

BIOMEDICAL PHOTONICS

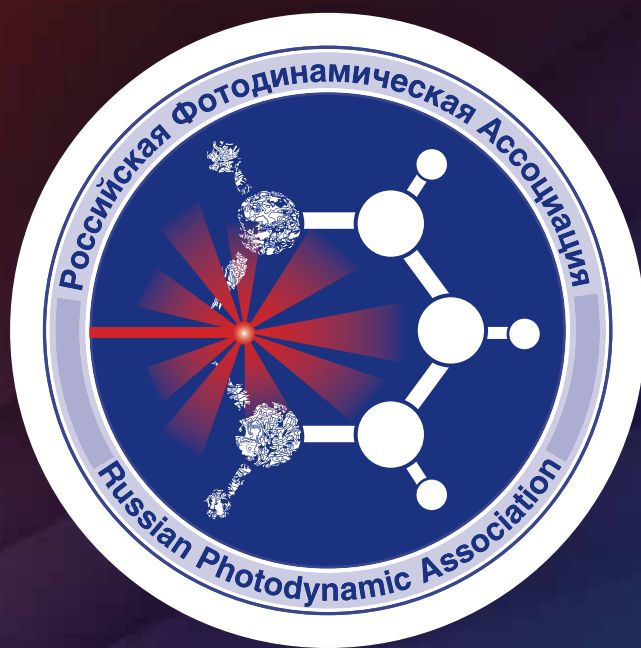
Volume 15, # 3, 2026

In the issue:

- Expression profiles of heme synthesis metabolite transporters as potential molecular determinants of sensitivity to 5-ALA-induced PDT
- Comparative evaluation of the efficiency of various modes of photodynamic and radiation therapy exposed to cells of transplanted Ehrlich's carcinoma in experiment
- Analysis of collagen presence in brain tumors using spontaneous Raman scattering spectroscopy
- Combination of ferroptosis and photodynamic therapy is an effective strategy for cancer treatment
- Potential of endogenous photosensitizers in photodynamic therapy of mycobacterial infections

BMP

Российская Фотодинамическая Ассоциация



www.pdt-association.com

BIOMEDICAL PHOTONICS

FOUNDERS:

Russian Photodynamic Association
P.A. Herzen Moscow Cancer Research Institute

EDITOR-IN-CHIEF:

Filonenko E.V., Dr. Sci. (Med.), Professor, Head of the Centre of Laser and Photodynamic Diagnosis and Therapy of Tumors in P.A. Herzen Moscow Cancer Research Institute (Moscow, Russia)

DEPUTY CHIEF EDITOR:

Grin M.A., Dr. Sci. (Chem.), Professor, Chief of Department of Chemistry and Technology of Biological Active Substances named after Preobragenskiy N.A. in Moscow Technological University (Moscow, Russia)

Loschenov V.B., Dr. Sci. (Phys. and Math.), Professor, Chief of Laboratory of Laser biospectroscopy in the Natural Sciences Center of General Physics Institute of the Russian Academy of Sciences (Moscow, Russia)

EDITORIAL BOARD:

Kaprin A.D., Academician of the Russian Academy of Sciences, Dr. Sci. (Med.), Professor, General Director of National Medical Research Radiological Centre of the Ministry of Health of the Russian Federation (Moscow, Russia)

Romanko Yu.S., Dr. Sci. (Med.), Professor of the Department of Oncology, Radiotherapy and Plastic Surgery named after L.L. Lyovshina in I.M. Sechenov First Moscow State Medical University (Moscow, Russia)

Stranadko E.Ph., Dr. Sci. (Med.), Professor, Chief of department of Laser Oncology and Photodynamic Therapy of State Research and Clinical Center of Laser Medicine named by O.K. Skobelcin of FMBA of Russia (Moscow, Russia)

Blondel V., PhD, Professor at University of Lorraine, Joint-Head of the Health-Biology-Signal Department (SBS) (Nancy, France)

Bolotine L., PhD, Professor of Research Center for Automatic Control of Nancy (Nancy, France)

Douplik A., PhD, Professor in Ryerson University (Toronto, Canada)

Steiner R., PhD, Professor, the Honorary Director of Institute of Laser Technologies in Medicine and Metrology at Ulm University (Ulm, Germany)

BIOMEDICAL PHOTONICS –

research and practice, peer-reviewed, multidisciplinary journal.

The journal is issued 4 times per year.

The circulation – 1000 copies., on a quarterly basis.

The journal is included into the List of peer-reviewed science press of the State Commission for Academic Degrees and Titles of Russian Federation.

The journal is indexed in the international abstract and citation database – Scopus.

The publisher "Agentstvo MORE".
Moscow, Khokhlovskiy lane, 9

Editorial Staff:

Chief of the editorial Staff	Ivanova-Radkevich V.I.
Science Editor	Prof. Mamontov A.S.
Literary Editor	Moiseeva R.N.
Translators	Kalyagina N.A.
Computer Design	Kreneva E.I.
Desktop Publishing	Shalimova N.M.

The Address of Editorial Office:

Russia, Moscow, 2nd Botkinskiy Proezd, 3
Tel. 8 (495) 945–86–60
www: PDT-journal.com
E-mail: PDT-journal@mail.ru

Corresponding to:

125284, Moscow, p/o box 13

Registration certificate (PI), No. FS 77–51995, issued on 29.11.2012 by the Federal Service for Supervision of Communications, Information Technology, and Mass Media of Russia.

The subscription index
of "Rospechat" agency – 70249

The editorial staff is not responsible for the content of promotional material. Articles represent the authors' point of view, which may be not consistent with view of the journal's editorial board. Editorial Board admits for publication only the articles prepared in strict accordance with guidelines for authors. Whole or partial presentation of the material published in the Journal is acceptable only with written permission of the Editorial board.

BIOMEDICAL PHOTONICS

BIOMEDICAL PHOTONICS –

научно-практический, рецензируемый,
мультидисциплинарный журнал.
Выходит 4 раза в год.
Тираж – 1000 экз., ежеквартально.

Входит в Перечень ведущих рецензируемых
научных журналов ВАК РФ.
Индексируется в международной
реферативной базе данных Scopus.

Издательство «Агентство МОРЕ».
Москва, Хохловский пер., д. 9

Редакция:

Зав. редакцией	Иванова-Радкевич В.И.
Научный редактор	проф. Мамонтов А.С.
Литературный редактор	Моисеева Р.Н.
Переводчик	Калягина Н.А.
Компьютерный дизайн	Кренева Е.И.
Компьютерная верстка	Шалимова Н.М.

Адрес редакции:

Россия, Москва, 2-й Боткинский пр., д. 3
Тел. 8 (495) 945–86–60
www: PDT-journal.com
E-mail: PDT-journal@mail.ru

Адрес для корреспонденции:

125284, Москва, а/я 13

Свидетельство о регистрации ПИ
№ ФС 77–51995, выдано 29.11.2012 г.
Федеральной службой по надзору в сфере
связи, информационных технологий
и массовых коммуникаций (Роскомнадзор).

Индекс по каталогу агентства

«Роспечать» – 70249

Редакция не несет ответственности за содержа-
ние рекламных материалов.

В статьях представлена точка зрения авторов,
которая может не совпадать с мнением редак-
ции журнала.

К публикации принимаются только статьи, под-
готовленные в соответствии с правилами для
авторов, размещенными на сайте журнала.

Полное или частичное воспроизведение
материалов, опубликованных в журнале,
допускается только с письменного разрешения
редакции.

УЧРЕДИТЕЛИ:

Российская Фотодинамическая Ассоциация,
Московский научно-исследовательский онкологический институт
им. П.А. Герцена

ГЛАВНЫЙ РЕДАКТОР:

Филоненко Е.В., доктор медицинских наук, профессор, руководитель
Центра лазерной и фотодинамической диагностики и терапии опухолей
Московского научно-исследовательского онкологического института
им. П.А. Герцена (Москва, Россия)

ЗАМ. ГЛАВНОГО РЕДАКТОРА:

Грин М.А., доктор химических наук, профессор, заведующий
кафедрой химии и технологии биологически активных соединений
им. Н.А. Преображенского Московского технологического университета
(Москва, Россия)

Лощенов В.Б., доктор физико-математических наук, профессор,
заведующий лабораторией лазерной биоспектроскопии в Центре
естественно-научных исследований Института общей физики
им. А.М. Прохорова РАН (Москва, Россия)

РЕДАКЦИОННАЯ КОЛЛЕГИЯ:

Каприн А.Д., академик РАН, доктор медицинских наук, профессор,
генеральный директор Национального медицинского исследовательского
центра радиологии Минздрава России (Москва, Россия)

Романко Ю.С., доктор медицинских наук, профессор кафедры онкологии,
радиотерапии и пластической хирургии им. Л.Л. Лёвшина Первого Москов-
ского государственного медицинского университета им. И.М. Сеченова
(Москва, Россия)

Странадко Е.Ф., доктор медицинских наук, профессор, руководитель Отде-
ления лазерной онкологии и фотодинамической терапии ФГБУ «Государствен-
ный научный центр лазерной медицины им. О.К.Скобелкина ФМБА России»

Blondel V., профессор Университета Лотарингии, руководитель отделения
Здравоохранение-Биология-Сигналы (SBS) (Нанси, Франция)

Bolotine L., профессор научно-исследовательского центра автоматизации
и управления Нанси (Нанси, Франция)

Douplik A., профессор Университета Райерсона (Торонто, Канада)

Steiner R., профессор, почетный директор Института лазерных технологий
в медицине и измерительной технике Университета Ульма (Ульм, Германия)

ORIGINAL ARTICLES

Expression profiles of heme synthesis metabolite transporters as potential molecular determinants of sensitivity to 5-ALA-induced PDT

Ivanova-Radkevich V.I., Filonenko E.V.

4

Comparative evaluation of the efficiency of various modes of photodynamic and radiation therapy exposed to cells of transplanted Ehrlich's carcinoma in experiment

Rostovtsev N.M., Polyakov V.G., Kurenkov E.L., Kuzmina N.E., Neizvestnykh E.A., Kuzmina A.V., Minko M.A.

11

Analysis of collagen presence in brain tumors using spontaneous Raman scattering spectroscopy

Antipova E.D., Romanishkin I.D., Savelieva T.A., Ryabova A.V., Shugay S.V., Kosyrkova A.V., Pavlova G.V., Pronin I.N., Loschenov V.B.

21

REVIEWS OF LITERATURE

Combination of ferroptosis and photodynamic therapy is an effective strategy for cancer treatment

Sanarova E.V., Lantsova A.V., Nikolaeva L.L., Kiseleva M.P., Borisova L.M.

30

Potential of endogenous photosensitizers in photodynamic therapy of mycobacterial infections

Shleeva M.O., Bagaeva D.I., Kasatkina S.I., Kaprelyants A.S., Savitsky A.P.

39

ОРИГИНАЛЬНЫЕ СТАТЬИ

Профили экспрессии транспортеров метаболитов синтеза гема, как потенциальные молекулярные детерминанты чувствительности к ФДТ с использованием 5-АЛК

В.И. Иванова-Радкевич, Е.В. Филоненко

4

Зависимость изменений сосудистого русла опухоли от различной дозы фотодинамического и лучевого воздействия на клетки карциномы Эрлиха в эксперименте

Н.М. Ростовцев, В.Г. Поляков, Е.Л. Куренков, Н.Е. Кузьмина, Е.А. Неизвестных, А.В. Кузьмина, М.А. Минко

11

Анализ присутствия коллагена в опухолях головного мозга методом спектроскопии спонтанного комбинационного рассеяния света

Е.Д. Антипова, И.Д. Романишкин, Т.А. Савельева, А.В. Рябова, С.В. Шугай, А.В. Косырькова, Г.В. Павлова, И.Н. Пронин, В.Б. Лощенов

21

ОБЗОРЫ ЛИТЕРАТУРЫ

Комбинация ферроптоза и фотодинамической терапии – эффективная стратегия для лечения рака

Е.В. Санарова, А.В. Ланцова, Л.Л. Николаева, М.П. Киселева, Л.М. Борисова

30

Потенциал эндогенных фотосенсибилизаторов в фотодинамической терапии микобактериальных инфекций

М.О. Шлеева, Д.И. Багаева, С.И. Касаткина, А.С. Капрельянц, А.П. Савицкий

39

EXPRESSION PROFILES OF HEME SYNTHESIS METABOLITE TRANSPORTERS AS POTENTIAL MOLECULAR DETERMINANTS OF SENSITIVITY TO 5-ALA-INDUCED PDT

Ivanova-Radkevich V.I.¹, Filonenko E.V.^{2,3}

¹Peoples' Friendship University of Russia (RUDN University), Moscow, Russia

²P.A. Herzen Moscow Oncology Research Center – branch of FSBI NMRC of the Ministry of Health of the Russian Federation, Moscow, Russia

³RTU MIREA, Moscow, Russia

Abstract

Expression profiles of transporters of heme synthesis metabolites can be used as potential molecular determinants defining the variable sensitivity of tumor cells to photodynamic therapy (PDT) using 5-aminolevulinic acid (5-ALA). The study included the JFCR39 panel of human cell lines. Transcriptional activity profiles of genes encoding nine key transporter proteins (*SLC15A1*, *SLC36A1*, *SLC6A6*, *SLC6A13*, *TSP01/2*, *ABCG2*, *ABCB6*, *FLVCR1*, *SLC25A37*) were obtained from The Human Protein Atlas (HPA) database. For quantitative assessment, the nTPM (normalized transcripts per million) metric was used. Data on protoporphyrin IX (PPIX) accumulation levels after incubation of cells with 1 mM 5-ALA were taken from the published screening by Kitajima Y. et al. (2019). Statistical significance of associations was assessed using Pearson correlation analysis. A pronounced tissue specificity of correlations between transporter expression and intracellular PPIX accumulation was established. In the brain tumor panel, a very strong negative association of PPIX levels with the expression of the iron transporter MFRN (*SLC25A37*; $R = -0.91$ to -0.96) and the ATP-binding cassette transporter ABCB6 (-0.94) was revealed, which may indicate a critical role of mitochondrial iron uptake and tetrapyrrole efflux in glioma resistance to PDT. At the same time, for the classic 5-ALA transporters themselves (*PEPT1*, *PAT1*, *TauT*, *GAT2*), no statistically significant positive association with the photosensitizer level was found either across the entire panel or in most nosologies. An exception was ovarian cancer lines, where PAT1 expression demonstrated a moderate positive correlation with PPIX accumulation ($R = 0.62$ – 0.75). Comparison of efflux transporters showed that the contribution of ABCB6 to the reduction of PPIX concentration is comparable to or exceeds that of the recognized marker ABCG2, especially in lines of neuroectodermal origin.

Key words: photodynamic therapy, 5-aminolevulinic acid, protoporphyrin IX, transmembrane transporters.

Contacts: Ivanova-Radkevich V.I., e-mail: ivanova-radkevich-vi@rudn.ru

For citations: Ivanova-Radkevich V.I., Filonenko E.V. Expression profiles of heme synthesis metabolite transporters as potential molecular determinants of sensitivity to 5-ALA-induced PDT, *Biomedical Photonics*, 2026, vol. 15, no. 3, pp. 4–10. doi: 10.24931/2413-9432-2026-15-3-4-10

ПРОФИЛИ ЭКСПРЕССИИ ТРАНСПОРТЕРОВ МЕТАБОЛИТОВ СИНТЕЗА ГЕМА, КАК ПОТЕНЦИАЛЬНЫЕ МОЛЕКУЛЯРНЫЕ ДЕТЕРМИНАНТЫ ЧУВСТВИТЕЛЬНОСТИ К ФДТ С ИСПОЛЬЗОВАНИЕМ 5-АЛК

В.И. Иванова-Радкевич¹, Е.В. Филоненко^{2,3}

¹Российский Университет дружбы народов, Москва, Россия

²«Московский научно-исследовательский онкологический институт им. П.А. Герцена – филиал ФГБУ «Национальный медицинский исследовательский центр радиологии» Министерства здравоохранения Российской Федерации, Москва, Россия

³РТУ МИРЭА, Москва, Россия

Резюме

Профили экспрессии транспортеров метаболитов синтеза гема могут быть использованы, как потенциальные молекулярные детерминанты, определяющие вариабельную чувствительность опухолевых клеток к фотодинамической терапии (ФДТ) с использова-

нием 5-аминолевулиновой кислоты (5-АЛК). В исследование была включена панель клеточных линий человека JFCR39. Профили транскрипционной активности генов девяти ключевых белков-транспортёров (*SLC15A1*, *SLC36A1*, *SLC6A6*, *SLC6A13*, *TSP01/2*, *ABCG2*, *ABCB6*, *FLVCR1*, *SLC25A37*) были получены из базы данных The Human Protein Atlas (HPA). Для количественной оценки был использован показатель nTPM (нормализованное количество транскриптов на миллион). Данные об уровне накопления протопорфирина IX (ППИХ) после инкубации клеток с 1 мМ 5-АЛК заимствованы из опубликованного скрининга Kitajima Y. et al. (2019). Статистическая значимость взаимосвязей оценивалась методом корреляционного анализа Пирсона. Установлена выраженная тканеспецифичность корреляций между экспрессией транспортёров и внутриклеточным накоплением ППИХ. На панели опухолей головного мозга выявлена очень сильная отрицательная связь уровня ППИХ с экспрессией транспортёра железа MFRN (*SLC25A37*; $R = -0,91$ до $-0,96$) и АТФ-связывающего кассетного транспортёра ABCB6 ($-0,94$), что может указывать на критическую роль митохондриального захвата железа и эффлюкса тетрапирролов в резистентности глиом к ФДТ. В то же время для классических транспортёров самой 5-АЛК (*PEPT1*, *PAT1*, *TauT*, *GAT2*) статистически значимой положительной связи с уровнем фотосенсибилизатора не обнаружено ни по всей панели, ни в большинстве нозологий. Исключением стали линии рака яичников, где экспрессия PAT1 продемонстрировала умеренную положительную корреляцию с накоплением ППИХ ($R = 0,62-0,75$). Сравнение транспортёров выведения показало, что вклад ABCB6 в снижение концентрации ППИХ сопоставим или превышает таковой у общепризнанного маркера ABCG2, особенно в линиях нейроэктодермального происхождения.

Ключевые слова: фотодинамическая терапия, 5-аминолевулиновая кислота, протопорфирин IX, трансмембранные переносчики, корреляция.

Контакты: Иванова-Радкевич В.И., e-mail: ivanova-radkevich-vi@rudn.ru

Для цитирования: Иванова-Радкевич В.И., Филоненко Е.В. Профили экспрессии транспортёров метаболитов синтеза гема, как потенциальные молекулярные детерминанты чувствительности к ФДТ с использованием 5-АЛК // Biomedical Photonics. – 2026. – Т. 15, № 3. – С. 4–10. doi: 10.24931/2413-9432-2026-15-3-4-10

Introduction

Photodynamic therapy (PDT) has established itself as an effective method for treating malignant neoplasms, widely applied in global clinical practice. The basis of antitumor PDT lies in the selective accumulation of a photosensitizer in pathological tissue, followed by its irradiation with light of a specific wavelength. This process initiates the generation of singlet oxygen and other cytotoxic reactive oxygen species, inducing structural damage to tumor cells and their death [1].

One possible approach involves the use of exogenous precursors, pro-photosensitizers, which include 5-aminolevulinic acid (5-ALA). 5-ALA is synthesized in virtually all cells from glycine and succinyl-CoA by the enzyme 5-aminolevulinate synthase. As an endogenous, non-photoactive intermediate metabolite of heme biosynthesis, 5-ALA, after administration into the body, is efficiently utilized in normal cells to the final product – non-photoactive heme, which is integrated into the structure of hemoproteins. In tumor cells, the biochemical cascade is blocked at the final stage due to a deficiency of the enzyme ferrochelatase. This leads to the abnormal accumulation of intracellular protoporphyrin IX (PPIX) – a highly active photosensitizer, whose fluorescent properties are also used for intraoperative navigation [1,2,3].

The efficiency of PPIX induction by exogenous 5-ALA demonstrates pronounced tissue specificity, depending on the degree of vascularization, microenvironment, and cell phenotype. One of the key factors determining the variability of PPIX accumulation

is considered to be the asymmetry of transmembrane transport: differences in the rate of 5-ALA uptake and the elimination of intermediate products of heme synthesis (uroporphyrinogens, coproporphyrinogens, protoporphyrinogens) [2,4].

Transport of exogenous 5-ALA across the plasma membrane is mediated by several carrier systems, including the peptide transporters PEPT1 and PEPT2, the proline transporter PAT1, as well as the taurine transporter TauT and the GABA transporter GAT2 [4,5]. It is logical to assume that overexpression of these proteins may contribute to enhanced uptake of the pro-sensitizer 5-ALA by malignant cells compared to healthy cells, thereby modulating PPIX concentration. The mechanisms of efflux of intermediate metabolites of heme synthesis play an equally significant role [4,5]. Furthermore, the intensity of PPIX conversion to heme is limited by the availability of iron ions within the cell. The balance between the rate of 5-ALA absorption and the kinetics of PPIX elimination may determine the final intracellular concentration of the photosensitizer [6].

The aim of this study was to evaluate differences in the expression of genes encoding major transporters of 5-ALA and intermediates of heme synthesis in a panel of JFCR39 cell lines characterized by varying abilities to accumulate PPIX upon exposure to exogenous 5-ALA and heterogeneous sensitivity to subsequent PDT. For systematic analysis of transcription profiles, The Human Protein Atlas (HPA, <https://www.proteinatlas.org> [7]) was used. To identify statistically significant correlations between the expression level of each

investigated transporter and the efficiency of PPIX accumulation, data from a large-scale screening by Kitajima Y. et al. (2019), conducted on the same cell line panel [8] (Fig. 1), were integrated. Identification of molecular determinants associated with selective PPIX accumulation may help clarify the mechanisms of differential tumor sensitivity to 5-ALA-induced PDT and potentially optimize personalized treatment protocols.

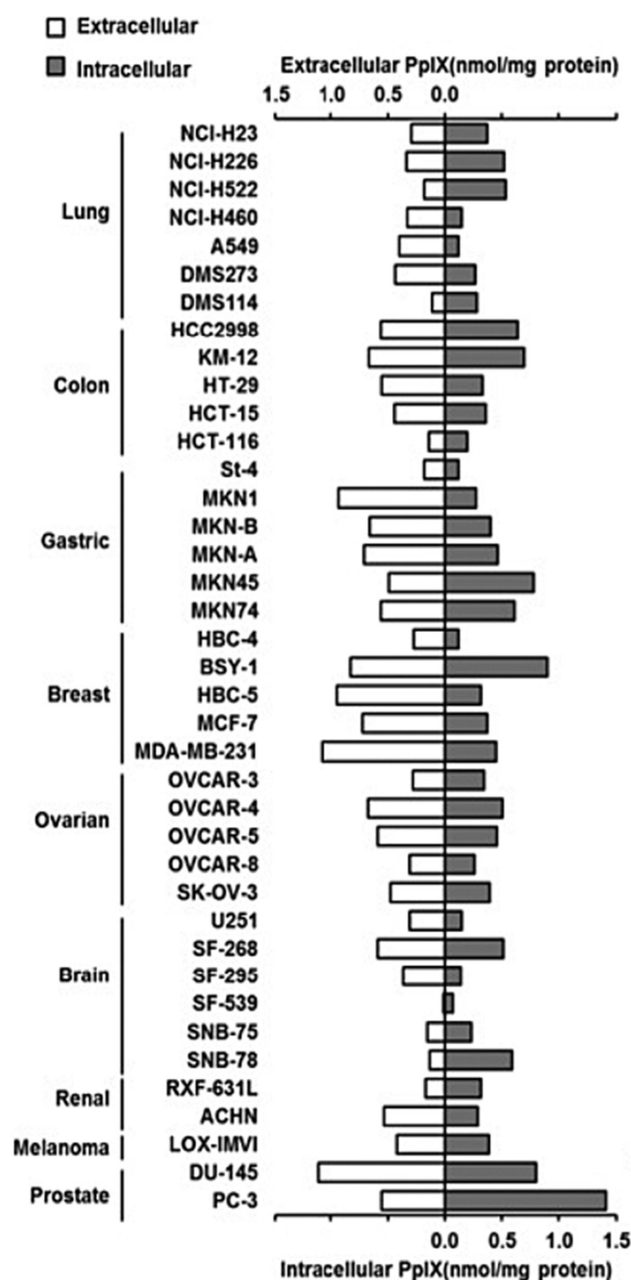


Рис. 1. Внутриклеточное и внеклеточное накопление ППІХ после инкубации клеток с 1 мМ 5-АЛК (клеточная панель JFCR39) [8].

