilitation levels among non-cancer patients; B – inducing growth and metastatic spread of existing MNs; C – contributing to the development of other primary tumors in patients with MNs. Drug-induced carcinogenesis develops in several directions.

In recent years, the approach to assessing the key characteristics of human carcinogens has changed. Most of them (85 %) have turned out to be genotoxic, 47 % change cell proliferation, and 40 % induce oxidative stress. Other mechanisms of carcinogenesis also retain their negative roles.

The monograph can be used in the course of lectures and practical classes on oncology for students, PhD students and residents of the faculty of physician promotion. It is of considerable interest to all oncologists.

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