Fig. 1. Intracellular and extracellular PpIX accumulation after incubation with 1 mM 5-ALA (JFCR39 cell panel) [8].

Materials and Methods

Expression Profiles of Transporter Proteins

The following transporter proteins were included in the analysis [4,5]:

- PEPT1 (*SLC15A1*) – di- and tripeptide transporter; proven involvement in the transport of exogenous 5-ALA into cells.
- PAT1 (*SLC36A1*) – amino acid and GABA transporter; presumed involvement in the transport of 5-ALA into cells.
- TauT (*SLC6A6*) – presumed involvement in the transport of 5-ALA into cells.
- GAT2 (*SLC6A13*) – presumed involvement in the transport of 5-ALA into cells.
- TSPO1/2 (*Tspo1*) – presumed involvement in the transport of 5-ALA into cells.
- ABCG2 (*ABCG2*) – one of the main transporters of PPIX and pheophorbide A.
- ABCB6 (*ABCB6*) – presumed involvement in the transport of various tetrapyrroles, such as heme, coproporphyrin III, PPIX, and pheophorbide A.
- FLVCR1 (*FLVCR1*) – presumed involvement in the transport of heme, PPIX, and coproporphyrin.
- MFRN (*SLC25A37*) – transports iron ions into mitochondria, where they are used for heme synthesis from PPIX, thereby reducing PPIX concentration.

To determine the expression profiles of these transporter proteins in various cell lines, the HPA database (The Human Protein Atlas, <https://www.proteinatlas.org>) was used.

Expression levels were assessed using the nTPM (normalized transcripts per million) metric. nTPM is a normalized value of the number of transcripts (RNA copies) for a specific gene, expressed as the number of molecules per million molecules in the sample. This metric is used for quantitative RNA expression analysis.

Analyzed Cell Lines

Assessment of the expression levels of the specified transporter proteins was performed in cell lines from the JFCR39 panel [8]; however, cell lines for which data were missing in the HPA database were excluded from the analysis: HCC2998, St-4, MKN-A, MKN-B, HBC-4, HBC-5, BSY-1, RXF-631L, SNB-75.

Thus, the analysis was performed on the following cell lines:

- Lung cancer: NCI-H23, NCI-H226, NCI-H522, NCI-H460, A549, DMS273, DMS114.
- Colon cancer: KM-12, HT-29, HCT-15, HCT-116.
- Gastric cancer: MKN1, MKN45, MKN74.
- Breast cancer: MCF-7, MDA-MB-231.
- Ovarian cancer: OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, SK-OV-3.
- Brain cancer: U251, SF-268, SF-295, SF-539, SNB-75.

- Renal cancer: ACHN.
- Melanoma: LOX-IMVI.
- Prostate cancer: DU-145, PC-3.

PPIX Accumulation in JFCR39 Panel Cell Lines After 5-ALA Treatment

We used data published by Kitajima Y. et al. [8] on PPIX accumulation in JFCR39 panel cell lines after 5-ALA treatment. The authors incubated cultured cells with a 10-1000 μM solution of 5-ALA for 4 hours. PPIX accumulation was determined based on fluorescence intensity using an EnVision 2103 microplate reader (PerkinElmer, Waltham, USA) (excitation wavelength: 405 nm, emission wavelength: 635 nm) [8].

Statistical Analysis

We determined the strength of the correlation (correlation coefficient R) between the expression level of transporter proteins for heme synthesis metabolites and the level of PPIX accumulation after 5-ALA treatment in various cell lines. Statistika and Excel software were used for data analysis, graph plotting, and correlation analysis.

Correlation coefficients between the expression of transporter proteins and the accumulation of intracellular and total (intra- and extracellular PPIX) after incubation with 5-ALA were determined for all cell lines included in the analysis.

A subgroup correlation analysis was also performed for each nosology comprising at least 5 cell lines (lung cancer, ovarian cancer, brain cancer).

Correlation coefficients were determined relative to the magnitude of intracellular and total (intracellular and extracellular) PPIX accumulation.

The interpretation of the obtained data regarding correlation strength is presented in Table 1.

Results and Discussion

Table 2 presents the expression profiles of transporter proteins for heme synthesis metabolites in the JFCR39 panel cell lines (HPA database). We evaluated the correlation of transporter protein expression levels with both intracellular PPIX levels and its total (intra- and extracellular) levels.

Table 3 presents the results of the correlation analysis of the strength of association between the expression levels of various transporter proteins and the levels of PPIX accumulation after 5-ALA treatment of cells.

Correlation analysis revealed a very strong negative association between the expression of the transporters ABCB6 (PPIX transport) and MFRN (iron ion transport) and intracellular PPIX accumulation in brain tumor cell lines (U251, SF-268, SF-295, SF-539, and SNB-75). The correlation coefficient R exceeded 0.9 in absolute value in both cases. Interestingly, the contribution of the MFRN transporter

Таблица 1
Интерпретация силы связи между переменными по коэффициенту корреляции

Table 1
Interpretation of the strength of the relationship between variables based on the correlation coefficient

R		Сила связи The strength of the connection
от +0,9 до +1,0	от -1,0 до -0,9	очень сильная very strong
от +0,7 до +0,9	от -0,9 до -0,7	сильная strong
от +0,5 до +0,7	от -0,7 до -0,5	заметная noticeable
от +0,3 до +0,5	от -0,5 до -0,3	умеренная moderate
от +0,1 до +0,3	от -0,3 до -0,1	слабая weak
от +0,0 до 0,1	от -0,1 до 0,0	очень слабая или отсутствие very weak or non-existent

to the overall PPIX accumulation metrics was the most significant among all transporters of heme synthesis metabolites. With high expression of this protein, iron ions actively enter the cell mitochondria, participating in the synthesis of non-photoactive heme from photosensitizing PPIX, thereby reducing the level of the latter and the potential efficacy of 5-ALA-induced PDT. However, it should be noted that in other cell cultures (lung, ovarian tumors) and across the entire cell panel, the negative association was significantly weaker (Table 2, Fig. 1).

It is also interesting to compare the contribution of ABCG2 and ABCB6 transporter activity to PPIX accumulation. As a rule, most studies attribute the primary role in PPIX efflux from tumor cells to the ABCG2 transporter [9,10,11]. For example, Cramer S. et al. [11] note that ABCG2 transporter expression is increased in glioblastoma cells. The authors conclude that this may be one of the reasons for the relatively low efficacy of 5-ALA-induced PDT specifically in patients with glioblastomas and the need for somewhat higher doses of 5-ALA (20-30 mg/kg) compared to other nosologies. Our analysis on a broad cell panel showed that, overall, the contribution of ABCG2 and ABCB6 to PPIX accumulation across the panel is similar, and for several nosologies (e.g., brain tumor cell lines), the negative correlation between ABCB6 expression (-0.94) and PPIX accumulation is even stronger than for ABCG2 (-0.28).

Another important observation relates to the role of proteins that, according to literature data [5,6,9,12,13], are involved in the transport of 5-ALA: PEPT1, PAT1, TauT, GAT2, TSPO1/2. As several authors show, increased expression of 5-ALA transporters should presumably lead to increased uptake of 5-ALA by cells and, consequently,

Таблица 2

Уровни экспрессии РНК белков транспортеров метаболитов синтеза гема (база данных HPA [7])

Table 2

RNA expression levels of heme synthesis metabolite transporter proteins (HRA database [7])

Нозология/ Nosology	Клеточная линия / Cell Line	Концентрация ППХ (нмоль/ мг белка) [5]/ PPH concen- tration (nmol/ mg protein) [5]		Уровень экс- прессии РНК белков-тран- спортеров (nTPM) [1] The level of RNA expres- sion of trans- porter proteins (nTPM) [1]								
		внутри клеточ- ная/ intracellular	внутри- и вне- клеточная (intra- and extracellular)	PEPT1 (SLC15A1)	PAT1 (SLC36A1)	TauT (SLC6A6)	GAT2 (SLC6A13)	TSPO1/2 (Tspo1/2)	ABCG2 (ABCG2)	ABCB6 (ABCB6)	FLVCR1 (FLVCR1)	MFRN (SLC25A37)
Легкие Lung	NCI-H23	0,38	0,67	0,2	8,3	4,6	0,3	156,7	22,4	13,5	7,8	11,9
	NCI-H226	0,53	0,85	0,1	6,9	4,9	0	139,4	4,1	21,6	4,7	15
	NCI-H522	0,54	0,7	0,2	10,1	4,8	0	66,1	0	0,6	16	1,8
	NCI-H460	0,15	0,48	0,1	13,3	24,3	0	207,4	32,6	51,4	18,3	22,7
	A549	0,12	0,52	0,1	8,4	41,1	0,2	119,3	56,9	26,8	12,6	10,7
	DMS273	0,27	0,7	0	10,8	6,7	0	135,7	0,8	16,3	12,1	7
	DMS114	0,28	0,38	0,2	13,9	1,9	0,1	87,4	0	19,7	8,8	5,5
Толстая кишка Colon	KM-12	0,68	1,13	0,1	5,6	4,1	0,1	328,2	41,1	5,4	14,1	7,8
	HT-29	0,33	0,88	0,1	4,1	13,2	0	235	25,4	21,3	8,9	8
	HCT-15	0,35	0,8	0,1	11,8	16	0	150	1,4	0,5	15,6	14,6
	HCT-116	0,2	0,32	1,5	10	11,4	0,2	120,3	1,7	10,1	11,9	9,6
Желудок Gastric	MKN1	0,27	1,2	0,3	14,6	12,8	0	134,7	9,2	19,6	4,8	7,5
	MKN45	0,77	1,25	8,3	9,5	18,8	0	128,4	0,4	0,3	22,2	4,4
	MKN74	0,6	1,15	0	10,3	6,6	0,5	46,8	3,7	0,4	7,9	3,5
Молочная железа Breast	MCF-7	0,37	1,08	0,1	7,1	10,7	0	86,8	21,9	19,5	3,9	3,1
	MDA-MB-231	0,45	1,52	0,1	8,6	10,2	0	222	9,9	8,1	4,5	21,8
Яичники Ovarian	OVCAR-3	0,35	0,62	1	7,8	2	0,2	29,1	0,2	17,8	6	4,6
	OVCAR-4	0,5	1,17	0,8	11	3,8	0,5	273,3	0,2	20,1	4,1	4,9
	OVCAR-5	0,45	1,03	28,2	12	64,1	0	168,3	68	10,5	6,3	12,5
	OVCAR-8	0,25	0,57	0,1	8,9	5,1	1,8	191,8	0,7	10,5	2,6	10,2
	SK-OV-3	0,4	0,87	0,7	7,9	0,9	0,2	176,3	1,1	9	4,1	8,3
Мозг Brain	U251	0,13	0,37	0,8	6,8	32,6	0,1	102	0,1	26,4	7,8	11,9
	SF-268	0,52	1,1	0,3	15,2	3,7	0	201,7	0,2	16,8	5,2	4,1
	SF-295	0,13	0,5	0,1	9,6	7,5	0,3	188,2	11,3	25,3	6,2	12,4
	SF-539	0,07	0,1	0,1	12,8	7,2	0	200,5	0,4	30,8	6,6	16,8
	SNB-75	0,23	0,47	0,2	13,3	28,1	0	259,4	0,8	26,9	4,4	14,3
Меланома Melanoma	LOX-IMVI	0,38	0,8	0,1	14,4	21,7	0,1	220	0	13,8	4,8	32,8
Предста- тельная железа Prostate	DU-145	0,8	1,9	0,3	8,3	26,4	0	178	2,9	17,7	9	35,7
	PC-3	1,4	1,87	0,7	7	7,9	0	232,2	0	7,5	7,5	5,9

Таблица 3
Корреляционный анализ силы связи между величиной экспрессии различных белков-транспортеров (база данных HPA [7]) и величинами накопления ППІХ после обработки клеток 5-АЛК [5]

Table 3
Correlation analysis of the strength of the relationship between the expression levels of various transporter proteins (NRA database [7]) and the accumulation levels of PPIX after treatment of cells with 5-ALA [5]

Нозология Nosology	Коэффициент корреляции уровня экспрессии РНК белков-транспортеров (R) относительно: – внутриклеточного накопления ППІХ – суммарно внутри- и внеклеточного накопления ППІХ The correlation coefficient of the level of RNA expression of transporter proteins (R) relative to: – intracellular accumulation of PPIX – total intracellular and extracellular accumulation of PPIX								
	PEPT1 (SLC15A1)	PAT1 (SLC36A1)	TauT (SLC6A6)	GAT2 (SLC6A13)	TSP01/2 (Tspo1/2)	ABCG2 (ABCG2)	ABCB6 (ABCB6)	FLVCR1 (FLVCR1)	MFRN (SLC25A37)
Все нозологии All nosologies	0,11 0,12	-0,26 -0,22	-0,15 -0,01	-0,17 -0,13	0,17 0,23	-0,12 0,00	-0,51 -0,42	0,10 -0,06	-0,10 0,10
Легкие Lung	0,37 -0,24	-0,44 -0,77	-0,73 -0,37	-0,23 -0,22	-0,43 -0,02	-0,69 -0,35	-0,69 -0,45	-0,41 -0,38	-0,38 -0,06
Яичники Ovarian	0,37 0,40	0,62 0,75	0,33 0,38	-0,73 -0,52	0,41 0,65	0,35 0,38	0,39 0,26	0,45 0,24	-0,18 0,00
Мозг Brain	0,01 0,04	0,64 0,46	-0,31 -0,32	-0,37 -0,08	0,26 0,15	-0,28 -0,03	-0,94 -0,99	-0,56 -0,47	-0,91 -0,96

its incorporation into the synthesis of photoactive PPIX. However, in our analysis, none of the listed transporters demonstrated a strong association between expression levels and PPIX accumulation (Table 2), either across the entire JFCR39 panel or for individual nosologies.

The maximum positive correlation values were obtained for the PAT1 transporter in ovarian cancer cell lines (OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, SK-OV-3) –

the correlation coefficient was 0.62 for intracellular PPIX accumulation and 0.75 for total PPIX accumulation. In brain cancer cell lines (U251, SF-268, SF-295, SF-539, SNB-75), the association was also positive, with the correlation coefficient reaching 0.64 for intracellular PPIX accumulation. For the same transporter protein in lung cancer cell lines, a strong negative correlation was shown for total PPIX ($R = -0.77$) and a moderate negative correlation for intracellular PPIX

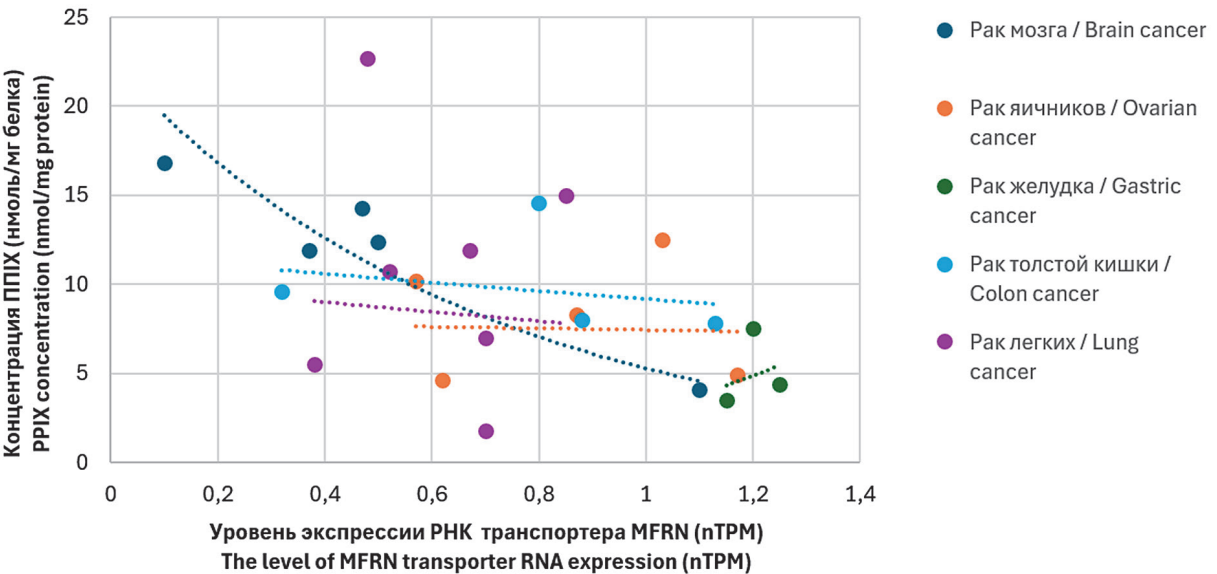


Рис. 1. Корреляция между уровнем экспрессии РНК транспортера MFRN в клеточных линиях панели [7] и накоплением ППІХ после экспозиции с 5-АЛК [8].
Fig. 1. Correlation between the level of MFRN transporter RNA expression in cell lines of panel [7] and the accumulation of PPIX after exposure to 5-ALA [8].

($R = -0.44$). For the other 5-ALA transporters, no significant correlation was demonstrated in the majority of cell lines of a single nosology.

Conclusion

Of all the transporters whose expression we analyzed, only the iron ion transporter MFRN showed a high correlation with PPIX accumulation.

Certainly, the obtained results of the correlation analysis require further confirmation on other cell panels. However, it can already be stated that the significant contribution of 5-ALA transporters to the ability of cells to accumulate photoactive PPIX after exogenous administration of 5-ALA, as indicated by many authors [12,14,15], is likely not valid for all cell lines and nosologies.

REFERENCES

1. Austin E. et al. Photodynamic therapy: overview and mechanism of action. *Journal of the American Academy of Dermatology*, 2025, Vol. 94(6), pp. 1619-1632.
2. Chandratte S., Olsen J., Howley R., Chen B. Targeting ABCG2 transporter to enhance 5-aminolevulinic acid for tumor visualization and photodynamic therapy. *Biochemical pharmacology*, 2023, Vol. 217, pp. 115851.
3. Ivanova-Radkevich V.I. Biochemical basis of selective accumulation and targeted delivery of photosensitizers to tumor tissues. *Biochemistry (Moscow)*, 2022, Vol. 87(11), pp. 1226-1242. <https://doi.org/10.1134/S0006297922110025>.
4. Lai H.W., Nakayama T., Ogura S. Key transporters leading to specific protoporphyrin IX accumulation in cancer cell following administration of aminolevulinic acid in photodynamic therapy/diagnosis. *International Journal of Clinical Oncology*, 2021, Vol. 26, pp. 26-33.
5. Ivanova-Radkevich V.I., Kuznetsova O.M., Filonenko E.V. The role of transmembrane transporters in the accumulation of 5-ALA-induced protoporphyrin IX in tumor cells. *Biomedical Photonics*, 2024, Vol. 13(2), pp. 43-48. doi.org/10.24931/2413-9432-2024-13-2-43-48
6. Hagiya Y., et al. Pivotal roles of peptide transporter PEPT1 and ATP-binding cassette (ABC) transporter ABCG2 in 5-aminolevulinic acid (ALA)-based photocytotoxicity of gastric cancer cells in vitro. *Photodiagnosis Photodyn Ther*, 2012, Vol. 9, pp. 204-214. [doi: 10.1016/j.pdpdt.2011.12.004](https://doi.org/10.1016/j.pdpdt.2011.12.004), <https://www.proteinatlas.org>
7. Kitajima Y., Ishii T., Kohda T., Ishizuka M., Yamazaki K., Nishimura Y., Tanaka T., Dan S., Nakajima M. Mechanistic study of PpIX accumulation using the JFCR39 cell panel revealed a role for dynamin 2-mediated exocytosis. *Sci Rep*, 2019, Vol. 179(1), pp. 8666. [doi: 10.1038/s41598-019-44981-y](https://doi.org/10.1038/s41598-019-44981-y). PMID: 31209282; PMCID: PMC6572817.
8. Hagiya Y. et al. Expression levels of PEPT1 and ABCG2 play key roles in 5-aminolevulinic acid (ALA)-induced tumor-specific protoporphyrin IX (PpIX) accumulation in bladder cancer. *Photodiagnosis and photodynamic therapy*, 2013, Vol. 10(3), pp. 288-295.
9. Morita M. et al. Fluorescence-based discrimination of breast cancer cells by direct exposure to 5-aminolevulinic acid. *Cancer medicine*, 2019, Vol. 8(12), pp. 5524-5533.
10. Cramer S.W., Chen C.C. Photodynamic therapy for the treatment of glioblastoma. *Frontiers in surgery*, 2020, Vol. 6, pp. 81.
11. Tran T.T. et al. Neurotransmitter Transporter Family Including SLC 6 A 6 and SLC 6 A 13 Contributes to the 5-Aminolevulinic Acid (ALA)-Induced Accumulation of Protoporphyrin IX and Photodamage, through Uptake of ALA by Cancerous Cells. *Photochemistry and photobiology*, 2014, Vol. 90(5), pp. 1136-1143.
12. Manceau H. et al. TSP02 translocates 5-aminolevulinic acid into human erythroleukemia cells. *Biology of the Cell*, 2020, Vol. 112(4), pp. 113-126.
13. Ebrahimi S. et al. Enhancing 5-ALA-PDT efficacy against resistant tumor cells: Strategies and advances. *Life Sciences*, 2024, Vol. 351, pp. 122808.
14. Bermudez Moretti M. et al. δ -aminolevulinic acid transport in murine mammary adenocarcinoma cells is mediated by BETA transporters. *British journal of cancer*, 2002, Vol. 87(4), pp. 471-474.

ЛИТЕРАТУРА

1. Austin E. et al. Photodynamic therapy: overview and mechanism of action // *Journal of the American Academy of Dermatology*. – 2025. – Vol. 94(6). – P. 1619-1632.
2. Chandratte S., Olsen J., Howley R., Chen B. Targeting ABCG2 transporter to enhance 5-aminolevulinic acid for tumor visualization and photodynamic therapy // *Biochemical pharmacology*. – 2023. – Vol. 217. – P. 115851.
3. Ivanova-Radkevich V.I. Biochemical basis of selective accumulation and targeted delivery of photosensitizers to tumor tissues // *Biochemistry (Moscow)*. – 2022. – Vol. 87(11). – P. 1226-1242. <https://doi.org/10.1134/S0006297922110025>.
4. Lai H.W., Nakayama T., Ogura S. Key transporters leading to specific protoporphyrin IX accumulation in cancer cell following administration of aminolevulinic acid in photodynamic therapy/diagnosis // *International Journal of Clinical Oncology*. – 2021. – Vol. 26. – P. 26-33.
5. Иванова-Радкевич В.И., Кузнецова О.М., Филоненко Е.В. Роль трансмембранных переносчиков в накоплении 5-АЛК-индуцированного протопорфирина IX в опухолевых клетках // *Biomedical Photonics*. – 2024. – Т. 13, №. 2. – С. 43-48. doi.org/10.24931/2413-9432-2024-13-2-43-48
6. Hagiya Y., et al. Pivotal roles of peptide transporter PEPT1 and ATP-binding cassette (ABC) transporter ABCG2 in 5-aminolevulinic acid (ALA)-based photocytotoxicity of gastric cancer cells in vitro // *Photodiagnosis Photodyn Ther*. – 2012. – Vol. 9. – P. 204-214. [doi: 10.1016/j.pdpdt.2011.12.004](https://doi.org/10.1016/j.pdpdt.2011.12.004), <https://www.proteinatlas.org>
7. Kitajima Y., Ishii T., Kohda T., Ishizuka M., Yamazaki K., Nishimura Y., Tanaka T., Dan S., Nakajima M. Mechanistic study of PpIX accumulation using the JFCR39 cell panel revealed a role for dynamin 2-mediated exocytosis // *Sci Rep*. – 2019. – Vol. 179(1). – P. 8666. [doi: 10.1038/s41598-019-44981-y](https://doi.org/10.1038/s41598-019-44981-y). PMID: 31209282; PMCID: PMC6572817.
8. Hagiya Y. et al. Expression levels of PEPT1 and ABCG2 play key roles in 5-aminolevulinic acid (ALA)-induced tumor-specific protoporphyrin IX (PpIX) accumulation in bladder cancer // *Photodiagnosis and photodynamic therapy*. – 2013. – Vol. 10(3). – P. 288-295.
9. Morita M. et al. Fluorescence-based discrimination of breast cancer cells by direct exposure to 5-aminolevulinic acid // *Cancer medicine*. – 2019. – Vol. 8(12). – P. 5524-5533.
10. Cramer S.W., Chen C.C. Photodynamic therapy for the treatment of glioblastoma // *Frontiers in surgery*. – 2020. – Vol. 6. – P. 81.
11. Tran T.T. et al. Neurotransmitter Transporter Family Including SLC 6 A 6 and SLC 6 A 13 Contributes to the 5-Aminolevulinic Acid (ALA)-Induced Accumulation of Protoporphyrin IX and Photodamage, through Uptake of ALA by Cancerous Cells // *Photochemistry and photobiology*. – 2014. – Vol. 90(5). – P. 1136-1143.
12. Manceau H. et al. TSP02 translocates 5-aminolevulinic acid into human erythroleukemia cells // *Biology of the Cell*. – 2020. – Vol. 112(4). – P. 113-126.
13. Ebrahimi S. et al. Enhancing 5-ALA-PDT efficacy against resistant tumor cells: Strategies and advances // *Life Sciences*. – 2024. – Vol. 351. – P. 122808.
14. Bermudez Moretti M. et al. δ -aminolevulinic acid transport in murine mammary adenocarcinoma cells is mediated by BETA transporters // *British journal of cancer*. – 2002. – Vol. 87(4). – P. 471-474.

COMPARATIVE EVALUATION OF THE EFFICIENCY OF VARIOUS MODES OF PHOTODYNAMIC AND RADIATION THERAPY EXPOSED TO CELLS OF TRANSPLANTED EHRlich'S CARCINOMA IN EXPERIMENT

Rostovtsev N.M.^{1,2}, Polyakov V.G.³, Kurenkov E.L.², Kuzmina N.E.^{1,2}, Neizvestnykh E.A.^{1,2}, Kuzmina A.V.², Minko M.A.²

¹Chelyabinsk Regional Children's Clinical Hospital, Chelyabinsk, Russia

²South-Urals State Medical University, Chelyabinsk, Russia

³National Medical Research Center of Oncology named after N. N. Blokhin, Moscow, Russia

Abstract

Photodynamic therapy (PDT) is the subject of numerous studies and has already proven its efficiency in the treatment of neoplastic and nonneoplastic diseases. PDT is a procedure involving the administration of a photosensitizing agent followed by activation of the drug with light of a certain wavelength and generation of active oxygen forms. Further toxic effects causing apoptotic, necrotic, or autophagic death of cancer cells result in serious vascular damage to tumor tissue, and activate systemic immune response. From the point of treatment optimization and individual approach, PDT can undoubtedly be considered as a method of personalized medicine. The aim of the study was to investigate vascular changes in tumor tissue depending on various modes of photodynamic and radiation exposure. The study was performed on 223 laboratory mice of strains CBA, C57BL and C3HA, and F1 hybrids (CBA × C57BL) with Ehrlich's adenocarcinoma transplanted into the subcutaneous tissue of the back from tumor-bearing mice. The photosensitizer radachlorine of the second-generation based on chlorin e6 (Russia) was administered intraperitoneally to animals. The degree of photosensitizer accumulation in tissues was determined using the "LESA-01-Biospec" fluorescence spectroscopic system (Russia). The source of laser irradiation was the therapeutic semiconductor laser "Lakhta-Milon" (Russia). It has been established that PDT at high light dose leads to a significant irreversible reduction in the volume and area of the vascular bed, a decrease in tumor vascular perfusion, and maximum suppression of tumor angiogenic activity during combined treatment – a combination of resection and intraoperative PDT at the resected tumor bed. The experimental data demonstrate the more powerful destructive effect of high-dose PDT and the advantages of laser therapy over radiation. The cytoreductive effects of photodamage can be successfully used in modern oncology during surgical treatment as an additional intraoperative treatment at the resected tumor bed, improving treatment outcomes.

Key words: photodynamic therapy, Ehrlich's adenocarcinoma, laser, photosensitizer, vascular epithelial growth factor, tumor vascular bed, angiogenesis.

Contacts: Kuzmina N.E., e-mail: K-natalya@inbox.ru

For citations: Rostovtsev N.M., Polyakov V.G., Kurenkov E.L., Kuzmina N.E., Neizvestnykh E.A., Kuzmina A.V., Minko M.A. Comparative evaluation of the efficiency of various modes of photodynamic and radiation therapy exposed to cells of transplanted Ehrlich's carcinoma in experiment, *Biomedical Photonics*, 2026, vol. 15, no. 3, pp. 11–20. doi: 10.24931/2413-9432-2026-15-3-11-20

ЗАВИСИМОСТЬ ИЗМЕНЕНИЙ СОСУДИСТОГО РУСЛА ОПУХОЛИ ОТ РАЗЛИЧНОЙ ДОЗЫ ФОТОДИНАМИЧЕСКОГО И ЛУЧЕВОГО ВОЗДЕЙСТВИЯ НА КЛЕТКИ КАРЦИНОМЫ ЭРЛИХА В ЭКСПЕРИМЕНТЕ

Н.М. Ростовцев^{1,2}, В.Г. Поляков³, Е.Л. Куренков², Н.Е. Кузьмина^{1,2}, Е.А. Неизвестных^{1,2}, А.В. Кузьмина², М.А. Минко²

¹Челябинская областная детская клиническая больница, Челябинск, Россия

²Южно-Уральский государственный медицинский университет, Челябинск, Россия

³Национальный медицинский исследовательский центр онкологии имени Н. Н. Блохина, Москва, Россия

Резюме

Фотодинамическая терапия (ФДТ) является предметом многочисленных исследований и уже доказала свою эффективность в лечении опухолевых и неопухолевых заболеваний. ФДТ представляет собой процедуру, включающую введение фотосенсибилизирующего агента с последующей активацией препарата светом определенной длины волны и генерацией активных форм кислорода. Дальнейшие токсические эффекты вызывают апоптотическую, некротическую или аутофагическую гибель раковых клеток, приводят к серьезным сосудистым повреждениям опухолевой ткани, активируют системные иммунные реакции. С точки зрения оптимизации лечения и индивидуального подхода ФДТ несомненно может рассматриваться как инструмент персонализированной медицины. Целью исследования стало изучение сосудистых изменений опухолевой ткани в зависимости от различных режимов фотодинамического и лучевого воздействия. Исследование выполнено на 223 лабораторных мышах линий СВА, С57Bl, СЗНА, гибридах F (CBA* C57Bl) с перевитой в подкожную клетчатку спины аденокарциномой Эрлиха от мышей-опухоленосителей. В работе использовался фотосенсибилизатор второго поколения на основе хлорина еб радахлорин (Россия), который вводили животным внутривенно. Степень накопления фотосенсибилизатора в тканях определяли с помощью флуоресцентно-спектроскопической установки «ЛЭСА-01-Биоспек» (Россия). Источником лазерного облучения явился терапевтический полупроводниковый лазер «Лакта-Милон» (Россия). Установлено, что высокие световые дозы при ФДТ ведут к выраженному необратимому уменьшению объема и площади сосудистого русла, снижению сосудистой перфузии опухоли, с максимальным подавлением ангиогенной активности опухоли при комбинированном лечении – сочетании резекции и интраоперационной ФДТ на ложе удаленной опухоли. Полученные в ходе эксперимента данные свидетельствуют о более мощном деструктивном воздействии высокодозной ФДТ, преимуществах лазерного воздействия в сравнении с лучевым. Циторедуктивные эффекты фотоповреждения могут успешно использоваться в современной онкологии на этапе хирургического лечения в качестве дополнительного интраоперационного воздействия на ложе удаленной опухоли, улучшая результаты терапии.

Ключевые слова: фотодинамическая терапия, аденокарцинома Эрлиха, лазер, фотосенсибилизатор, фактор роста эпителия сосудов, сосудистое русло опухоли, ангиогенез.

Контакты: Кузьмина Н.Е., e-mail: K-natalya@inbox.ru

Для цитирования: Ростовцев Н.М., Поляков В.Г., Куренков Е.Л., Кузьмина Н.Е., Неизвестных Е.А., Кузьмина А.В., Минко М.А. Зависимость изменений сосудистого русла опухоли от различной дозы фотодинамического и лучевого воздействия на клетки карциномы Эрлиха в эксперименте // Biomedical Photonics. – 2026. – Т. 15, № 3. – С. 11–20. doi: 10.24931/2413-9432-2026-15-3-11-20

Introduction

According to the International Agency for Research on Cancer, approximately 20 million new cases of this disease are registered worldwide annually. Cancer is the cause of 10 million deaths per year, making it the second leading cause of mortality [1].

The main clinical method of cancer treatment currently is cytoreductive or suppressive therapy based on surgical resection, chemotherapy, radiotherapy, or a combination of these treatment methods. Due to the risk of recurrence, tumor invasiveness, and metastasis caused by the lack of specific action on the tumor, the effectiveness of these treatment methods remains insufficient. Therefore, clinical necessity dictates the introduction of new alternative methods, which include anticancer photodynamic therapy (PDT) [2]. Low invasiveness and toxicity, high selectivity of damage, and the absence of risk of local and systemic complications are advantageous benefits of PDT over traditional methods in oncology. At the same time, the use of combined and complex therapy of cancer diseases is steadily expanding, since the multimodal approach allows overcoming the disadvantages of one method through the advantages of another. The combination of PDT with chemotherapy, surgery, and

radiation therapy leads to an additive effect, expressed in increased treatment effectiveness [3, 4].

Photodynamic therapy is based on the photochemical reaction between a photosensitizer (PS) activated by light and molecular oxygen. Harmless individually, these components in combination lead to the formation of reactive oxygen species capable of directly causing cell damage [4]. Although PDT is the subject of ongoing research, it is generally accepted that the damaging effect is realized through three mechanisms. First, due to the localization and activation of PS in tumor tissue, the generated reactive singlet oxygen can directly destroy target cells by inducing necrosis and apoptosis. Second, PDT can affect the vascular network of the tumor, thereby disrupting the supply of oxygen and essential nutrients. The third mechanism is the activation of the immune system by PDT, causing an inflammatory and immune response against tumor cells [2, 4–7].

The relative contribution of these mechanisms to effective destruction of tumor cells depends on the PS used, the time between PS administration and light exposure, the total light dose and fluence rate, the oxygen concentration in the tumor, and possibly other, still insufficiently studied, variables. Consequently, determining the optimal conditions for the use of

PDT requires coordinated interdisciplinary efforts [8]. Modern PS under the influence of visible radiation with different wavelengths (from blue to green and red) allow a selective approach to the treatment of tumor formations at various depths of occurrence, since by selecting the PS and the corresponding wavelength, it is possible to regulate the penetrating ability of light into biological tissues.

The biological behavior of cancer is characterized by mechanisms that ensure its survival primarily through two most important processes: tumor neoangiogenesis and evasion from immune surveillance [7]. The outcome of photodynamic destruction of the tumor is hemorheological disorders and impaired blood supply to tumor tissue, therefore targeted action on tumor vessels represents a logical therapeutic approach. Excessive vascularization of tumor tissues is one of the features of the tumor that allows it to survive in the body and serves as the main pathway for metastasis. This occurs due to increased secretion by tumor cells of vascular endothelial growth factor (VEGF), which initiates neovascularization in the tissue. Such a vascular network ensures the viability of the tumor by increasing blood perfusion into the growing tissue, supplying cancer cells with sufficient oxygen and nutrients and eliminating metabolic byproducts. Destruction of the already existing vascular network or suppression of new vessel formation is an important factor in the effectiveness of PDT. Damage to endothelial and subendothelial cells triggers a physiological cascade of thrombotic reactions, including platelet aggregation, release of vasoactive molecules, leukocyte adhesion, increased vascular permeability, and their constriction. As a result, vascular obstruction and collapse lead to hypoxia, deprivation of the tumor of nutrients with its subsequent destruction. Vascular damage occurring after completion of PDT treatment also contributes to long-term tumor control [6, 9–13].

Consequently, experimental analysis of the mechanisms of photodamage carries valuable information. In this regard, the aim of our study was to investigate the severity of changes in the tumor vascular network—Ehrlich adenocarcinoma—depending on different regimens of photodynamic exposure and radiation therapy.

Materials and methods

The work was performed in compliance with International Recommendations (Ethical Code) for conducting biomedical research using animals, on laboratory mice of strains CBA, C57BL, C3HA, and F1 hybrids (CBA* C57Bl) weighing 18–25 g with Ehrlich adenocarcinoma transplanted subcutaneously into the back area. Ascitic fluid from donor mice for reproducing the solid tumor was injected subcutaneously into the

back area. On the 7th day after tumor transplantation, when the linear size of tumor nodes reached a maximum value of 0.5–0.6 mm, the animals were taken into the experiment, distributed into 6 groups: experimental and control.

Animals of the experimental groups were intraperitoneally administered the second-generation PS radachlorin (LLC “RADA-PHARMA”, Russia) at an equivalent dose for the animal of 15 mg/kg. The photosensitizer dose was selected according to the generally accepted recalculation from the recommended human dose and the results of our own preliminary experiments. The use of this PS was chosen taking into account previously obtained data on its preferential accumulation in the endothelium of tumor vessels [14]. The degree of photosensitizer accumulation in tissues was determined using the fluorescence-spectroscopic device “LESA-01-Biospec” (Russia).

For PDT, a laser diode module “ML662-SP” (ZAO “Milon Laser”, Russia) with radiation at a wavelength of 662 nm and a power of 3 W was used. Irradiation was started 2–3 h after PS administration during the period of maximum tumor/healthy tissue contrast index. The light guide was positioned at the center of the tumor with capture of the peritumoral zone by 0.5 cm.

For radiation therapy, a modified “Luch-1” unit used for γ -irradiation of small laboratory animals was employed. Animals were exposed at a dose regimen of 5 Gy.

The study included 48 mice of the control group and 175 experimental animals. Animals with transplanted Ehrlich carcinoma not subjected to specific treatment constituted the control group. Their removal from the experiment was carried out at established time points (7th, 14th, 21st, 28th days).

Experimental studies were performed on five groups of animals: group I – PDT at a light dose of 100 J/cm²; group II – PDT at a light dose of 200 J/cm²; group III – radiation therapy at a dose of 5 Gy; group IV – PDT at a light dose of 400 J/cm²; group V – animals underwent removal of half the tumor volume followed by intraoperative PDT at a dose of 400 J/cm².

Animals of the experimental groups were removed from the experiment on the 14th, 21st, 28th days from the moment of transplantation, which corresponded to the 7th, 14th, 21st days after the treatment.

Sampling of material for histological evaluation was performed on the 7th, 14th, 21st days after the treatment (correspondingly on the 14th, 21st, 28th days after tumor transplantation).

Microslides were stained with hematoxylin and eosin, picrofuchsin by Van Gieson, and combined staining was also used: PAS – Alcian blue [15, 16].

The tumor vascular bed was detected using an endothelial marker to CD34 of endothelial cells

(QBEnd-10, 1:50, Dako, Denmark). On a conventional unit area, phases were identified by stereometric methodology, their measurement and calculation were performed; in images of histological preparations, the area of the tumor vascular bed was determined using the licensed image analysis software for microobjects "Videotest-Morphology 5.0" ("Videotest", Russia). On a section with an area of $600043.21 \mu\text{m}^2$, the area of the tumor vascular bed was determined in 3–6 fields of view.

Angiogenic activity of tumor cells was assessed by the level of expression of vascular endothelial growth factor (Vascular Endothelial Growth Factor, VEGF; DAKO, VG, 1:40), a stimulator of angiogenesis. The results of immunohistochemical reactions for VEGF were evaluated using a semiquantitative method. Interpretation of VEGF expression: negative reaction – no staining or <10% of tumor cells stained (0 points); weak reaction – 10–20% of tumor cells stained (1 point); moderate reaction – 20–50% of tumor cells stained (2 points); intense reaction – >50% of tumor cells stained (3 points). The relative volume of the vascular bed in the tumor was determined using ocular grids by the point counting method in 3–10 randomly selected fields of view of each observation [17].

Automated statistical processing of the obtained results was performed using the licensed application software package Statistica 6.0 (StatSoft, Inc., USA) [18]. Parametric and nonparametric research methods were used. Statistical significance of differences of compared characteristics in groups was determined using Student's t-test and Mann-Whitney U-test. Differences were considered statistically significant at $p < 0.05$, which corresponds to 95% probability of error-free prediction.

Results

Morphological characteristics of the vascular bed in the tumor of animals of the control and experimental groups

It is known that the morphofunctional and diagnostic indicator reflecting the proportion of tumor tissue volume occupied by vessels is the relative volume of the vascular bed in the tumor node. This parameter directly characterizes the intensity of tumor angiogenesis necessary to provide the growing neoplasm with oxygen and nutrients. The vascular bed area indicator reflects tumor aggressiveness; high values of vascular bed area in the tumor directly correlate with increased risk of metastasis and lower survival prognosis.

In the control group of animals, in the intact tumor, the vascular bed was represented by a developed network of capillaries and a small number of arterioles and venules. Immunomorphological study showed that the capillary endothelium contacts a large number of tumor epitheliocytes, the endothelial lining is thin, and in certain areas discontinuous (Fig. 1). After 1 week from tumor transplantation, the relative volume

of the vascular bed was $(14.40 \pm 0.76)\%$, reaching a maximum value on the 14th day – $(15.00 \pm 0.91)\%$ ($p > 0.05$). No significant increase in vascular bed volume and vascularization area was observed by the 21st–28th days ($p > 0.05$).

The obtained data on the relative volume and area of the vascular bed in the control and experimental groups are presented in Table 1.

In group I, after photodynamic exposure at a light dose of 100 J/cm^2 , the construction of the tumor microcirculatory bed on the 7th day almost did not differ from the untreated tumor. A narrow-looped network of anastomosing capillaries and a few larger vessels occupied a volume of $(12.20 \pm 0.87)\%$ and an area of $(8674.90 \pm 1439.06) \mu\text{m}^2$ (Table 1). With increasing duration of the experiment, differences in vascularization indicators from the control group were absent.

In group II, after PDT at a light dose of 200 J/cm^2 , the appearance of changes in the vascular network was noted in the form of individual areas of paretic dilated and emptied vessels (Fig. 2). Compared with the control group, the relative volume of vessels was significantly lower – $(10.20 \pm 0.77)\%$ ($p < 0.05$), the vascular bed area was $(8251.00 \pm 1892.75) \mu\text{m}^2$. The values of area and relative volume of vessels after PDT on the 21st day slightly decreased compared to the 7th and 14th days. Lower values of vessel volume than in the control and group I were recorded on the 14th and 21st days ($p < 0.05$). The values of vessel area in groups I–II had no significant differences from the control (Table 1).

In group III of animals, after radiation therapy at a dose of 5 Gy, a more pronounced decrease in tumor vascularization was observed. This was manifested by reduction of the capillary network in it along with dilation

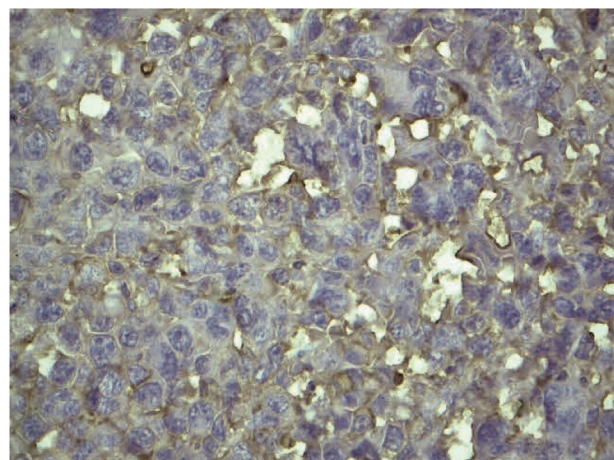


Рис. 1. Развитая капиллярная сеть опухоли. Экспрессия CD34 в эндотелиальных клетках. Иммуногистохимический метод, увеличение $\times 400$.

Fig. 1. Developed capillary network of the tumor. CD34 expression in endothelial cells. Immunohistochemistry, magnification $\times 400$.

Таблица 1
Относительный объем и площадь сосудистого русла в контрольной и опытных группах

Table 1
Relative volume and area of the vascular bed in the control and experimental groups

Группы Groups	Сутки наблюдения Day of observation					
	7-е 7th		14-е 14th		21-е 21st	
	Относительный объем сосуди- стого русла, % Relative volume of the vascular bed, %	Площадь сосу- дистого русла, мкм ² Surface area of the vascular bed, μm ²	Относительный объем сосуди- стого русла, % Relative volume of the vascular bed, %	Площадь сосу- дистого русла, мкм ² Surface area of the vascular bed, μm ²	Относительный объем сосуди- стого русла, % Relative volume of the vascular bed, %	Площадь сосу- дистого русла, мкм ² Surface area of the vascular bed, μm ²
Группа контроля Control group	14,40 ± 0,76	9106,70 ± 1101,17	15,00 ± 0,91	9476,80 ± 1081,34	13,30 ± 0,65	9027,70 ± 1348,43
I	12,20 ± 0,87 °	8674,90 ± 1439,06	13,40 ± 0,84	8951,90 ± 1015,50	12,50 ± 0,77	8793,40 ± 1390,31
II	10,20 ± 0,77 °	8251,00 ± 1892,75	10,30 ± 0,80 ° ¹	8430,80 ± 1269,57	9,30 ± 0,76 ° ¹	7842,00 ± 932,00
III	7,00 ± 0,79 ° ¹²	7361,70 ± 1829,73	7,30 ± 0,64 ° ¹²	7584,70 ± 1937,62	6,50 ± 0,56 ° ¹²	5183,90 ± 2829,59
IV	0,60 ± 0,13 ° ¹²³	456,50 ± 70,54 ° ¹²³	0,70 ± 0,14 ° ¹²³	538,30 ± 67,99 ° ¹²³	0,70 ± 0,13 ° ¹²³	376,20 ± 75,44 ° ¹²³
V	0,30 ± 0,09 ° ¹²³	154,60 ± 116,58 ° ¹²³	–	–	–	–

° – $p < 0,05$ по сравнению с контрольной группой; ¹ – $p < 0,05$ по сравнению с I группой; ² – $p < 0,05$ по сравнению со II группой; ³ – $p < 0,05$ по сравнению с III группой.
° – $p < 0.05$ compared to the control group; ¹ – $p < 0.05$ compared to group I; ² – $p < 0.05$ compared to group II; ³ – $p < 0.05$ compared to group III.

of the lumen of large vessels. The relative volume of the vascular network after X-ray therapy was: on the 7th day – $(7.00 \pm 0.79)\%$, on the 14th day – $(7.30 \pm 0.64)\%$, on the 21st day – $(6.50 \pm 0.56)\%$ ($p < 0.05$ compared with control, groups I and II at analogous time points). The vascular bed area in group III was also smaller than in the control, groups I and II, but no significant differences in indicators were established (Table 1).

A significant decrease in vascularization area and relative volume of vessels was noted with an increase in the PDT dose to 400 J/cm^2 in groups IV–V.

In group IV, with an increase in the light dose to 400 J/cm^2 , on the 7th day the vascular bed was represented by individual vessels filled with thrombotic masses with organization phenomena, surrounded by perivascular infiltrates; reduction of the capillary bed was noted (Fig. 3). The capillary network was practically not detected. At the final time point of the experiment, no proliferation of endothelial cells or formation of new microvessels in the tumor was found. The relative volume of single functioning vessels at all observation time points in group IV remained practically unchanged – $0.60\text{--}0.70\%$. Significant differences in the indicators of vascular bed

volume were established on the 7th, 14th, and 21st days between group IV and groups I, II, III, and control. The

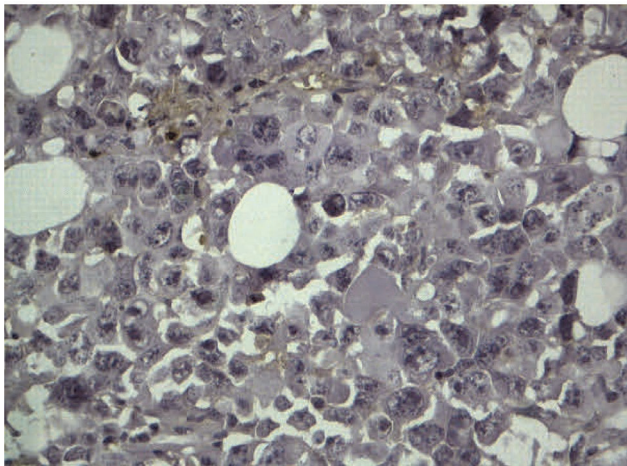


Рис. 2. Спазм и парез на отдельных участках сосуда после ФДТ. Экспрессия CD34 в эндотелии. Иммуногистохимический метод, увеличение $\times 400$.
Fig. 2. Spasm and paresis in individual sections of the vessel after PDT. CD34 expression in the endothelium. Immunohistochemical method, magnification $\times 400$.

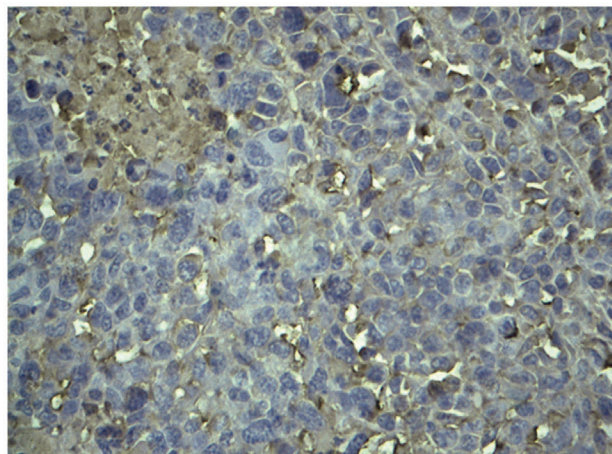


Рис. 3. Редукция капиллярного русла после ФДТ. Экспрессия CD34 в эндотелиальных клетках. Иммуногистохимический метод, увеличение $\times 400$.

Fig. 3. Capillary reduction after PDT. CD34 expression in the endothelium. Immunohistochemical method, magnification $\times 400$.

vascular bed area in group IV was $(456.50 \pm 70.54) \mu\text{m}^2$ on the 7th day, decreasing to $(376.20 \pm 75.44) \mu\text{m}^2$ by the 21st day ($p > 0.05$) (Table 1).

The indicators of relative volume of the vascular bed in group V were lower than in the control and groups I–III ($p < 0.05$). The vascularization area was $(154.60 \pm 116.58) \mu\text{m}^2$ and was minimal compared to all compared groups ($p < 0.05$) (Table 1).

Assessment of expression of angiogenesis factor (VEGF) in the tumor of animals of the control and experimental groups

The degree of expression of VEGF—vascular endothelial growth factor—in tumor cells reflects the activity of new vessel formation by the tumor and is

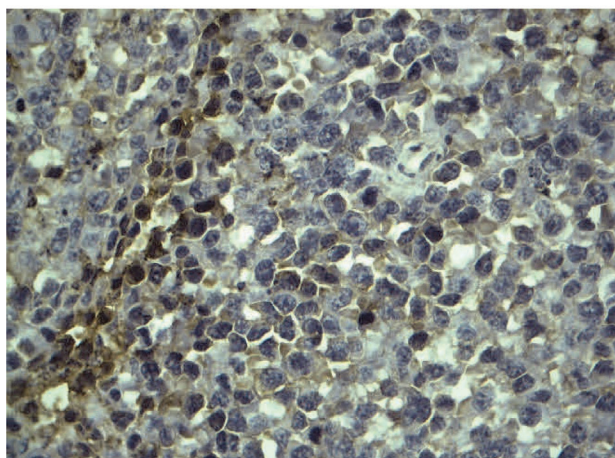


Рис. 4. Неоангиогенез в опухоли. Формирование эндотелиальной выстилки сосуда. Экспрессия CD34 в эндотелии. Иммуногистохимический метод, увеличение $\times 400$.

Fig. 4. Tumor neoangiogenesis. Formation of the endothelial lining of the vessel. CD34 expression in the endothelium. Immunohistochemistry, magnification $\times 400$.

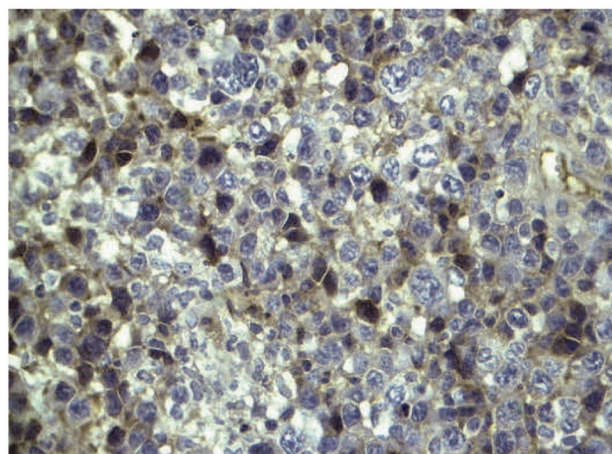


Рис. 5. Выраженная экспрессия VEGF клетками опухоли (3 балла). Иммуногистохимический метод, увеличение $\times 400$.

Fig. 5. Pronounced VEGF expression by tumor cells (3 points). Immunohistochemical method, magnification $\times 400$.

directly related to the aggressiveness and growth rate of the tumor.

In the control group, the maximum severity of expression of the angiogenic growth factor was detected on the 14th day from tumor transplantation (Fig. 4); in this group, pronounced and moderately pronounced expression of the marker dominated. In tumor tissue, expression of endothelial growth factor was more often detected in poorly differentiated cells of loose connective tissue, located in groups or in the form of chains.

In tumor tissue of mice of group I, on the 7th day, an intense immunomorphological reaction with the VEGF marker was noted. Only high and moderately pronounced expression of the angiogenesis factor was detected (Fig. 5, 6). The number of VEGF-positive cells decreased on the 14th and 21st days after PDT (difference from the 7-day time point: $p < 0.05$). There were no statistically significant differences in VEGF expression by tumor cells compared with the control group.

Tumor cells of animals of group II were characterized predominantly by moderate VEGF expression on the 7th day after PDT (Fig. 5). At later time points, weak intensity of the reaction with antibodies to VEGF was significantly more often observed ($p < 0.05$). With an increase in the light dose to 200 J/cm^2 , differences in the intensity of VEGF expression by tumor cells of group II compared with intact and group I were absent. VEGF expression was detected predominantly in endothelial cells and in stromal fibroblasts, very rarely in tumor epithelium.

In group III, after X-ray irradiation, the level of VEGF expression by cells on the 7th day averaged (1.70 ± 0.17) points (more often moderate expression). At the same time, pronounced expression of the angiogenesis

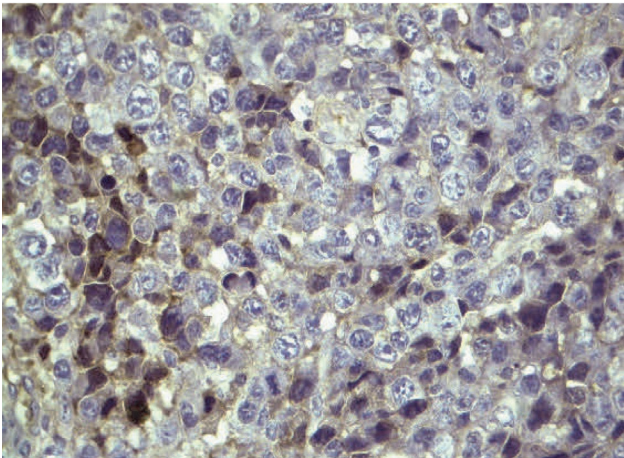


Рис. 6. Умеренная экспрессия VEGF клетками опухоли (2 балла). Иммуногистохимический метод, увеличение $\times 400$.
Fig. 6. Moderate expression of VEGF by tumor cells (2 points). Immunohistochemical method, magnification $\times 400$.

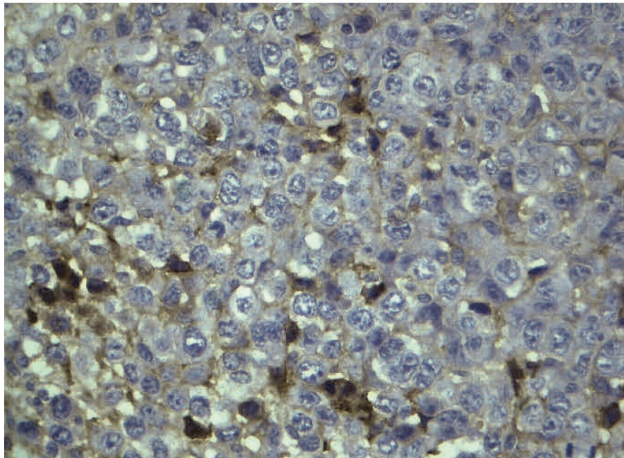


Рис. 7. Слабая экспрессия VEGF клетками опухоли (1 балл). Иммуногистохимический метод, увеличение $\times 400$.
Fig. 7. Weak expression of VEGF by tumor cells (1 point). Immunohistochemical method, magnification $\times 400$.

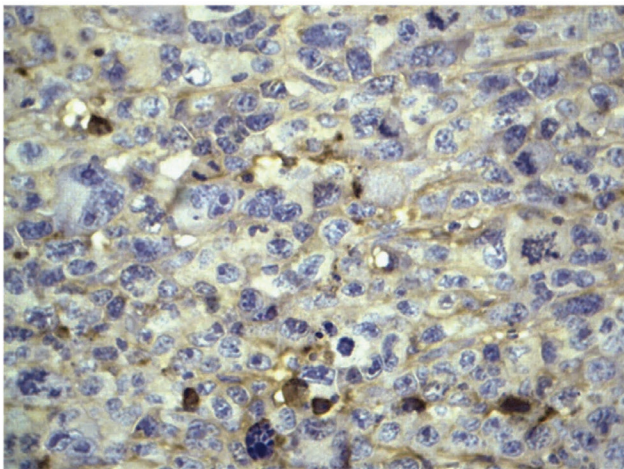


Рис. 8. Единичные клетки с экспрессией VEGF (0 баллов). Иммуногистохимический метод, увеличение $\times 400$.
Fig. 8. Single cells with VEGF expression (0 points). Immunohistochemical method, magnification $\times 400$.

Таблица 3
Экспрессии фактора ангиогенеза (VEGF) в опухоли животных контрольной и опытных групп в разные сутки наблюдения

Table 3
Expression of angiogenesis factor (VEGF) in tumors of control and experimental group on different days of observation in points

Группы Groups	Экспрессии фактора ангиогенеза (VEGF) в опухоли животных контрольной и опытных групп в разные сроки наблюдения в баллах Expression of angiogenesis factor (VEGF) in tumors of control and experimental group on different days of observation in points		
	7-е 7th	14-е 14th	21-е 21st
Группа контроля Control group	3	3	3
I	3	2	2
II	2	1	1
III	2	2	2
IV	1*	1**	0**
V	0-1*	0*	0*

* – $p < 0,05$ по сравнению с контрольной, I, II, III группами;
** – $p < 0,05$ по сравнению с контрольной, I, III группами.
* – $p < 0.05$ compared to the control, I, II, and III groups;
** – $p < 0.05$ compared to the control, I, and III groups.

factor after X-ray therapy was not observed, in contrast to the control and group I ($p < 0.05$). The absence of pronounced reaction with VEGF-positive cells persisted on the 14th day after radiation therapy ($p < 0.05$ compared with control and group I). No statistically significant differences in the level of expression of the angiogenesis factor activator with group II at all observation time points, as well as on the 21st day between groups I–III and the control group, were established.

In group IV, the most substantial decrease in VEGF expression by carcinoma cells was recorded. Thus, on the 7th day, only weakly pronounced VEGF expression was registered ($p < 0.05$ compared with control, groups I, II, III) (Fig. 7). These differences persisted on the 14th day, which indicates significant differences between group IV and intact, groups I and III ($p < 0.05$). On the 21st day in group IV, unlike groups I–III and the control group, a negative reaction with antibodies to VEGF predominated in the surviving tumor cells.

After combined therapy (group V), on the 7th day, a weak immunohistochemical reaction with antibodies to the angiogenesis factor was noted in single cells of tumor vessels. VEGF expression in cells of the adjacent stroma and in tumor epitheliocytes was absent (Fig. 8).

The level of VEGF expression by tumor cells in the animals of the study groups, expressed in points, is presented in Table 3.

Discussion

The study of pathomorphosis of the tumor process under the influence of photodynamic therapy is of important practical significance for improving the effectiveness of treatment of malignant tumors. Studies conducted on small animals in recent decades indicate that complete regression of carcinomas and sarcomas with PDT reaches 89% of cases. Treatment of unresectable tumors and metastatic lesions with PDT also indicates the achievement of partial regressions and an increase in the duration and quality of life of patients [19]. Thus, the present experimental study, performed in a series of experiments with the study of tumor angiogenesis, allows evaluating the immediate results of PDT.

Targeting the tumor vascular network represents a convincing therapeutic approach, since it is the vascular network that provides oxygen and nutrients necessary for the survival of tumor cells and serves as the main pathway for tumor metastasis. PDT leads to damage to microvessels, namely endothelial cells and the basement membrane, and creates thrombogenic areas in the vessel lumen. A physiological cascade of thrombotic reactions is triggered, including platelet aggregation, release of vasoactive molecules, leukocyte adhesion, increased vascular permeability, and their constriction. The resulting vascular obstruction and blood flow stasis lead to hypoxia, in which VEGF expression increases [9–13, 20].

At the same time, abnormal vascular structures in the tumor microenvironment may impede sufficient accumulation of photosensitizers, thereby weakening the therapeutic effectiveness of PDT [21]. According to available literature data, PDT at low doses not only fails to eliminate tumor cells but also activates signaling pathway molecules crucial for tumor survival, thereby promoting its recurrence [22, 23].

The study of the tumor vascular bed during our experiment showed sequentially proceeding changes after photodynamic exposure: irreversible stasis with reduction of the capillary network, thrombosis with subsequent organization of thrombi, thrombovasculitis. Circulatory disorders, the consequence of which was a decrease in the volume and area of vessels, occurred already at a light dose of 200 J/cm². An increase in the dose to 400 J/cm² caused more extensive disturbances of capillary blood flow: the tumor vascular bed was represented by individual vessels filled with thrombotic masses, and a pronounced decrease in the area of the vascular bed was noted. Unlike PDT, with X-ray irradiation, disturbances associated with increased

permeability and destruction of vessel walls were more often detected, which was accompanied by a decrease in the volume of the vascular network predominantly only in the central part of the tumor ($p < 0.05$). The preservation of the vascular component as a communication system in the peripheral zones of breast cancer after radiation therapy is also indicated in the works of other authors [24]. A significant limitation of local X-ray therapy, moreover, is radiation damage to surrounding soft tissues. Radiation-induced tissue injury (RITI) is the most common complication of clinical radiation therapy of tumors, which significantly limits the clinical application of radiation therapy [25, 26].

A necessary condition for the existence and progression of a tumor is angiogenesis, in which different types of cells, proangiogenic and antiangiogenic factors participate. The leading stimulator of neoangiogenesis is VEGF [11, 20]. A feature of the rapid growth of Ehrlich carcinoma is the increase of spontaneous necroses caused by a decrease in its capillarization and low cell proliferative activity in the terminal period. Thus, on the one hand, necrosis in the tumor develops as a result of hypoxia due to insufficient development of vessels in it. On the other hand, hypoxia and tumor decay products stimulate angiogenesis and vascularization. In the course of our experiment, a decrease in VEGF expression by tumor cells was found after PDT with a density of 400 J/cm² and after X-ray therapy ($p < 0.05$). However, with further comparison of these methods of exposure, it was established that after radiation exposure on the 21st day, the level of VEGF production by tumor cells did not differ from the control tumor. Consequently, despite the decrease in the density of the vascular bed and the area of vascularization, after radiation therapy the potential of tumor tissue for new vessel formation was preserved. A similar tendency with preservation of the ability for neoangiogenesis in the tumor was observed with PDT at low energy density (100 and 200 J/cm²).

Thus, PDT at a dose of 400 J/cm² leads to a pronounced irreversible decrease in the volume and area of the vascular bed, a decrease in vascular perfusion of the tumor, with maximum suppression of tumor angiogenic activity in combined treatment—a combination of resection and intraoperative PDT on the bed of the removed tumor.

Conclusion

The data obtained during the experiment indicate a more powerful destructive effect of high-dose PDT and the advantages of photodynamic exposure compared with radiation. The cytoreductive effects of photodamage can be successfully used in modern oncology at the stage of surgical treatment as an additional intraoperative effect on the bed of the removed tumor, improving treatment results.

REFERENCES

1. Bray F, Laversanne M, Weiderpass E, Soerjomataram I. The ever-increasing importance of cancer as a leading cause of premature death worldwide. *Cancer*, 2021, vol. 127, no. 16, pp. 3029–3030. doi: 10.1002/cncr.33587.
2. Zhao W., Wang L., Zhang M., Liu Z., Wu C., Pan X., et al. Photodynamic therapy for cancer: mechanisms, photosensitizers, nanocarriers, and clinical studies. *MedComm* (2020), 2024, vol. 5, no. 7, pp. e603. doi: 10.1002/mco2.603.
3. Grin M., Suvorov N., Ostroverkhov P., Pogorilyy V., Kirin N., Popov A., et al. Advantages of combined photodynamic therapy in the treatment of oncological diseases. *Biophys. Rev*, 2022, vol. 14, no. 4, pp. 941–963. doi: 10.1007/s12551-022-00962-6.
4. Mroz P., Yaroslavsky A., Kharkwal G.B., Hamblin M.R. Cell death pathways in photodynamic therapy of cancer. *Cancers (Basel)*, 2011, vol. 3, no. 2, pp. 2516–2539. doi: 10.3390/cancers3022516.
5. Korshunova O.V., Plekhova N.G. Photodynamic therapy in oncology: present and future. *Pacific Medical Journal*, 2020, no. 4, pp. 15–19. (In Russ.) doi: 10.34215/1609-1175-2020-4-15-19.
6. van Straten D., Mashayekhi V., de Bruijn H.S., Oliveira S., Robinson D.J. Oncologic photodynamic therapy: basic principles, current clinical status and future directions. *Cancers (Basel)*, 2017, vol. 9, no. 2, pp. 19. doi: 10.3390/cancers9020019.
7. Papa V., Furci F., Minciullo P.L., Casciaro M., Allegra A., Gangemi S. Photodynamic therapy in cancer: insights into cellular and molecular pathways. *Curr. Issues Mol. Biol*, 2025, vol. 47, no. 2, pp. 69. doi: 10.3390/cimb47020069.
8. Agostinis P., Berg K., Cengel K.A., Foster T.H., Girotti A.W., Gollnick S.O., et al. Photodynamic therapy of cancer: an update. *CA Cancer J. Clin*, 2011, vol. 61, no. 4, pp. 250–281. doi: 10.3322/caac.20114.
9. Mokoena D.R., George B.P., Abrahamse H. Photodynamic therapy induced cell death mechanisms in breast cancer. *Int. J. Mol. Sci*, 2021, vol. 22, no. 19, pp. 10506. doi: 10.3390/ijms221910506.
10. Allison R.R., Moghissi K. Photodynamic therapy (PDT): PDT mechanisms. *Clin. Endosc*, 2013, vol. 46, no. 1, pp. 24–29. doi: 10.5946/ce.2013.46.1.24.
11. Schmidt-Erfurth U., Schlötzer-Schrehard U., Cursiefen C., Michels S., Beckendorf A., Naumann G.O. Influence of photodynamic therapy on expression of vascular endothelial growth factor (VEGF), VEGF receptor 3, and pigment epithelium-derived factor. *Invest. Ophthalmol. Vis. Sci*, 2003, vol. 44, no. 10, pp. 4473–4480. doi: 10.1167/iovs.02-1115.
12. Castano A.P., Demidova T.N., Hamblin M.R. Mechanisms in photodynamic therapy: Part three-Photosensitizer pharmacokinetics, biodistribution, tumor localization and modes of tumor destruction. *Photodiagnosis Photodyn. Ther*, 2005, vol. 2, no. 2, pp. 91–106. doi: 10.1016/S1572-1000(05)00060-8.
13. Chen D., Yuan W., Park H.C., Li X. *In vivo* assessment of vascular-targeted photodynamic therapy effects on tumor microvasculature using ultrahigh-resolution functional optical coherence tomography. *Biomed. Opt. Express*, 2020, vol. 11, no. 8, pp. 4316–4325. doi: 10.1364/BOE.397602.
14. Filonenko, E.V. The history of development of fluorescence diagnosis and photodynamic therapy and their capabilities in oncology. *Russ J Gen Chem*, 2015, vol. 85, pp. 211–216. doi: org/10.1134/S1070363215010399
15. Lillie R.D. *Pathohistological technique and practical histochemistry*. Moscow: Mir; 1969. 645 p. (In Russ.)
16. Merkulov G.A. *Course in pathohistological technique*. 4th ed. Leningrad: Medgiz. Leningrad department; 1961. 340 p. (In Russ.)
17. Avtandilov G.G. *Medical morphometry: Manual*. Moscow: Meditsina; 1990. 384 p. (In Russ.) ISBN 5-225-00753-8.
18. Rebrova O.Yu. *Statistical analysis of medical data. Application of the STATISTICA software package*. Moscow: Mediasfera; 2002. 312 p. (In Russ.) ISBN 5-89084-013-4.

ЛИТЕРАТУРА

1. Bray F, Laversanne M, Weiderpass E, Soerjomataram I. The ever-increasing importance of cancer as a leading cause of premature death worldwide // *Cancer*. – 2021. – Vol. 127, № 16. – P. 3029–3030. doi: 10.1002/cncr.33587
2. Zhao W., Wang L., Zhang M., Liu Z., Wu C., Pan X., et al. Photodynamic therapy for cancer: mechanisms, photosensitizers, nanocarriers, and clinical studies // *MedComm* (2020). – 2024. – Vol. 5, № 7. – P. e603. doi: 10.1002/mco2.603.
3. Grin M., Suvorov N., Ostroverkhov P., Pogorilyy V., Kirin N., Popov A., et al. Advantages of combined photodynamic therapy in the treatment of oncological diseases // *Biophys. Rev*. – 2022. – Vol. 14, № 4. – P. 941–963. doi: 10.1007/s12551-022-00962-6.
4. Mroz P., Yaroslavsky A., Kharkwal G.B., Hamblin M.R. Cell death pathways in photodynamic therapy of cancer // *Cancers (Basel)*. – 2011. – Vol. 3, № 2. – P. 2516–2539. doi: 10.3390/cancers3022516.
5. Коршунова О.В., Плехова Н.Г. Фотодинамическая терапия в онкологии: настоящее и будущее // *Тихоокеанский медицинский журнал*. – 2020. – № 4. – С. 15–19. doi: 10.34215/1609-1175-2020-4-15-19.
6. van Straten D., Mashayekhi V., de Bruijn H.S., Oliveira S., Robinson D.J. Oncologic photodynamic therapy: basic principles, current clinical status and future directions // *Cancers (Basel)*. – 2017. – Vol. 9, № 2. – P. 19. doi: 10.3390/cancers9020019.
7. Papa V., Furci F., Minciullo P.L., Casciaro M., Allegra A., Gangemi S. Photodynamic therapy in cancer: insights into cellular and molecular pathways // *Curr. Issues Mol. Biol*. – 2025. – Vol. 47, № 2. – P. 69. doi: 10.3390/cimb47020069.
8. Agostinis P., Berg K., Cengel K.A., Foster T.H., Girotti A.W., Gollnick S.O., et al. Photodynamic therapy of cancer: an update // *CA Cancer J. Clin*. – 2011. – Vol. 61, № 4. – P. 250–281. doi: 10.3322/caac.20114.
9. Mokoena D.R., George B.P., Abrahamse H. Photodynamic therapy induced cell death mechanisms in breast cancer // *Int. J. Mol. Sci*. – 2021. – Vol. 22, № 19. – P. 10506. doi: 10.3390/ijms221910506.
10. Allison R.R., Moghissi K. Photodynamic therapy (PDT): PDT mechanisms // *Clin. Endosc*. – 2013. – Vol. 46, № 1. – P. 24–29. doi: 10.5946/ce.2013.46.1.24.
11. Schmidt-Erfurth U., Schlötzer-Schrehard U., Cursiefen C., Michels S., Beckendorf A., Naumann G.O. Influence of photodynamic therapy on expression of vascular endothelial growth factor (VEGF), VEGF receptor 3, and pigment epithelium-derived factor // *Invest. Ophthalmol. Vis. Sci*. – 2003. – Vol. 44, № 10. – P. 4473–4480. doi: 10.1167/iovs.02-1115.
12. Castano A.P., Demidova T.N., Hamblin M.R. Mechanisms in photodynamic therapy: Part three-Photosensitizer pharmacokinetics, biodistribution, tumor localization and modes of tumor destruction // *Photodiagnosis Photodyn. Ther*. – 2005. – Vol. 2, № 2. – P. 91–106. doi: 10.1016/S1572-1000(05)00060-8.
13. Chen D., Yuan W., Park H.C., Li X. *In vivo* assessment of vascular-targeted photodynamic therapy effects on tumor microvasculature using ultrahigh-resolution functional optical coherence tomography // *Biomed. Opt. Express*. – 2020. – Vol. 11, № 8. – P. 4316–4325. doi: 10.1364/BOE.397602.
14. Filonenko, E.V. The history of development of fluorescence diagnosis and photodynamic therapy and their capabilities in oncology. // *Russ J Gen Chem*. – 2015. – Vol. 85. – P. 211–216. doi:org/10.1134/S1070363215010399.
15. Лилли Р.Д. Патогистологическая техника и практическая гистохимия. – Москва: Мир, 1969. – 645 с.
16. Меркулов Г.А. Курс патологистологической техники. – 4-е изд. – Ленинград: Медгиз. Ленингр. отд-ние, 1961. – 340 с.
17. Автандилов Г.Г. Медицинская морфометрия: руководство. – Москва: Медицина, 1990. – 384 с. ISBN 5-225-00753-8.
18. Реброва О.Ю. Статистический анализ медицинских данных. Применение пакета прикладных программ STATISTICA. – Москва: МедиаСфера, 2002. – 312 с. ISBN 5-89084-013-4.

19. Spichenkova I.S., Kaplan M.A., Gulidov I.A., Sokol N.I., Ponomarev H.V., Bandurko L.N. Combination of photodynamic therapy and rentgenotherapy in treatment of sarcoma M1 in rats. *Rossiiskij bioterapevticeskij zurnal (Russian Journal of Biotherapy)*, 2005, vol. 4, no. 3, pp. 62–68. (In Russ.)
20. dos Santos A.F., de Almeida D.R.Q., Terra L.F., Baptista M.S., Labriola L. Photodynamic therapy in cancer treatment – an update review. *J. Cancer Metastasis Treat*, 2019, vol. 5, pp. 25. doi: 10.20517/2394-4722.2018.83.
21. Yue D., Cai X., Fan M., Zhu J., Tian J., Wu L., et al. An alternating irradiation strategy-driven combination therapy of PDT and RNAi for highly efficient inhibition of tumor growth and metastasis. *Adv. Healthc. Mater*, 2021, vol. 10, no. 8, pp. e2001850. doi: 10.1002/adhm.202001850.
22. Wan Y., Fu L.H., Li C., Lin J., Huang P. Conquering the hypoxia limitation for photodynamic therapy. *Adv. Mater*, 2021, vol. 33, no. 48, pp. e2103978. doi: 10.1002/adma.202103978.
23. Lv Y., Pu L., Ran B., Xiang B. Targeting tumor angiogenesis and metabolism with photodynamic nanomedicine. *Front. Cell Dev. Biol*, 2025, vol. 13, pp. 1558393. doi: 10.3389/fcell.2025.1558393.
24. Gil Marques F., Poli E., Malaquias J., Carvalho T., Portêlo A., Ramires A., et al. Low doses of ionizing radiation activate endothelial cells and induce angiogenesis in peritumoral tissues. *Radiother. Oncol*, 2020, vol. 151, pp. 322–327. doi: 10.1016/j.radonc.2020.06.038.
25. Simman R., Bach K., Abbas F., Klomparens K., Brickman B.J. Management of radiation-induced tissue injuries: A review of current treatment strategies. *Plast. Reconstr. Surg. Glob. Open*, 2023, vol. 11, no. 6, pp. e5043. doi: 10.1097/GOX.0000000000005043.
26. Zhou L., Zhu J., Liu Y., Zhou P.K., Gu Y. Mechanisms of radiation-induced tissue damage and response. *MedComm (2020)*, 2024, vol. 5, no. 10, pp. e725. doi: 10.1002/mco2.725.
19. Спиченкова И.С., Катан М.А., Гулидов И.А., Сокол Н.К., Пономарев Г.В., Бандурко Л.Н. Сочетанная фотодинамическая и рентгенотерапия саркомы М1 у крыс // *Российский биотерапевтический журнал*. – 2005. – Т. 4, № 3. – С. 62–68.
20. dos Santos A.F., de Almeida D.R.Q., Terra L.F., Baptista M.S., Labriola L. Photodynamic therapy in cancer treatment – an update review // *J. Cancer Metastasis Treat*. – 2019. – Vol. 5. – P. 25. doi: 10.20517/2394-4722.2018.83.
21. Yue D., Cai X., Fan M., Zhu J., Tian J., Wu L., et al. An alternating irradiation strategy-driven combination therapy of PDT and RNAi for highly efficient inhibition of tumor growth and metastasis // *Adv. Healthc. Mater*. – 2021. – Vol. 10, № 8. – P. e2001850. doi: 10.1002/adhm.202001850.
22. Wan Y., Fu L.H., Li C., Lin J., Huang P. Conquering the hypoxia limitation for photodynamic therapy // *Adv. Mater*. – 2021. – Vol. 33, № 48. – P. e2103978. doi: 10.1002/adma.202103978.
23. Lv Y., Pu L., Ran B., Xiang B. Targeting tumor angiogenesis and metabolism with photodynamic nanomedicine // *Front. Cell Dev. Biol*. – 2025. – Vol. 13. – P. 1558393. doi: 10.3389/fcell.2025.1558393.
24. Gil Marques F., Poli E., Malaquias J., Carvalho T., Portêlo A., Ramires A., et al. Low doses of ionizing radiation activate endothelial cells and induce angiogenesis in peritumoral tissues // *Radiother. Oncol*. – 2020. – Vol. 151. – P. 322–327. doi: 10.1016/j.radonc.2020.06.038.
25. Simman R., Bach K., Abbas F., Klomparens K., Brickman B.J. Management of radiation-induced tissue injuries: A review of current treatment strategies // *Plast. Reconstr. Surg. Glob. Open*. – 2023. – Vol. 11, № 6. – P. e5043. doi: 10.1097/GOX.0000000000005043.
26. Zhou L., Zhu J., Liu Y., Zhou P.K., Gu Y. Mechanisms of radiation-induced tissue damage and response // *MedComm (2020)*. – 2024. – Vol. 5, № 10. – P. e725. doi: 10.1002/mco2.725.

ANALYSIS OF COLLAGEN PRESENCE IN BRAIN TUMORS USING SPONTANEOUS RAMAN SCATTERING SPECTROSCOPY

Antipova E.D.¹, Romanishkin I.D.¹, Savelieva T.A.^{1,2}, Ryabova A.V.^{1,2,3},
Shugay S.V.⁴, Kosyrkova A.V.⁴, Pavlova G.V.^{4,5}, Pronin I.N.⁴, Loschenov V.B.^{1,2}

¹Prokhorov General Physics Institute of Russian Academy of Sciences, Moscow, Russia

²National Research Nuclear University MEPhI (Moscow Engineering Physics Institute), Moscow, Russia

³RUDN University, Moscow, Russia

⁴N.N. Burdenko National Medical Research Center of Neurosurgery, Moscow, Russia

⁵Institute of Higher Nervous Activity and Neurophysiology of the Russian Academy of Sciences, Moscow, Russia

Abstract

As brain tumors progress, excess collagen increases the rigidity of the extracellular matrix, which promotes metastasis, impedes drug penetration, and reduces the effectiveness of therapy. A detailed study of collagen as a promising biomarker of malignancy is highly relevant for complementing histopathology for accurate diagnosis and treatment planning. This study proposes an approach to detect collagen in intracranial tumor tissues using spontaneous Raman spectroscopy with excitation at a wavelength 785 nm.

Key words: intracranial tumors, gliomas, meningiomas, optical spectroscopy, Raman scattering, collagen, extracellular matrix

Contacts: Antipova E.D. e-mail: Antipova.ED@mail.ru

For citations: Antipova E.D., Romanishkin I.D., Savelieva T.A., Ryabova A.V., Shugay S.V., Kosyrkova A.V., Pavlova G.V., Pronin I.N., Loschenov V.B. Analysis of collagen presence in brain tumors using spontaneous Raman scattering spectroscopy, Biomedical Photonics, 2026, vol. 15, no. 3, pp. 21–29. doi: 10.24931/2413–9432–2026–15–3–21–29

АНАЛИЗ ПРИСУТСТВИЯ КОЛЛАГЕНА В ОПУХОЛЯХ ГОЛОВНОГО МОЗГА МЕТОДОМ СПЕКТРОСКОПИИ СПОНТАННОГО КОМБИНАЦИОННОГО РАССЕЯНИЯ СВЕТА

Е.Д. Антипова¹, И.Д. Романишкин¹, Т.А. Савельева^{1,2}, А.В. Рябова^{1,2,3}, С.В. Шугай⁴,
А.В. Косырькова⁴, Г.В. Павлова^{4,5}, И.Н. Пронин⁴, В.Б. Лощенов^{1,2}

¹Институт общей физики им. А.М. Прохорова Российской академии наук, Москва, Россия

²Национальный исследовательский ядерный университет «МИФИ» (Московский инженерно-физический институт), Москва, Россия

³Российский Университет дружбы народов, Москва, Россия

⁴Национальный медицинский исследовательский центр нейрохирургии имени академика Н. Н. Бурденко, Москва, Россия

⁵Институт высшей нервной деятельности и нейрофизиологии Российской академии наук, Москва, Россия

Резюме

При прогрессировании опухолей головного мозга избыточный коллаген повышает жёсткость внеклеточного матрикса, что способствует метастазированию, препятствует проникновению препаратов и снижает эффективность терапии. Детальное изучение

коллагена как перспективного биомаркера злокачественности весьма актуально для дополнения к гистопатологии в точной диагностике и планировании лечения. В данном исследовании предлагается подход к детекции коллагена в тканях внутричерепных опухолей методом спектроскопии спонтанного комбинационного рассеяния с возбуждением на длине волны 785 нм.

Ключевые слова: внутричерепные опухоли, глиомы, менингиомы, оптическая спектроскопия, комбинационное рассеяние, коллаген, внеклеточный матрикс

Контакты: Антипова Е.Д. e-mail: Antipova.ED@mail.ru

Для цитирования: Антипова Е.Д., Романишкин И.Д., Савельева Т.А., Рябова А.В., Шугай С.В., Косырькова А.В., Павлова Г.В., Пронин И.Н., Лощенов В.Б. Анализ присутствия коллагена в опухолях головного мозга методом спектроскопии спонтанного комбинационного рассеяния света // Biomedical Photonics. – 2026. – Т. 15, № 3. – С. 21–29. doi: 10.24931/2413-9432-2026-15-3-21-29

Introduction

The extracellular matrix (ECM) is a complex network of macromolecules (proteins and heteropolysaccharides) secreted by cells and forming an ordered structure in the intercellular space. It performs several key functions: it serves as a framework for organs and tissues, acts as a “biological glue”, participates in the regulation of water-salt balance, and forms specialized structures (bone, cartilage, basement membranes). The main components of the ECM are structural proteins such as collagen and elastin, glycosaminoglycans, proteoglycans, and non-collagen proteins (fibronectin, laminin, tenascin), which determine the mechanical properties of tissues. Collagen can be divided into several groups depending on the type of structure it forms. There are 28 different types of collagen, but the most common are types I–IV. Type I collagen is the most prevalent in the human body and typically accounts for approximately 80–90% of the total collagen content [1] [2]. In a normal brain, the ECM is “soft” and contains a limited amount of proteins. A key feature of the ECM of a healthy brain is its low collagen content: it is present primarily in the basement membranes of blood vessels and glia-associated structures. This distinguishes the brain's ECM from most other body tissues, where collagen is the main structural component [3]. However, its expression is increased in human glioblastoma and glioma cell lines [4]. The dense ECM promotes fibrosis and the formation of a physical barrier to both T-cell penetration and drug infiltration, leading to immunosuppression and drug resistance [5] [6]. Recent in vitro studies have shown that collagen density is an important regulator of the immunosuppressive phenotype of tumor-associated macrophages (TAMs). This mechanism may be critical for the ability of tumors enriched in M2-polarized macrophages, such as glioblastoma, to evade immune destruction and may represent a limiting factor in the effectiveness of immunotherapy [7].

The ECM of the central nervous system (CNS), compared to the ECM of other organs, has a number of features and contains, among other things, non-fibrous components and specialized structural forms [8]. The

main specialized form of ECM is type IV collagen in the basement membrane (BM) of blood vessels, which plays a key role in maintaining the integrity of the blood-brain barrier (BBB). Another type of collagen (collagen XIX) is found in the perineuronal nets (PNN). It is a rare non-fibrillar ECM protein belonging to the FACIT (fibril-associated collagens with Interrupted Triple helices) family [9]. The authors of the study [10] showed that physical compaction (a process in which cells are collected and packed together, causing associated changes in cell shape and size) of human glioblastoma cell lines U87MG, U251 and LN229 induces the expression of collagen types IV and VI and the collagen-cross-linking enzyme lysyl oxidase, and also increases in vitro expression of the angiogenic factor vascular endothelial growth factor. A study [11] demonstrated that the collagen type V $\alpha 1$ chain gene (COL5A1) plays a key role in the development of glioblastoma. Researchers examined the involvement of COL5A1 in the remodeling of extracellular structures and the regulation of actin filaments during glioblastoma metastasis. In [12], an increase in collagen XVI mRNA and protein levels was found in glioblastomas compared to normal cerebral cortex and de novo expression of collagen XVI was shown in glioblastomas as part of a tumor-specific remodeling of the ECM.

Glioblastoma undergoes ECM remodeling: tumor cells actively alter the ECM composition, increasing the expression of components such as hyaluronic acid, fibronectin, tenascin-C, and various proteoglycans. These changes contribute to increased tumor cell migration, their invasion of surrounding tissue, and the development of resistance to therapy. Hyaluronic acid plays a key role in glioblastoma progression: its elevated levels increase with tumor malignancy and promote cell migration through interaction with the CD44 receptor, while fibronectin and tenascin-C enhance tumor cell adhesion, proliferation, and invasive properties. Thus, the ECM not only serves a structural function but also actively regulates signaling pathways that influence tumor cell survival and behavior [3].

Meningiomas with mesenchymal differentiation are characterized by active production of ECM components.

Collagen plays a fundamental role in organizing the microenvironment of these tumors. According to study [3], normal arachnoid cells and meningioma cells synthesize various types of collagen, including type IV collagen, which is a component of basement membranes, as well as type III procollagen. These proteins, along with fibronectin and laminin, form a unique stroma that provides a scaffold for tumor cells. Matrix metalloproteinases (MMPs), particularly gelatinases MMP-2 and MMP-9, play a key role in collagen matrix remodeling in meningiomas. These enzymes degrade collagen: MMP-2 cleaves type V collagen, while MMP-9 is capable of cleaving types I, III, and V collagen. High expression levels of these proteinases, along with their tissue inhibitor TIMP-1, indicate a delicate balance between collagen synthesis and degradation. The authors of the study [13] also note that MMP expression levels were higher in recurrent and atypical meningiomas, suggesting a link between collagenolytic enzyme activity and the aggressive course of the disease.

The high frequency of expression of ECM components, including collagen-degrading enzymes, makes them potential targets for therapeutic intervention: inhibition of MMP-2 and MMP-9 activity is considered a promising strategy for reducing the risk of tumor recurrence. Thus, studying the collagen profile and metalloproteinase activity is not only of theoretical importance for understanding the biology of meningiomas but also of great practical significance for the development of new therapeutic methods [13].

In [14], the possibility of using Raman microspectroscopy to distinguish between two types of collagen—I and IV—and to analyze the structural features of the skin was investigated. Both types of collagen play a key role in the structure of the skin: collagen I predominates in the dermis and provides its mechanical strength, while collagen IV is the main component of the basement membrane of the dermal-epidermal junction and is responsible for the integrity of this layer. Both proteins are susceptible to changes during aging and tumor transformation. Despite their similar amino acid composition, collagens I and IV have different spatial organizations: collagen I forms fibrils, while collagen IV molecules assemble into sheet-like chicken-wire meshworks via specialized terminal domains rather than forming rigid parallel fibers. These structural differences are reflected in the spectra. The authors found that the most informative spectral bands are Amide I and Amide III, as well as regions associated with proline, hydroxyproline, and phenylalanine. For example, a shift in the Amide I band and changes in the intensity of the proline-associated bands indicate differences in protein conformation. The study [15] examines the use of Raman spectroscopy for breast cancer diagnostics. Ex vivo breast tissue samples from 58 patients were examined, including

normal tissue, fibrocystic lesions, fibroadenomas, and infiltrating carcinoma. A total of 130 Raman spectra were obtained. The MCR-ALS (multivariate curve resolution) method was then applied. Particular attention was paid to collagen, a key structural protein of the ECM. Analysis showed that spectral parameters associated with collagen and fat possessed the greatest diagnostic significance. In a pilot study [16], Raman spectroscopy was used to diagnose cartilaginous tumors (from benign enchondroma (BE) to malignant chondrosarcoma (MC) with increasing degrees of aggressiveness (G1, G2, G3)). One of the important findings was the degradation of type II collagen with increasing tumor malignancy. In Raman spectra, this was manifested by the weakening of characteristic collagen bands (at 728, 830, and 1206 cm^{-1}). The cause of collagen degradation turned out to be closely related to changes in the metabolism of proline (a key amino acid stabilizing the collagen helix), which consequently indicates the destruction of the ECM as a central process in tumor progression.

In [17], the question of whether Raman spectroscopy can detect and distinguish between different collagen types (I–VI) in pure form and within the ECM produced by MG63 osteoblast-like cells was investigated. The authors measured the spectra of pure human collagen types I–VI in dry, liquid, and compressed powder form. The key conclusion is that Raman spectroscopy combined with multivariate statistical analysis (PCA-LDA) successfully distinguishes between different collagen types if the samples are in the same physical state, because this has a stronger influence on the spectrum than the collagen type. Among all the types, type II collagen stood out, as it demonstrated an intense peak at 954 cm^{-1} (C–C bond in proline). This is explained by the unique structure of its α -chain and its less frequent cross-linking compared to type I collagen. The spectra of collagens IV and VI were the most similar (their confidence intervals overlapped), while collagens I, II, III, and V formed distinct clusters. Another important result was that the spectra of natural, non-mineralized ECM from MG63 cells matched the pattern of pure dried collagen type I (and partially type III), confirming that pure substances adequately reflect the actual collagen in the human body. Thus, a reference spectral library for collagens I–VI was created, which has diagnostic value. The authors emphasize that this method allows for the detection of collagen changes not only in bone diseases but also in any connective tissue pathology.

The aim of the work presented in this article was to develop a computational approach to analyzing Raman spectra of intracranial tumor tissue samples ex vivo for spectral detection of collagen in the extracellular matrix of meningioma, glioblastoma, and white matter of the brain. This included recording and analyzing spectra of the main biochemical components of the extracellular

matrix of the studied biological tissues, retrospective analysis of Raman spectra of glioblastoma, meningioma, and normal brain tissue, and identification of the most informative Raman spectral ranges in which samples belonging to different tissue types differ in collagen content.

Materials and methods

Spectral data

In this study, a retrospective analysis of spectral data recorded *ex vivo* in the cryopreservation laboratory of the N.N. Burdenko National Medical Research Center of Neurosurgery was conducted, as part of studies published in the previous works of the research team [18, 19]. For the initial assessment of the contribution of collagen, 6 glioblastoma samples (18 spectra), 4 meningioma samples, and 5 healthy tissue samples (the samples were balanced in volume) were analyzed.

Also, as part of the study, a reference database was created: Raman spectra of compounds that are close analogues of the main biochemical components of the tissues studied were recorded: gelatin (a collagen model), human albumin, cholesterol, and hyaluronic acid.

To record Raman spectra, we used a system consisting of a Raman-HR-TEC-785 Raman spectrometer (StellarNet, USA), a Ramulaser™ 785 narrowband laser source with a wavelength of 785 nm (StellarNet, USA), a computer with StellarNet SpectraWiz software, and a fiber-optic confocal probe for delivering laser radiation and the Raman signal. This system enables recording spectra in the range of 200–2750 cm^{-1} . The spectral resolution of the system was 4 cm^{-1} .

Raman spectra were recorded to form the reference database using a series of measurements at several points on each sample. For gelatin, measurements were performed at 10 different points on the sample; three spectra were recorded at each point with a 10 s exposure

due to high autofluorescence. For human albumin and hyaluronic acid, recording was performed similarly (three spectra at 10 points) with a 30 s exposure. For cholesterol, ten spectra were recorded at one point with a 30 s exposure. For brain tumor samples, recording was performed as follows: for glioblastoma, three spectra were recorded at different points with a 30 s exposure; for meningioma and healthy tissue, ten spectra were recorded with a 30 s exposure. Before each series of measurements, a background signal was recorded with the laser turned off. All measurements were performed in a darkened room.

Spectral data preprocessing

Data processing was performed in MATLAB (MathWorks, R2023) and included the following steps: to reduce random noise, the spectra were pre-averaged, then smoothed using a modified Savitzky–Golay filter for a non-uniform spectral grid (window size: 21 points, third-order polynomial), background fluorescence was removed using the Vancouver algorithm (the degree of the polynomial was selected in accordance with the sample characteristics), and the spectra were normalized (to the maximum intensity value). For the spectra prepared for analysis in this manner, spectral approximation by a combination of Gaussians was selected as the decomposition method.

Method for detecting collagen using Raman spectra

Spectra of simple biochemical components were recorded to find collagen-specific peaks among collagen and non-collagen spectra. However, the reference spectra themselves do not provide a comprehensive basis for decomposing the Raman spectra of intracranial tumor tissue. To quantitatively analyze spectral data and identify hidden biochemical components, a method of approximating experimental spectra with a sum of

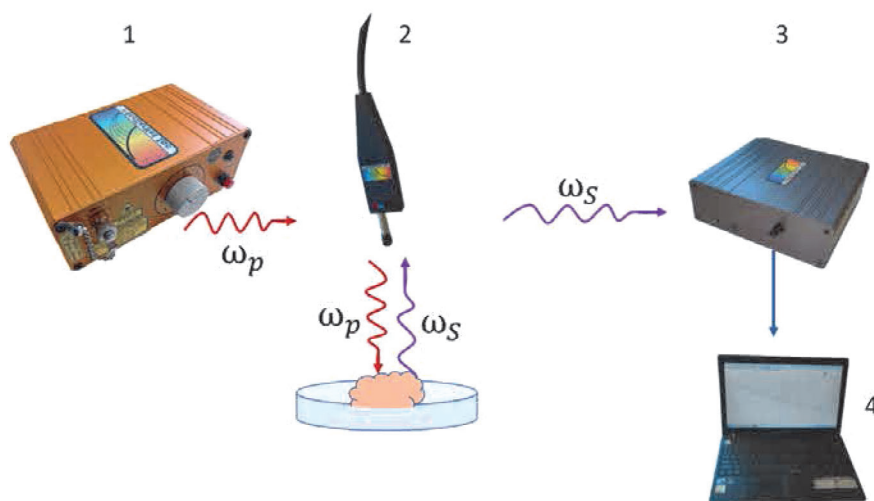


Рис. 1. Схема спектроскопической системы для регистрации КР-спектров: 1 – лазер Ramulaser™ ($\lambda = 785$ нм); 2 – оптоволоконный зонд; 3 – спектрометр Raman-HR-TEC-785; 4 – ноутбук с программным обеспечением.

Fig. 1. Scheme of a spectroscopic system for recording Raman spectra: 1 – laser Ramulaser™ ($\lambda = 785$ nm); 2 – fiber optic probe; 3 – spectrometer Raman-HR-TEC-785; 4 – PC with special software.

Gaussian functions was used. The number of components was manually determined based on visual analysis of the spectral profile. For the spectra of glioblastoma, meningioma, and normal brain tissue, 34 peaks, uniformly distributed across the spectral range, were used.

Optimization of the parameters of each Gaussian peak (center, amplitude, and width) was performed using the least-squares method using the lsqcurvefit function. To prevent convergence to unphysical solutions, the following constraints were used: the peak center position was no more than $\pm 10 \text{ cm}^{-1}$ from the initial approximation; the amplitude was from 0 to 2; the width was from 1 to 50 cm^{-1} .

The quality of the approximation was assessed using the root mean square error. For each isolated peak, the following were determined: center position (cm^{-1}); amplitude (A); width (σ); and peak area. After approximation, peaks with amplitudes less than 0.03 (3% of the maximum spectral intensity) were excluded from further analysis, as such peaks are comparable to the noise level and may be approximation artifacts.

Since the same biochemical component can appear in different spectra as peaks with similar but not identical positions, clustering of the resulting peaks was performed. Peaks were assigned to one cluster if the distance between their centers did not exceed 5 cm^{-1} . The positions of known Raman lines in the vicinity of those lines that appeared in the recorded spectra were used as starting positions. Since relatively simple substances were used as reference spectra, a threshold of 5 cm^{-1} was chosen based on an analysis of the scatter of the recorded line positions from the expected peak positions for clustering. For each cluster, the average center position was calculated. A cluster was included in the final list of target peaks only if it was present in at least 50% of all studied spectra, which made it possible to exclude random outliers and increase the reliability of component identification.

For each target peak, the areas of all Gaussian components located within a range of $\pm 5 \text{ cm}^{-1}$ relative to the center of the corresponding cluster were summed. The resulting value was used as a quantitative characteristic of the contribution of a given biochemical component to the sample spectrum.

Since a retrospective analysis of random spectra from an existing sample of spectral data obtained in previous years in the biobank was carried out [18], we used information about the normal distribution of the areas of individual peaks in these spectra to apply parametric methods of statistical data analysis. For each target peak, a one-way analysis of variance (ANOVA) was performed to compare three groups of samples: glioblastoma; meningioma; and normal brain tissue. Differences were considered statistically significant at a significance level of $p < 0.05$. For significant peaks, the following were determined: p value; mean peak area values in each group; and standard deviations of peak area in each group.

Results and discussion

Analysis of reference Raman spectra of the main biochemical components

The processed reference Raman spectra of the main tissue components are shown in Fig. 2. These substances form a reliable model for the analysis of Raman spectra, since in total they cover the spectral "fingerprints" of almost all macromolecules of biological tissues: albumin is a model of globular proteins (its spectrum clearly shows the vibrational bands of the peptide backbone (Amide I and Amide III), as well as phenylalanine and tryptophan), gelatin is a model of fibrous proteins of the ECM, in particular collagen, hyaluronic acid is a model of carbohydrate components of connective tissue and intercellular substance (glycosaminoglycans) (allows for the detection of specific glycosidic bonds, acetyl groups and carbohydrate rings), cholesterol is a basic marker of lipid membranes and fat inclusions.

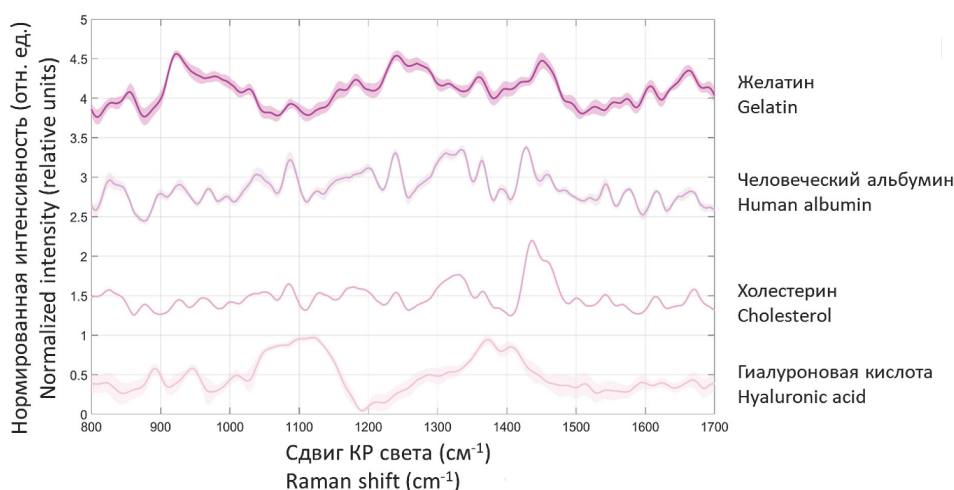


Рис. 2. Обработанные референсные спектры основных компонентов ткани. Представлены средние значения и стандартное отклонение (σ).
Fig. 2. Processed reference spectra of the main tissue components. Mean values and standard deviations are presented (σ).

Analysis of Raman spectra of intracranial tumor tissues

To illustrate the approximation method, Fig. 4 shows the averaged spectra of each sample type (glioblastoma, normal tissue, meningioma) with superimposed approximating curves obtained as a sum of Gaussian functions. Spectra were averaged for visualization purposes and were not used in further quantitative analysis. All peak parameter calculations (center position, amplitude, width, area) were performed for each spectrum individually, without preliminary averaging. The averaged spectra in Fig. 4 are shown to demonstrate the quality of the approximation. As can be seen from the figure, the approximating curves provide a good description of the experimental data over the entire studied range of 800–1700 cm^{-1} . The root-mean-square error values were in the range of 0.0032–0.0034, indicating good agreement between the model and the experimental data.

Search for characteristic collagen peaks in intracranial tumor tissues

During the analysis of the spectra, statistically significant differences were revealed between the

samples of glioblastoma, meningioma, and normal brain tissue for a number of characteristic peaks (see Table 1). The most pronounced differences were observed in the region of 881.2 cm^{-1} ($p = 0.01$), which may be associated with changes in the content of hydroxyproline, tryptophan, and protein structures. For the peak of 968.9 cm^{-1} ($p = 0.046928$), differences corresponding to lipid components and calcium triphosphate were noted. The peak of 992 cm^{-1} ($p = 0.000022$), associated with phosphate ions, turned out to be especially significant: in meningioma tissue, its intensity was significantly lower compared to glioblastoma and normal tissue.

Changes associated with fluctuations in the tryptophan ring, glycogen, phospholipids, and nucleic acids were recorded in the 1016.4–1096.7 cm^{-1} range. Thus, the peaks at 1042.6 cm^{-1} ($p = 0.000058$) and 1096.7 cm^{-1} ($p = 0.000001$) demonstrated a significant increase in intensity in meningioma, which may reflect increased metabolic activity and changes in the content of membrane components.

Significant differences were also found for the 1124 cm^{-1} ($p = 0.000089$) and 1207.7 cm^{-1} ($p = 0.000057$) bands

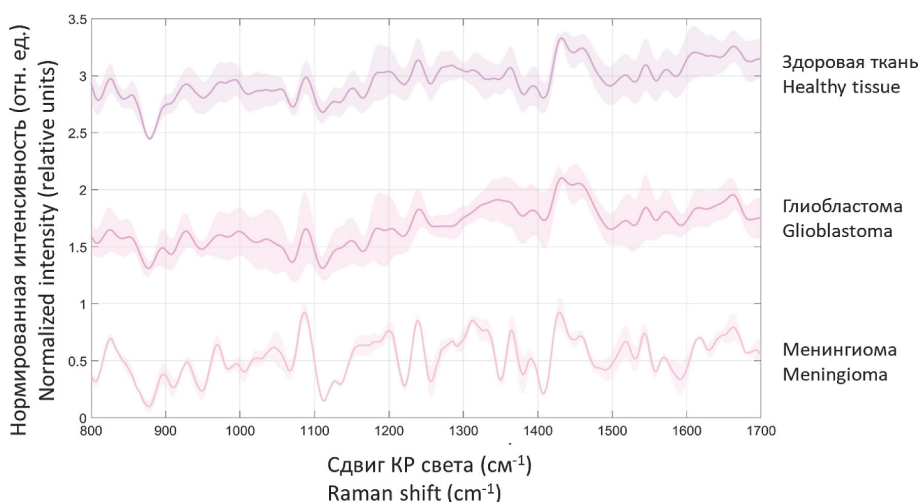


Рис. 3. Обработанные спектры опухолей головного мозга основных компонентов ткани. Представлены средние значения и стандартное отклонение.
Fig. 3. Processed spectra of brain tumors' main tissue components. Mean values and standard deviations are presented.

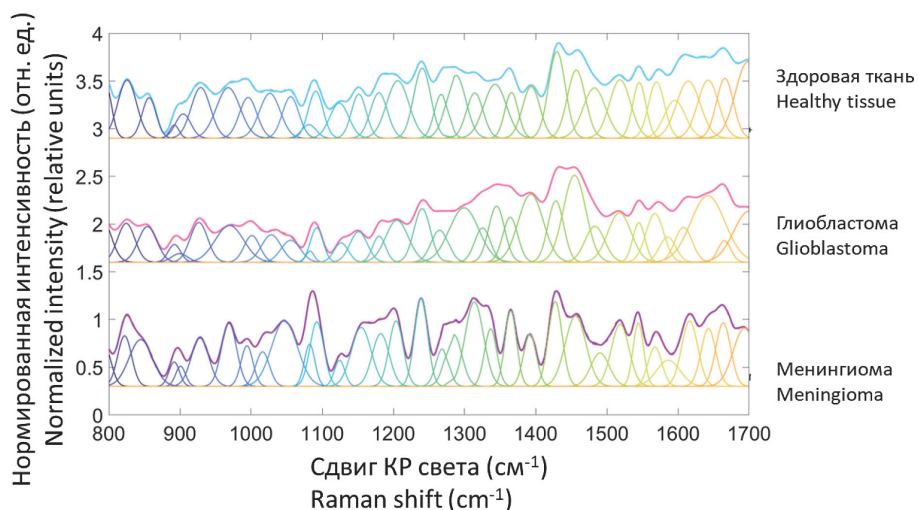


Рис. 4. Спектры КР света исследованных образцов: экспериментальные данные нормированы по максимуму и представлены в виде суммы гауссовых кривых, аппроксимирующие спектры опухолей.
Fig. 4. Raman spectra of the studied samples: The experimental data are normalized to the maximum and presented as a sum of Gaussian curves approximating the tumor spectra.

Таблица 1

Характерные пики и соответствующие им предполагаемые биохимические корреляты [20], по которым были выявлены статистически значимые различия между группами образцов глиобластомы, менингиомы и нормальной ткани головного мозга

Table 1

Characteristic peaks with their suggested biochemical correlates [20], that revealed statistically significant differences between groups of glioblastoma, meningiomas and normal brain tissue samples

Пик (cm^{-1}) Peak (cm^{-1})	Площади под кривой для глиобластомы/ менингиомы/нормы Areas under the curve for glioblastoma/ meningioma/ norma	P-value	Предполагаемая интерпретация Suggested interpretation
881.2	26.7± 1.8 22.6± 2.1 25.8± 1.6	0.01	Гидроксипролин / триптофан $\delta(\text{кольцо})$ / $\rho(\text{CH}_2)$ (белки) Hydroxyproline/tryptophan $\delta(\text{ring})/\rho(\text{CH}_2)$ (proteins)
968.9	22.5±1.2 28.3±5.9 25.3 ± 1.5	0.047	Липиды/Трифосфат кальция $\text{Ca}_3(\text{PO}_4)_3$ Lipids/Calcium triphosphate $\text{Ca}_3(\text{PO}_4)_3$
992	22.4±4.0 6.1 ± 3.7 20.7±3.3	0.00002	Фосфат-ионы Phosphate ions
1016.4	21.5±0.5 23.5±2.0 20.8±1.3	0.031	Триптофановое кольцо/растягивание C-O рибозы/ гликоген Tryptophan ring/C-O stretch of ribose/glycogen
1042.6	22.4±1.1 27.5±1.3 22.8±0.7	0.00006	Колебания C-O и C-N связей в белке Vibrations of C-O and C-N bonds in proteins
1096.7	20.4±1.2 28.4±2.2 19.9±1.7	0.000001	Колебания C-C и симметричные колебания PO_2 фосфолипидов и нуклеиновых кислот/ колебания C=O сложного эфира/альдегида C-C vibrations and symmetric PO_2 vibrations of phospholipids and nucleic acids/C=O vibrations of ester/aldehyde
1124	21.9±4.0 9.2 ± 2.5 18.6±2.7	0.00009	Гликоген/C-C-связь фосфолипидов (боковых цепей), холестерин Glycogen/C-C linkage of phospholipids (side chains), cholesterol
1207.7	22.4±3.5 36.1±8.0 22.4±2.0	0.00006	Колебания $\nu(\text{C}-\text{C}_6\text{H}_5)$ в молекулах тирозина, гидроксипролина (аминокислоты коллагена) и фенилаланина Vibrations of $\nu(\text{C}-\text{C}_6\text{H}_5)$ in molecules of tyrosine, hydroxyproline (an amino acid of collagen), and phenylalanine
1238	19.7±3.3 6.6 ± 1.0 20.0±4.9	0.00014	Амид III Amide III
1314.4	15.9±0.8 27.9±0.0 14.8±2.1	0.000066	Деформация CH_3/CH_2 липидов и белков CH_3/CH_2 deformation of lipids and proteins
1366.7	15.1±5.8 7.3± 2.2 14.8±3.0	0.03615	Колебания CH_3 (фосфолипиды)/триптофан/гуанин CH_3 (phospholipids)/tryptophan/guanine vibrations
1395.1	19.5±4.7 25.3± 1.5 17.3± 2.2	0.04912	Деформация CH_2/CH_3 липидов и белков CH_2/CH_3 deformation of lipids and proteins
1513.5	17.4±3.2 36.3±0.0 15.6±1.8	0.00006	Каротиноид/ колебания кольца в основаниях ДНК Carotenoid/ ring vibrations in DNA bases
1542.7	9.9± 3.9 4.7± 3.3 10.9±2.3	0.02743	Нуклеиновые кислоты Nucleic acids
1567.0	11.3 ± 2.8 7.6 ± 2.2 12.2 ± 1.7	0.02691	Гемоглобин Hemoglobin
1616.1	11.6±1.7 17.40±4.1 10.7±1.5	0.00053	Ароматические аминокислоты (белок) Aromatic amino acids (protein)

associated with glycogen, cholesterol, and collagen aromatic amino acids. In particular, for meningioma, an increase in the intensity of the 1207.7 cm^{-1} peak was observed, which may indicate an increased content of ECM protein structures.

Statistically significant changes were also detected in the protein regions of the spectrum. The peak at 1238 cm^{-1} , related to the Amide III band ($p=0.000143$), was characterized by a decrease in intensity in meningioma samples. Similarly, for the peak at 1314.4 cm^{-1} ($p=0.000066$), corresponding to CH_3/CH_2 deformation vibrations of lipids and proteins, a significant increase in values was observed in meningioma tissue compared to glioblastoma and normal tissue.

In the high-frequency region of the spectrum, differences were associated with lipid components, nucleic acids, and proteins. Peaks at 1366.7 cm^{-1} ($p = 0.036148$) and 1395.1 cm^{-1} ($p = 0.049117$) reflected changes in phospholipids, tryptophan, and lipid structures. In addition, for the 1513.5 cm^{-1} band ($p = 0.000061$), associated with carotenoids and DNA bases, a sharp increase in intensity was observed in meningioma tissue.

For the peaks at 1542.7 cm^{-1} ($p = 0.027431$) and 1567.0 cm^{-1} ($p = 0.026910$), associated with nucleic acids and hemoglobin, a decrease in intensity was observed in tumor tissues compared to normal tissue. Finally, the peak at 1616.1 cm^{-1} ($p = 0.000525$), corresponding to aromatic amino acids of proteins, was significantly higher in meningioma samples, which may indicate structural changes in the protein composition of the tissue.

Thus, the obtained results demonstrate the presence of significant biochemical differences between glioblastoma, meningioma, and normal brain tissue. The most informative peaks were those associated with phospholipids, proteins, nucleic acids, and ECM components, confirming the potential of spectral analysis for the differential diagnosis of brain tumors.

As a result of the analysis, spectral characteristics were found that statistically significantly differentiated the tissue groups under consideration ($p<0.05$). Among the protein peaks were selected the listed below.

- 1) The peak at 1042.6 cm^{-1} (C-O and C-N bonds in the protein) in the meningiomas group showed high values (27.51 ± 1.28), in the glioblastomas group and the norm the values were comparable (22.40 ± 1.09 and 22.76 ± 0.72 , respectively).
- 2) The peak at 1207.7 cm^{-1} , corresponding to $\nu(\text{C-C6H5})$ vibrations in the molecules of tyrosine, hydroxyproline (an amino acid of collagen) and phenylalanine, showed higher values in the group of meningiomas (36.07 ± 8.00) compared to glioblastomas (22.44 ± 3.53) and normal tissue (22.37 ± 1.92).
- 3) The peak at 1238.0 cm^{-1} (Amide III, reflecting the secondary structure of proteins) was significantly reduced in meningiomas (6.64 ± 0.98) relative to glioblastomas (19.70 ± 3.32) and normal (19.97 ± 4.89).

- 4) The peak at 1616.1 cm^{-1} (aromatic amino acids, protein) was the highest in meningiomas (17.40 ± 4.07), while in glioblastomas and normal cells its values were (11.56 ± 1.65 and 10.67 ± 1.05), respectively.

The analysis revealed characteristic spectral differences between tumors of different types. It is important to pay attention to the peak associated with collagen, which indicates an increased content of collagen in meningioma; this corresponds to literature data, according to which meningiomas, which are mesenchymal in nature, actively synthesize ECM proteins, including collagen [3]. At the same time, glioblastoma tissue, like normal brain tissue, is characterized by a limited presence of collagen, localized mainly in the basement membranes of blood vessels [8].

Conclusion

The spectra of the main components of biological tissues, such as gelatin, albumin, hyaluronic acid, and cholesterol, were recorded. Methods for their analysis were proposed, revealing the "spectroscopic signatures" of these components, by which the corresponding biochemical features can be identified in the spectra of biological tissue samples. A retrospective analysis of Raman spectra obtained *ex vivo* from glioblastoma, meningioma, and healthy brain tissue samples was conducted. A spectral processing algorithm was developed and implemented, including smoothing, background signal subtraction, spectral peak approximation with Gaussian functions, peak center clustering, and statistical analysis of the obtained parameters. An automatic search for characteristic collagen peaks in the spectra of the studied tissues was performed, and a quantitative assessment of their spectral characteristics was performed. Using analysis of variance (ANOVA), spectral peaks were identified for which statistically significant differences were found between the glioblastoma, meningioma, and normal brain tissue groups ($p < 0.05$). The most informative protein spectral peaks were identified, allowing differentiation between glioblastoma, meningioma, and normal brain tissue, opening up the possibility of using them as spectral markers in the diagnosis of brain tumors. The obtained results demonstrate differences in the composition and structural organization of collagen in the ECM of the studied tissues and confirm the feasibility of using Raman spectroscopy for their detection. Raman spectroscopy, combined with automated spectral data analysis, is shown to be a promising tool for collagen research and the search for diagnostically significant brain tumor markers.

Funding

This work was supported by the Ministry of Science and Higher Education of the Russian Federation (the Federal Scientific-technical program for genetic technologies development for 2019–2030), agreement No 075-15-2025-559.

REFERENCES

1. Chakraborty R., Asthana A., Singh A.K., Adhikari R., Bin Hasan A. Chapter 20 - Collagen - a highly developed and abundant fibrous protein: synthesis and characterization. *Handbook of Natural Polymers, Elsevier*, 2023, pp. 489-508. ISBN 9780323998536. doi: 10.1016/B978-0-323-99853-6.00013-9.
2. Naomi R., Ridzuan P.M., Bahari H. Current Insights into Collagen Type I. *Polymers (Basel)*, 2021, vol. 16, pp. 2642. doi: 10.3390/polym13162642
3. Mohiuddin E., Wakimoto H. Extracellular matrix in glioblastoma: opportunities for emerging therapeutic approaches. *American Journal of Cancer Research*, 2021, vol. 11, pp. 3742-3754.
4. Di Vito A., Donato A., Bria J., Conforti F., La Torre D., Malara N., Donato G. Extracellular Matrix Structure and Interaction with Immune Cells in Adult Astrocytic Tumors. *Cell Mol Neurobiol*, 2024, vol. 1, pp. 54. doi: 10.1007/s10571-024-01488-z
5. Ge H., Tian M., Pei Q., Tan F., Pei H. Extracellular matrix stiffness: new areas affecting cell metabolism. *Front Oncol*, 2021, vol. 11, pp. 631991. doi: 10.3389/fonc.2021.631991.
6. Flies D.B., Langermann S., Jensen C., Karsdal M.A., Willumsen N. Regulation of tumor immunity and immunotherapy by the tumor collagen extracellular matrix. *Front Immunol*, 2023, vol.14, pp. 1199513. doi: 10.3389/fimmu.2023.1199513.
7. Larsen A.M.H., Kuczek D.E., Kalvisa A., Siersbæk M.S., Thorseth M.L., Johansen A.Z., Carretta M., Grøntved L., Vang O., Madsen D.H. Collagen density modulates the immunosuppressive functions of macrophages. *J Immunol*, 2020, vol. 205, pp. 1461-1472. doi: 10.4049/jimmunol.1900789.
8. Soles A., Selimovic A., Sbrocco K., Ghannoum F., Hamel K., Moncada E.L., Gilliat S., Cvetanovic M. Extracellular Matrix Regulation in Physiology and in Brain Disease. *Int J Mol Sci*, 2023, vol.8, pp. 7049. doi: 10.3390/ijms24087049.
9. Oudart J.B., Monboisse J.C., Maquart F.X., Brassart B., Brassart-Pasco S., Ramont L. Type XIX collagen: A new partner in the interactions between tumor cells and their microenvironment. *Matrix Biol*, 2017, vol. 57-58, pp. 169-177. doi: 10.1016/j.matbio.2016.07.010.
10. Mammoto T., Jiang A., Jiang E., Panigrahy D., Kieran M.W., Mammoto A. Role of collagen matrix in tumor angiogenesis and glioblastoma multiforme progression. *Am J Pathol*, 2013, vol. 183, pp. 1293-1305. doi: 10.1016/j.ajpath.2013.06.026.
11. Tsai H.F., Chang Y.C., Li C.H. et al. Type V collagen alpha 1 chain promotes the malignancy of glioblastoma through PPRC1-ESM1 axis activation and extracellular matrix remodeling. *Cell Death Discov*, 2021, vol. 7, pp. 313. doi: 10.1038/s41420-021-00661-3.
12. Senner V., Ratzinger S., Mertsch S., Grässel S., Paulus W. Collagen XVI expression is upregulated in glioblastomas and promotes tumor cell adhesion. *FEBS Lett*, 2008, vol. 582, pp. 3293-3300. doi: 10.1016/j.febslet.2008.09.017.
13. Das A., Tan W.-L., Smith D.R. Expression of extracellular matrix markers in benign meningiomas. *Neuropathology*, 2003, vol. 23, pp. 275-281.
14. Nguyen T.T., Gobinet C., Feru J., Brassart-Pasco S., Manfait M., Piot O. Characterization of Type I and IV Collagens by Raman Microspectroscopy: Identification of Spectral Markers of the Dermo-Epidermal Junction. *Spectroscopy: An International Journal*, 2012, vol. 27, pp. 421-427. doi: 10.1155/2012/686183.
15. Haka A.S., Shafer-Peltier K. E., Fitzmaurice M., Crowe J., Dasari R.R., Feld M.S. Diagnosing breast cancer by using Raman spectroscopy. *Proceedings of the National Academy of Sciences of the United States of America*, 2005, vol. 102, pp. 12371-12376. doi: 10.1073/pnas.0501390102.
16. D'Acunto M., Gaeta R., Capanna R. et al. Contribution of Raman Spectroscopy to Diagnosis and Grading of Chondrogenic Tumors. *Scientific Reports*, 2020, vol. 10, pp. 2155. doi: 10.1038/s41598-020-58848-0.
17. Ingle I., Kerns J.G., Shepherd R.F. Multivariate analysis of Raman spectra for discriminating human collagens: In vitro identification of extracellular matrix collagens produced by an osteosarcoma cell line. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2025, vol. 328, pp. 125434. doi: 10.1016/j.saa.2024.125434.
18. Romanishkin I.D., Savelieva T.A., Ospanov A., Linkov K.G., Shugay S.V., Goryajnov S.A., Pavlova G.V., Pronin I.N., Loschenov V.B. Classification of intracranial tumors based on optical-spectral analysis. *Biomedical Photonics*, 2023, vol.12, pp. 4-10. doi: 10.24931/2413-9432-2023-12-3-4-10.
19. Ospanov A., Romanishkin I.D., Savelieva T.A., Shugay S.V., Kosyrkova A.V., Pavlova G.V., Pronin I.N., Loschenov V.B. Search for correlations in raman, diffuse reflectance, and fluorescence spectroscopy data from intracranial tumors. *Biomedical Photonics*, 2025, vol.14, pp. 22-33. doi: 10.24931/2413-9432-2025-14-4-22-33.
20. Brusatori M., Auner G., Noh T., et al. Intraoperative Raman Spectroscopy. *Neurosurg Clin N Am*, 2017, vol. 28, pp. 633-652. doi: 10.1016/j.nec.2017.05.014.

ЛИТЕРАТУРА

1. Chakraborty R., Asthana A., Singh A.K., Adhikari R., Bin Hasan A. Chapter 20 - Collagen - a highly developed and abundant fibrous protein: synthesis and characterization // *Handbook of Natural Polymers, Elsevier*. – 2023. – P. 489-508. ISBN 9780323998536. doi: 10.1016/B978-0-323-99853-6.00013-9.
2. Naomi R., Ridzuan P.M., Bahari H. Current Insights into Collagen Type I. // *Polymers (Basel)*. – 2021. – Vol. 16. – P. 2642. doi: 10.3390/polym13162642
3. Mohiuddin E., Wakimoto H. Extracellular matrix in glioblastoma: opportunities for emerging therapeutic approaches // *American Journal of Cancer Research*. — 2021. — Vol. 11, No. 8. — P. 3742–3754.
4. Di Vito A., Donato A., Bria J., Conforti F., La Torre D., Malara N., Donato G. Extracellular Matrix Structure and Interaction with Immune Cells in Adult Astrocytic Tumors. // *Cell Mol Neurobiol*. – 2024. – Vol. 1, No. 44. – P. 54. doi: 10.1007/s10571-024-01488-z
5. Ge H., Tian M., Pei Q., Tan F., Pei H. Extracellular matrix stiffness: new areas affecting cell metabolism // *Front Oncol*. – 2021. – Vol. 11. – P. 631991. doi: 10.3389/fonc.2021.631991.
6. Flies D.B., Langermann S., Jensen C., Karsdal M.A., Willumsen N. Regulation of tumor immunity and immunotherapy by the tumor collagen extracellular matrix // *Front Immunol*. – 2023. – Vol.14. – P. 1199513. doi: 10.3389/fimmu.2023.1199513.
7. Larsen A.M.H., Kuczek D.E., Kalvisa A., Siersbæk M.S., Thorseth M.L., Johansen A.Z., Carretta M., Grøntved L., Vang O., Madsen D.H. Collagen density modulates the immunosuppressive functions of macrophages // *J Immunol*. – 2020. – Vol. 205. – P. 1461–1472. doi: 10.4049/jimmunol.1900789.
8. Soles A., Selimovic A., Sbrocco K., Ghannoum F., Hamel K., Moncada E.L., Gilliat S., Cvetanovic M. Extracellular Matrix Regulation in Physiology and in Brain Disease // *Int J Mol Sci*. – 2023. – Vol.8, No. 24. – P. 7049. doi: 10.3390/ijms24087049.
9. Oudart J.B., Monboisse J.C., Maquart F.X., Brassart B., Brassart-Pasco S., Ramont L. Type XIX collagen: A new partner in the interactions between tumor cells and their microenvironment // *Matrix Biol*. – 2017. – Vol. 57-58. – P. 169-177. doi: 10.1016/j.matbio.2016.07.010.
10. Mammoto T., Jiang A., Jiang E., Panigrahy D., Kieran M.W., Mammoto A. Role of collagen matrix in tumor angiogenesis and glioblastoma multiforme progression // *Am J Pathol*. – 2013. – Vol. 183. – P. 1293-1305. doi: 10.1016/j.ajpath.2013.06.026.
11. Tsai H.F., Chang Y.C., Li C.H. et al. Type V collagen alpha 1 chain promotes the malignancy of glioblastoma through PPRC1-ESM1 axis activation and extracellular matrix remodeling // *Cell Death Discov*. – 2021. – Vol. 7. – P. 313. doi: 10.1038/s41420-021-00661-3.
12. Senner V., Ratzinger S., Mertsch S., Grässel S., Paulus W. Collagen XVI expression is upregulated in glioblastomas and promotes tumor cell adhesion // *FEBS Lett*. – 2008. – Vol. 582. – P. 3293-3300. doi: 10.1016/j.febslet.2008.09.017.
13. Das A., Tan W.-L., Smith D.R. Expression of extracellular matrix markers in benign meningiomas // *Neuropathology*. – 2003. – Vol. 23. – P. 275–281.
14. Nguyen T.T., Gobinet C., Feru J., Brassart-Pasco S., Manfait M., Piot O. Characterization of Type I and IV Collagens by Raman Microspectroscopy: Identification of Spectral Markers of the Dermo-Epidermal Junction // *Spectroscopy: An International Journal*. – 2012. – Vol. 27. – P. 421–427. doi: 10.1155/2012/686183.
15. Haka A.S., Shafer-Peltier K. E., Fitzmaurice M., Crowe J., Dasari R.R., Feld M.S. Diagnosing breast cancer by using Raman spectroscopy // *Proceedings of the National Academy of Sciences of the United States of America*. – 2005. – Vol. 102. – P. 12371–12376. doi: 10.1073/pnas.0501390102.
16. D'Acunto M., Gaeta R., Capanna R. et al. Contribution of Raman Spectroscopy to Diagnosis and Grading of Chondrogenic Tumors // *Scientific Reports*. – 2020. – Vol. 10. – P. 2155. doi: 10.1038/s41598-020-58848-0.
17. Ingle I., Kerns J.G., Shepherd R.F. Multivariate analysis of Raman spectra for discriminating human collagens: In vitro identification of extracellular matrix collagens produced by an osteosarcoma cell line // *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. – 2025. – Vol. 328. – P. 125434. doi: 10.1016/j.saa.2024.125434.
18. Романшикин И.Д., Савельева Т.А., Оспанов А., Линьков К.Г., Шугай С.В., Горайнов С.А., Павлова Г.В., Пронин И.Н., Лощенов В.Б. Классификация внутричерепных опухолей на основе оптико-спектрального анализа // *Biomedical Photonics*. – 2023. – Vol.12. – P. 4-10. doi: 10.24931/2413-9432-2023-12-3-4-10.
19. Оспанов А., Романшикин И.Д., Савельева Т.А., Шугай С.В., Косырькова А.В., Павлова Г.В., Пронин И.Н., Лощенов В.Б. Поиск корреляций в данных спектроскопии комбинационного рассеяния, диффузного отражения и флуоресценции тканей внутричерепных опухолей // *Biomedical Photonics*. – 2025. – Vol.14. – P. 22-33. doi: 10.24931/2413-9432-2025-14-4-22-33.
20. Brusatori M., Auner G., Noh T., et al. Intraoperative Raman Spectroscopy // *Neurosurg Clin N Am*. – 2017. – Vol. 28. – P. 633-652. doi: 10.1016/j.nec.2017.05.014.

COMBINATION OF FERROPTOSIS AND PHOTODYNAMIC THERAPY IS AN EFFECTIVE STRATEGY FOR CANCER TREATMENT

Sanarova E.V.¹, Lantsova A.V.¹, Nikolaeva L.L.^{1,2}, Kiseleva M.P.¹, Borisova L.M.¹

¹N.N. Blokhin National Medical Research Center of Oncology of the Ministry of Health of Russia, Moscow, Russia

²Sechenov University, Moscow, Russia

Abstract

This review examines in detail the theoretical foundations and practical aspects of using a combination of ferroptosis inducers and photosensitizers in experimental cancer therapy. Particular attention is given to comprehensive studies focusing on the combined use of these two therapeutic approaches. The results confirm the promise of further development in this research area.

Based on a combination of theoretical data and experimental studies, the effectiveness of a combined approach combining ferroptosis and photodynamic therapy (PDT) as an antitumor treatment strategy is convincingly demonstrated. A key factor determining the effectiveness of this combination is the synergistic antitumor effect, driven by interconnected biochemical processes, primarily the Fenton reaction. Ferroptosis involves a biochemical transformation capable of providing an additional source of oxygen, which is essential for effective PDT. In turn, PDT generates reactive oxygen species, which potentiate the Fenton reaction, thereby enhancing the processes leading to ferroptotic cell death in tumor cells. This creates a vicious cycle that greatly enhances the cytotoxic effect on malignant cells.

An analysis of experimental studies revealed that most studies used chlorin e6 and porphyrin derivatives as photosensitizers – compounds with proven photophysical properties. Erastin and its derivatives, as well as various iron compounds capable of effectively triggering and maintaining iron-dependent cell death, were most frequently used as ferroptosis inducers.

These data demonstrate the high potential of a combined strategy based on the coupling of ferroptosis and PDT and substantiate the need for further research in this area to develop new approaches to cancer treatment.

Key words: ferroptosis, photodynamic therapy, ferroptosis inducer, photosensitizer, antitumor activity.

Contacts: Lantsova A.V., e-mail: lantsova1979@mail.ru

For citation: Sanarova E.V., Lantsova A.V., Nikolaeva L.L., Kiseleva M.P., Borisova L.M. Combination of ferroptosis and photodynamic therapy is an effective strategy for cancer treatment, *Biomedical Photonics*, 2026, vol. 15, no. 3, pp. 30–38. doi: 10.24931/2413-9432-2026-15-3-30-38

КОМБИНАЦИЯ ФЕРРОПТОЗА И ФОТОДИНАМИЧЕСКОЙ ТЕРАПИИ – ЭФФЕКТИВНАЯ СТРАТЕГИЯ ДЛЯ ЛЕЧЕНИЯ РАКА

Е.В. Санарова¹, А.В. Ланцова¹, Л.Л. Николаева^{1,2}, М.П. Киселева¹, Л.М. Борисова¹

¹Национальный медицинский исследовательский центр онкологии им. Н.Н. Блохина Минздрава России, Москва, Россия

²Первый Московский государственный медицинский университет им. И.М. Сеченова Минздрава России (Сеченовский Университет), Москва, Россия

Резюме

В рамках проведенного обзора детально рассмотрены теоретические основы и практические аспекты применения комбинации индукторов ферроптоза и фотосенсибилизаторов в экспериментальной терапии злокачественных опухолей. Особое внимание в работе уделено комплексным исследованиям, фокусирующимся на совместном использовании двух терапевтических подходов. Полученные результаты подтверждают перспективность дальнейшего развития данного научного направления.

На основании совокупности теоретических данных и экспериментальных исследований убедительно продемонстрирована эффективность комбинированного подхода, объединяющего ферроптоз и фотодинамическую терапию (ФДТ), в качестве стратегии противоопухолевого лечения. Ключевым фактором, определяющим результативность такой комбинации, выступает синергетический противоопухолевый эффект, обусловленный взаимосвязанными биохимическими процессами, в первую очередь реакцией Фентона. В ходе ферроптоза реализуется биохимическое превращение, способное обеспечивать дополнительный источник кислорода,

необходимого для эффективного протекания ФДТ. В свою очередь, ФДТ генерирует активные формы кислорода, которые потенцируют реакцию Фентона, тем самым усиливая процессы, ведущие к ферроптотической гибели опухолевых клеток. В результате формируется замкнутый цикл, многократно повышающий цитотоксическое воздействие на злокачественные клетки.

Анализ экспериментальных работ показал, что в большинстве исследований в качестве фотосенсибилизатора используют хлорин е6 и производные порфирина – соединения, хорошо зарекомендовавшие себя в ФДТ благодаря оптимальным фотофизическим характеристикам. В роли индукторов ферроптоза наиболее часто применяют эрастин и его производные, а также различные соединения железа, способные эффективно запускать и поддерживать процессы железозависимой клеточной гибели.

Представленные данные свидетельствуют о высоком потенциале комбинированной стратегии, основанной на сопряжении ферроптоза и ФДТ, и обосновывают необходимость дальнейших исследований в этой области для разработки новых подходов к лечению онкологических заболеваний.

Ключевые слова: ферроптоз, фотодинамическая терапия, индуктор ферроптоза, фотосенсибилизатор, противоопухолевая активность.

Контакты: Ланцова А.В., e-mail: lantsova1979@mail.ru

Для цитирования: Санарова Е.В., Ланцова А.В., Николаева Л.Л., Киселева М.П., Борисова Л.М. Комбинация ферроптоза и фотодинамической терапии – эффективная стратегия для лечения рака // Biomedical Photonics. – 2026. – Т. 15, № 3. – С. 30–38. doi: 10.24931/2413–9432–2026–15–3–30–38

Introduction

Modern oncology has achieved significant success in developing comprehensive treatment regimens that combine surgery, chemotherapy, and radiotherapy. However, problems such as recurrence, metastasis, the development of drug resistance, and the high toxicity of existing methods continue to have a negative impact on the prognosis and quality of life of cancer patients. This necessitates the search for and implementation of new therapeutic strategies characterized by high selectivity and lower toxicity.

Among promising approaches, photodynamic therapy (PDT) occupies a special place. It is based on the selective accumulation of a photosensitizer (PS) in tumor tissue followed by its activation with laser radiation of an appropriate wavelength. The photochemical reaction results in the generation of reactive oxygen species (ROS), which lead to tumor cell death through several mechanisms and also activate the immune response. An important advantage of PDT is the possibility of preserving the anatomical and functional integrity of organs, which is particularly valuable in clinical practice [1–2].

At the same time, the practical application of PDT is limited by a number of factors, including insufficient PS selectivity, limited depth of light penetration into tissues, and the need for high-tech equipment. To overcome these limitations, PS delivery systems based on nanocarriers are being actively developed; these systems make it possible to increase the drug concentration in the tumor and minimize its effect on healthy tissues [3–5].

Of particular interest in the context of enhancing PDT efficacy is ferroptosis, a type of iron-dependent programmed cell death characterized by excessive ROS generation and lipid peroxidation of cell membranes. Currently, the induction of ferroptosis is regarded as

a promising antitumor strategy, and the identified molecular intersections between PDT and ferroptosis open up opportunities for creating combined therapeutic approaches [6–8]. It is assumed that the combined use of PDT and ferroptosis inducers can provide a synergistic antitumor effect by enhancing oxidative stress in tumor cells [9–10].

This review systematizes current data on the combined use of PDT and ferroptosis induction. Particular attention is paid to research results obtained by scientific teams both abroad and in the Russian Federation. The work identifies certain trends characteristic of the current stage of development of this research area: the predominant use of already known photosensitizers and ferroptosis inducers, as well as a focus on traditional therapeutic targets such as breast cancer, gliomas, and glioblastomas. These patterns necessitate the development of new vectors for improving the method, in particular through the introduction of promising substances and the expansion of its therapeutic application.

Mechanism of the synergistic effect of photodynamic therapy and ferroptosis in cancer treatment

The mechanism of the synergistic effect of PDT and ferroptosis is very interesting but still insufficiently studied, due to the relatively recent discovery of the phenomenon of ferroptosis itself. Ferroptosis is a complex form of cell death associated with iron metabolism and characterized by the development of oxidative stress due to increasing lipid peroxidation of cellular lipids. The involvement of ferroptosis has been noted in the development of many pathological processes, such as fibrosis, inflammation, and tumors. The most important characteristics of ferroptosis, which have recently received considerable attention,

include the accumulation of ROS, an imbalance of the antioxidant system, disturbances in lipid peroxidation (LPO) metabolism, and iron homeostasis [11]. Ferroptosis is regulated by the fine interplay of three molecular pathways involving glutathione depletion, iron metabolism, and LPO. A number of substances are currently known to be ferroptosis inducers, some of which have previously been used as medicinal agents [12–14].

In turn, the cytotoxic mechanism of PDT is based on the use of PSs—dyes activated by laser light in the visible or near-infrared range. Their excitation by photons triggers, as in ferroptosis, the production of ROS and subsequently tumor cell death. The targeted nature and high efficacy of this therapy are due to the targeted delivery of the PS to the tumor area and the provision of a sufficient amount of oxygen for ROS generation.

Recent studies have changed the view of the PDT mechanism from a simple induction of oxidative stress leading to necrosis or apoptosis, adding to this already complex mechanism ferroptosis, pyroptosis, and other types of cell death [15]. For example, in GL261 glioblastoma cells subjected to PDT with the well-known and clinically used second-generation PS Photosens (aluminum hydroxide trisulfophthalocyanine), an interesting phenomenon was discovered: cell death induced by irradiation is blocked by the ferroptosis inhibitors ferrostatin-1 and deferoxamine (DFO), which indirectly indicates that this PS induces ferroptosis in GL261 cells upon photoactivation [16]. In Japan, ferroptotic activity was demonstrated for the widely known and clinically used sodium talaporfin (a second-generation PS), which was found to induce

ferroptosis in gastric and esophageal cancer cells [17]. Ferroptotic activity is also found in newly synthesized PSs with enhanced photodynamic efficiency, good biocompatibility, and lysosomal targeting [18]. There are studies demonstrating the contribution of ferroptosis to antitumor PDT using an activatable, adaptable nanocarrier based on a metal-organic framework. Thus, a group of Chinese scientists formed a metal-organic framework based on an imidazole ligand coordinated with zinc, into which chlorin e6 (Ce6, a second-generation PS) was encapsulated. The high antitumor efficacy of the nanocarrier in vivo was confirmed in mice with murine breast carcinoma in terms of suppression of tumor growth and prolongation of animal survival. Simultaneous administration of an iron chelator weakened the antitumor activity of the nanocarrier due to suppression of ferroptosis, as evidenced by enhanced tumor growth and restoration of glutathione peroxidase 4 (GPX4) activity [19].

Hypoxia in the tumor microenvironment, caused by high intracellular oxygen consumption and deformation of tumor blood vessels, hinders PDT. In this regard, increasing oxygen production in the tumor microenvironment is a promising direction for enhancing PDT efficacy [20, 21]. Recently, a number of researchers have shifted their focus to an updated concept of ferroptosis-stimulated PDT. This advantageous combination is based on the biochemical characteristics of ferroptosis, which is expected to enhance the antitumor efficacy of PDT through ROS generation and sustained oxygen supply via the Fenton reaction (Fig. 1). Practical confirmation of this is the synergistic effect of ferroptosis inducers and PDT in experiments on tumor cells [22].

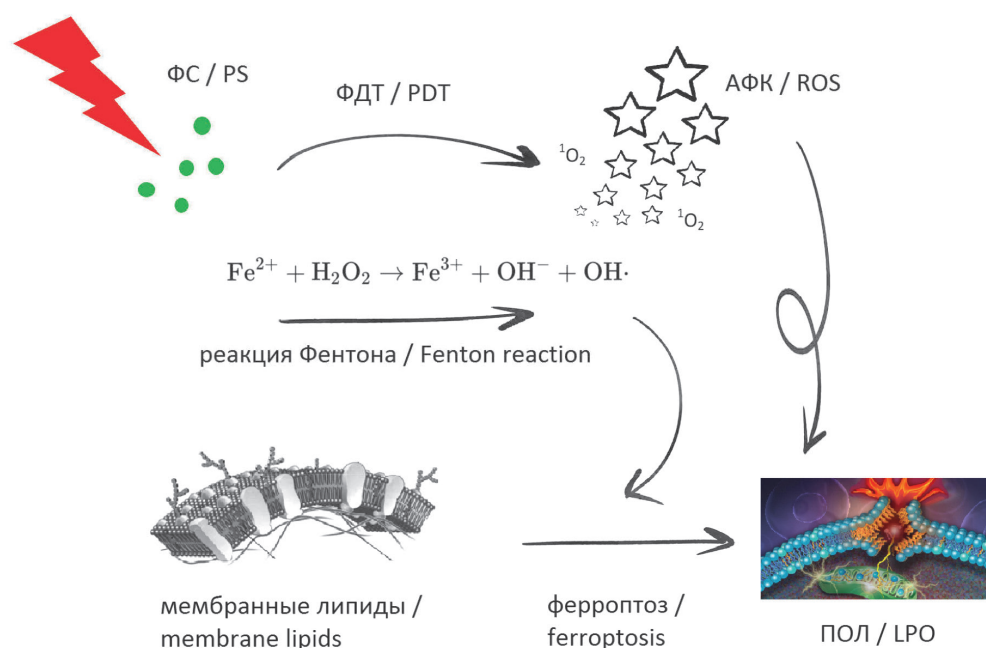


Рис. 1. Схематическое изображение взаимосвязи процессов, происходящих при ФДТ и ферроптозе в опухолевой клетке (АФК – активные формы кислорода, ФС – фотосенсибилизатор, ФДТ – фотодинамическая терапия, ПОЛ – перекисное окисление липидов).

Fig. 1. Schematic representation of the interconnection processes occurring during PDT and ferroptosis in a tumor cell (ROS – reactive oxygen species, PS – photosensitizer, PDT – photodynamic therapy, LPO – lipid peroxidation).

A key role in the processes occurring during ferroptosis activation and the photodynamic reaction is played by ROS, which are by-products of aerobic respiration under physiological conditions and cause DNA damage and genomic instability, thereby leading to the accumulation of genetic alterations in tumors and promoting cancer progression [23]. Tumor cells require a significant amount of ROS to maintain their invasive development [24]. However, excessive amounts of ROS cannot be *давя* and cause tumor cell death due to DNA damage and increasing LPO. This phenomenon is widely known as "oxidative stress" [25]. To overcome and modify these adverse conditions, the cell's antioxidant system maintains redox homeostasis. One of the important components of the intracellular antioxidant system is glutathione (a tripeptide consisting of the three amino acids cysteine, glycine, and glutamine), which participates in many metabolic and physiological processes in the human body. One of the functions of this substance is antioxidant. Glutathione suppresses the excess of ROS generated in the cell by oxidation to glutathione disulfide either directly or via an enzymatic reaction [26].

Tumor tissue contains four times more glutathione than normal tissue. This is necessary to adapt to rapid cell growth and survive under conditions of oxidative stress [27]. Another function of this tripeptide is the detoxification of drugs, which creates obstacles both for chemotherapy and for the implementation of photodynamic activity. It is reliably known that glutathione mediates the detoxification of doxorubicin, cisplatin, and other antitumor agents, which largely determines tumor resistance [28]. Although PDT stimulates an increase in intracellular ROS, intracellular glutathione has a direct suppressive effect on the development of oxidative stress, thereby reducing the efficacy of this therapy [29]. Cancer treatment methods aimed at suppressing glutathione metabolism are being studied, modified, and are extremely relevant.

It has already been proven that the mechanisms of ferroptosis and photodynamic action can work in fine synergy with each other; therefore, it is reasonable to assume that the induction of ferroptosis during PDT is a powerful alternative strategy for increasing the efficacy of antitumor therapy, in particular for tumors resistant to apoptosis and necrosis.

In scientific publications, ferroptosis is mentioned in connection with such nosologies as melanoma, colon cancer, breast carcinoma, glioblastoma, and prostate cancer [30–35]. Most of the presented studies are devoted to melanoma. Thus, Tsoi J. et al. demonstrated that ferroptosis-inducing agents (erastin, RSL3, ML162, and ML210) represent an alternative therapeutic approach aimed at the plasticity of melanoma cell differentiation to enhance the efficacy of targeted

therapy and immunotherapy [36]. A group of Chinese scientists obtained an expression profile of microRNA (miR-21-3p) in melanoma cells during ferroptosis induced by interferon- γ and proved that increased miR-21-3p expression promotes interferon- γ -mediated ferroptosis by enhancing LPO. In addition, this microRNA increased sensitivity to ferroptosis by directly targeting thioredoxin reductase 1 and enhancing ROS production. In turn, elevated miR-21-3p expression in the tumor enhanced the effect of anti-PD-1 antibodies by promoting ferroptosis of tumor cells [37]. Another study examined the combined use of sorafenib and vemurafenib. Vemurafenib is a BRAF kinase inhibitor approved for the treatment of melanoma; it suppresses kinase activity through competitive binding to the ATP-binding site, but resistance to it develops rather quickly. Tang F. et al. discovered an interesting fact: sorafenib (which exhibits ferroptotic activity) increases the sensitivity of melanoma cells to vemurafenib. Treatment with sorafenib alone did not significantly affect iron content, glutathione, malondialdehyde, or ROS production in vemurafenib-resistant cells, but the combined use of sorafenib and vemurafenib significantly increased ROS production, malondialdehyde and iron content, while decreasing glutathione levels. Interestingly, sorafenib significantly enhanced vemurafenib-induced cell death, which was blocked by the LPO inhibitor ferrostatin-1 but not by necrosulfonamide [38]. A study by Turubanova V.D. et al. showed that PDT with Photosens, which localizes in lysosomes, induces a type of regulated cell death combining features of apoptosis and ferroptosis. This phenomenon is explained by the release of hydrolytic enzymes and ionic iron upon photodynamic damage to the lysosomal membrane [39].

In 2020, a joint international study by scientists from the Institute of Biology and Biomedicine of Lobachevsky State University of Nizhny Novgorod (Russia), Ghent University (Belgium), and the University of Bologna (Italy) under the leadership of Prof. D. Krysko established that ferroptosis has immunogenic properties. In the course of this work, it turned out that PDT with Photosens induces a mixed type of cell death in GL261 glioma cells, with features characteristic of both apoptotic and ferroptotic death; in addition, a pronounced immunogenic effect of the therapy was detected [40]. The results obtained allowed the team of authors to create a model dendritic cell vaccine based on glioma cells induced by Photosens-PDT [40].

In another study, during investigation of the efficacy of PDT based on 5-aminolevulinic acid (5-ALA) in SiHa cervical cancer cells, increased intracellular ROS generation was observed, accompanied by decreased GPX4 expression, which indicates ferroptosis induced by PDT. It was also established that the ferroptosis inducer sorafenib potentiates 5-ALA-based PDT in vitro.

The antitumor effect of the combination of ferroptosis and PDT was confirmed in vivo on cervical cancer xenografts [41].

Enhancement of ferroptosis during PDT with ferroptosis inducers

Currently, studies devoted to the combination of PDT and ferroptosis-stimulating therapy are actively emerging [42, 43], including through the creation of combined particles [44]. It is assumed that such bifunctional particles will be able to generate large amounts of ROS, which can effectively inhibit glutathione activity and thereby demonstrate high antitumor efficacy [45]. In these studies, Ce6 is most often used as the PS, along with porphyrin derivatives—protoporphyrin IX and 5,10,15,20-tetrakis(4-aminophenyl)porphyrin [46–48], among others. As for ferroptosis-stimulating therapy, most studies have focused on ferroptosis inducers, among which the most frequently used are erastin and its derivatives (piperazine erastin, imidazole ketone erastin (IKE)), sorafenib, sulfasalazine, RAS-selective lethal 3 (RSL3), and iron compounds [49] (Table 1). However, ferroptotic activity, expressed to varying degrees, has also been found in many other compounds, including metformin, curcumin, metamizole sodium, simvastatin, sunitinib, lapatinib, disulfiram, quercetin, and others [50].

The simplest way to combine PDT and ferroptosis induction is the simultaneous administration of iron compounds and a PS. For example, nanoparticles

with a PLGA framework loaded with Fe_3O_4 and Ce6 (Fe_3O_4 -PLGA-Ce6) were obtained [51]. The use of these combined particles in vitro in murine 4T1 breast cancer cells showed that when Fe_3O_4 -PLGA-Ce6 is added to cells and subsequently internalized, Ce6 and $\text{Fe}^{3+}/\text{Fe}^{2+}$ are released; ionic iron stimulates the Fenton reaction and induces ferroptosis through a decrease in glutathione and GPX4 levels and an increase in malondialdehyde levels. In addition, upon laser irradiation, the nanoparticles release free Ce6, which enhances ROS accumulation, potentiating the iron-mediated cell death pathway. In vivo experiments on the 4T1 tumor model demonstrated the biocompatibility, antitumor efficacy of the developed particles, and the presence of additional useful properties for MRI due to the magnetic properties of Fe_3O_4 .

In scientific practice, a combination of a PS with erastin—the “gold standard” in ferroptosis induction—is used. The synergy between PDT and ferroptosis is being actively studied by Chinese scientists, who discovered an interesting technical fact: Ce6 and erastin molecules are capable of self-assembling into a “nanodrug” through hydrogen bonds and π - π interactions. Compared with monotherapy, this “nanodrug” (Ce6-erastin) exhibited greater antitumor efficacy against tongue squamous cell carcinoma both in vitro and in vivo. Zhu T. et al. calculated the combination index for Ce6-erastin and mathematically proved the presence of a synergistic effect [52].

Таблица 1

Примеры совместного успешного применения ФС и индукторов ферроптоза в эксперименте

Table 1

Examples of the joint successful use of photosensitizers and inducers of ferroptosis in an experiment

Фотосенсибилизатор Photosensitizer	Индуктор ферроптоза Inducer of ferroptosis	Носитель Carrier	Источник Source
Хлорин е6 Chlorin e6	Эрастин Erastin	Без носителя Without a carrier	[52]
Бенгальский розовый Rose Bengal	Эрастин Erastin	Экзосомы с функционализацией поверхности CD47 (Exos ^{CD47}) Exosomes with CD47 surface functionalization	[53]
Талапорфин натрия	IKE или сорафениб	Без носителя Without a carrier	[17]
Хлорин е6 Chlorin e6	Fe_3O_4	PLGA-частицы PLGA particles	[51]
Хлорин е6 Chlorin e6	RSL3	Нейтрофильно-миметические гибридные липосомы Neutrophil-mimetic hybrid liposomes	[56]
Протопорфилин IX Protoporphyrin IX	Эрастин Erastin	Амфифильные полимерные наночастицы Amphiphilic polymer nanoparticles	[46]
5,10,15,20-тетракис(4-аминофенил)порфирин 5,10,15,20-tetrakis(4-aminophenyl) porphyrin	Fe^{3+}	Наноконкомплекс из ПЭГилированной полигалактуроновой кислоты Nanocomplex of PEGylated polygalacturonic acid	[48]

Some researchers seek to create platforms for delivering erastin and a PS to tumor tissues with high specificity. The use of exosomes with a CD47-functionalized surface loaded with erastin and the PS Rose Bengal allowed the resulting modified exosomes to effectively evade phagocytosis by the mononuclear phagocyte system and to induce ferroptosis in tumor cells after laser irradiation at 532 nm both in vitro and in vivo. Moreover, compared with control "empty" exosomes, the "loaded" exosomes exerted less toxic effect on the liver. The proposed exosomes may become a promising strategy for the treatment of various types of malignant tumors [53].

A study of the biochemical mechanism of PDT using the PS sodium talaporfin led to the conclusion that this therapy induces ferroptosis by inhibiting cystine uptake via system Xc⁻, and when combined with ferroptosis inducers such as IKE or RSL3, an enhancement of tumor cell death induction occurs. Combining PDT with sodium talaporfin and ferroptosis inducers (IKE or sorafenib) in vivo significantly reduced tumor volume in the experiment [17]. Sun X. et al. confirmed that sorafenib in combination with PDT exerts a synergistic antitumor effect with activation of immune mechanisms [54]. Another group of Chinese researchers demonstrated the efficacy of combining surufatinib (a novel multikinase inhibitor with ferroptotic activity) with PDT in the treatment of such an aggressive disease as cholangiocarcinoma [55].

PDT and ferroptosis therapy have become widespread in studies devoted to the treatment of breast cancer, because ROS produced by PDT can promote ferroptosis in tumor cells, and ferroptosis therapy is expected to eliminate tumor cells resistant to apoptosis. Considering that PDT is characterized by a high level of ROS production and that neutrophils have a natural tendency to generate ROS, a neutrophil-mimetic hybrid system for the co-delivery of the PS Ce6 and the ferroptosis inducer RSL3 was theoretically proposed and practically developed. This hybrid system suppressed tumor cell growth through the natural ability of neutrophils to induce LPO and the enhanced permeability and retention (EPR)

effect, and then induced both apoptosis and ferroptosis, which led to a significant increase in peroxide levels and ultimately to potentiation of the LPO cascade. Synergistic ferroptosis-PDT demonstrated significant therapeutic efficacy in mice bearing 4T1 tumors, which indicates the promise of this type of breast cancer treatment [48].

As we have already described, the use of exosomes as a combined delivery system is also applicable in ferro-PDT. There are studies on loading macrophage exosomes with the PS SR780, Fe³⁺, and the antioxidant enzyme catalase, with subsequent modification of the nanoplatform with RS17 peptides to improve tumor targeting and block the CD47 signaling regulatory protein checkpoint. Upon laser activation, the created nanoparticles demonstrated high antitumor efficacy both against a subcutaneous Lewis lung carcinoma xenograft model and against an orthotopic lung metastasis model and a liver metastasis model [57].

Conclusion

The combination of ferroptosis and PDT is a new but undoubtedly effective and promising strategy for cancer treatment. Currently, research in this area proceeds along three main directions. The first includes the study of mechanisms of ferroptosis potentiation by PDT. The second direction is associated with the search for effective combinations of PSs and ferroptosis inducers. The third and most high-tech direction is the creation of combined nanoplatforms and nanodelivery systems to increase selectivity and reduce toxicity. All these directions are interrelated and developing in parallel, which has allowed scientists to prove the efficacy of ferro-photodynamic therapy in experiments. The body of primary scientific and practical knowledge obtained will further contribute to preclinical and clinical studies using this innovative type of tumor therapy.

The scientific review was carried out within the framework of the state assignment on the topic "Preclinical development of a new drug—a ferroptosis inducer for the treatment of oncological diseases" 2026–2027.

The authors declare no conflict of interest.

REFERENCES

1. Li X., Lovell J.F., Yoon J., Chen X. Clinical development and potential of photothermal and photodynamic therapies for cancer. *Nat Rev Clin Oncol*, 2020, Vol. 17(11), pp. 657–674. doi:10.1038/s41571-020-0410-2
2. Ji B., Wei M., Yang B. Recent advances in nanomedicines for photodynamic therapy (PDT)-driven cancer immunotherapy. *Theranostics*, 2022, Vol. 12(1), pp. 434–458. doi: 10.7150/thno.67300
3. Sanarova E.V., Smirnova Z.S., Polozkova A.P., Ignatjeva E.V., Borisova L.M., Kiseleva M.P., Meerovich G.A., Meerovich I.G., Oborotova N.A. Biopharmaceutical investigations of new liposomal drug form of tirosens. *Russian Journal of Biopharmaceuticals*, 2011, Vol. 3(6), pp. 33–36.
4. Sanarova E., Meerovich I., Lantsova A., Kotova E., Shprakh Z., Polozkova A., Orlova O., Borisova L., Smirnova Z., Oborotova N.,

ЛИТЕРАТУРА

1. Li X., Lovell J.F., Yoon J., Chen X. Clinical development and potential of photothermal and photodynamic therapies for cancer // *Nat Rev Clin Oncol*. – 2020. – Vol. 17, No. 11. – P. 657–674. DOI: 10.1038/s41571-020-0410-2
2. Ji B., Wei M., Yang B. Recent advances in nanomedicines for photodynamic therapy (PDT)-driven cancer immunotherapy // *Theranostics*. – 2022. – Vol. 12, No. 1. – P. 434–458. DOI: 10.7150/thno.67300
3. Sanarova E.V., Smirnova Z.S., Polozkova A.P., Ignatjeva E.V., Borisova L.M., Kiseleva M.P., Meerovich G.A., Meerovich I.G., Oborotova N.A. Biopharmaceutical investigations of new liposomal drug form of tirosens // *Russian Journal of Biopharmaceuticals*. – 2011. – Vol. 3, No. 6. – P.33–36.
4. Sanarova E., Meerovich I., Lantsova A., Kotova E., Shprakh Z., Polozkova A., Orlova O., Borisova L., Smirnova Z., Oborotova N.,

- Baryshnikov A., Meerovich G., Lukyanets E. Thiosens liposomal dosage form technology development and photodynamic efficiency assessment. *Journal of Drug Delivery Science and Technology*, 2014, Vol. 24(4), pp. 315-319.
5. Sanarova E.V., Lantsova A.V., Nikolaeva L.L., Oborotova N.A., Litvinenko Ya.E., Solov'eva N.L. Creation of a Model of a Complex Delivery Nanosystem Containing a Tyrosine Kinase Inhibitor and a Photosensitizer. *Pharm Chem J*, 2023, Vol. 57(7), pp. 1075-1079. doi: 10.1007/s11094-023-02986-y
 6. Zhao L., Zhou X., Xie F., Zhang L., Yan H., Huang J., Zhang C., Zhou F., Chen J., Zhang L. Ferroptosis in cancer and cancer immunotherapy. *Cancer Commun (Lond)*, 2022, Vol. 42(2), pp. 88-116. doi: 10.1002/cac2.12250
 7. Hassannia B., Vandenabeele P., Vanden Berghe T. Targeting ferroptosis to iron out cancer. *Cancer Cell*, 2019, Vol. 35(6), pp. 830-849. doi: 10.1016/j.ccell.2019.04.002
 8. Jiang X., Stockwell B.R., Conrad M. Ferroptosis: mechanisms, biology and role in disease. *Nat Rev Mol Cell Biol*, 2021, Vol. 22(4), pp. 266-282. doi: 10.1038/s41580-020-00324-8
 9. Wang Y., Xu Y., Qu Y., Jin Y., Cao J., Zhan J., Li Z., Chai C., Huang C., Li M. Ferroptosis: a novel cell death modality as a synergistic therapeutic strategy with photodynamic therapy. *Photodiagnosis Photodyn Ther*, 2025, Vol. 51, pp. 104463. doi: 10.1016/j.pdpdt.2024.104463
 10. Chang Q., Wang P., Zeng Q., Wang X. A review on ferroptosis and photodynamic therapy synergism: Enhancing anticancer treatment. *Heliyon*, 2024, Vol. 10(7), pp. e28942. doi: 10.1016/j.heliyon.2024.e28942
 11. Zhou Q., Meng Y., Li D., Yao L., Le J., Liu Y., Sun Y., Zeng F., Chen X., Deng G. Ferroptosis in cancer: From molecular mechanisms to therapeutic strategies. *Signal Transduct Target Ther*, 2024, Vol. 9(1), pp. 55. doi: 10.1038/s41392-024-01769-5
 12. Sanarova E.V., Lantsova A.V., Nikolaeva L.L., Oborotova N.A., Borisova L.M. Ferroptosis inducers – erastin and analogues (review). *Drug development & registration*, 2024, Vol. 13(2), pp. 77-83. doi: 10.33380/2305-2066-2024-13-2-1786
 13. Sanarova E.V., Lantsova A.V., Nikolaeva L.L., Osipov V.N., Gusev D.V., Borisova L.M. Solubilization of 3-hydroxyquinazoline derivative with antitumor activity. *Russian Journal of Biotherapy*, 2023, Vol. 22(4), pp. 60-67. doi: 10.17650/1726-9784-2023-22-4-60-67
 14. Li Y., Liu M., Zheng Y., Wang Z., Zhao Y. Coenzyme-depleting nanocarriers for enhanced redox cancer therapy under hypoxia. *J Colloid Interface Sci*, 2023, Vol. 641, pp. 135-145. doi: 10.1016/j.jcis.2023.03.057
 15. Mishchenko T., Balalaeva I., Gorokhova A., Vedunova M., Krysko D.V. Which cell death modality wins the contest for photodynamic therapy of cancer? *Cell Death Dis*, 2022, Vol. 13(5), pp. 455. doi: 10.1038/s41419-022-04851-4
 16. Turubanova V.D., Balalaeva I.V., Mishchenko T.A., Catanzaro E., Alzeibak R., Peskova N.N., Efimova I., Bachert C., Mitroshina E.V., Krysko O., Vedunova M.V., Krysko D.V. Immunogenic cell death induced by a new photodynamic therapy based on photosens and photodithazine. *J Immunother Cancer*, 2019, Vol. 7(1), pp. 350. doi: 10.1186/s40425-019-0826-3
 17. Kojima Y., Tanaka M., Sasaki M., Ozeki K., Shimura T., Kubota E., Kataoka H. Induction of ferroptosis by photodynamic therapy and enhancement of antitumor effect with ferroptosis inducers. *J Gastroenterol*, 2024, Vol. 59(2), pp. 81-94. doi: 10.1007/s00535-023-02054-y
 18. Chen Z., Wang Y., Li Z., Chen M., Li Y., Lu C., Lin Z., Zheng H., Chen L., Zhang Q. Improving ferroptosis-mediated immunotherapy for colorectal cancer through lysosome-targeted photodynamic therapy. *Mater Today Bio*, 2025, Vol. 31, pp. 101552. doi: 10.1016/j.mtbio.2025.101552
 19. Meng X., Deng J., Liu F., Guo T., Liu M., Dai P., Fan A., Wang Z., Zhao Y. Triggered All-Active Metal Organic Framework: Ferroptosis Machinery Contributes to the Apoptotic Photodynamic Antitumor Therapy. *Nano Lett*, 2019, Vol. 19(11), pp. 7866-7876. doi: 10.1021/acs.nanolett.9b02904
 20. Yi M., Xiong B., Li Y., Guo W., Huang Y., Lu B. Manipulate tumor hypoxia for improved photodynamic therapy using nanomaterials. *Eur J Med Chem*, 2023, Vol. 247, pp. 115084. doi: 10.1016/j.ejmech.2022.115084
 21. Moloudi K., Abrahamse H., George B.P. Nanotechnology-mediated photodynamic therapy: Focus on overcoming tumor hypoxia. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*, 2024, Vol. 16(1), pp. e1937. doi: 10.1002/wnan.1937
 22. Mishchenko T.A., Balalaeva I.V., Vedunova M.V., Krysko D.V. Ferroptosis and Photodynamic Therapy Synergism: Enhancing Anticancer Treatment. *Trends Cancer*, 2021, Vol. 7(6), pp. 484-487. doi: 10.1016/j.trecan.2021.01.013
 5. Sanarova E.V., Lantsova A.V., Nikolaeva L.L., Oborotova N.A., Litvinenko Ya.E., Solov'eva N.L. Creation of a Model of a Complex Delivery Nanosystem Containing a Tyrosine Kinase Inhibitor and a Photosensitizer. *Pharm Chem J*. – 2023. – Vol. 57, No. 7. – P.1075–1079. DOI: 10.1007/s11094-023-02986-y
 6. Zhao L., Zhou X., Xie F., Zhang L., Yan H., Huang J., Zhang C., Zhou F., Chen J., Zhang L. Ferroptosis in cancer and cancer immunotherapy. *Cancer Commun (Lond)*. – 2022. – Vol. 42, No. 2. – P.88-116. DOI: 10.1002/cac2.12250
 7. Hassannia B., Vandenabeele P., Vanden Berghe T. Targeting ferroptosis to iron out cancer. *Cancer Cell*. – 2019. – Vol. 35, No. 6. – P.830-849. DOI: 10.1016/j.ccell.2019.04.002
 8. Jiang X., Stockwell B.R., Conrad M. Ferroptosis: mechanisms, biology and role in disease. *Nat Rev Mol Cell Biol*. – 2021. – Vol. 22, No. 4. – P.266-282. DOI: 10.1038/s41580-020-00324-8
 9. Wang Y., Xu Y., Qu Y., Jin Y., Cao J., Zhan J., Li Z., Chai C., Huang C., Li M. Ferroptosis: a novel cell death modality as a synergistic therapeutic strategy with photodynamic therapy. *Photodiagnosis Photodyn Ther*. – 2025. – Vol. 51. – P. 104463. DOI: 10.1016/j.pdpdt.2024.104463
 10. Chang Q., Wang P., Zeng Q., Wang X. A review on ferroptosis and photodynamic therapy synergism: Enhancing anticancer treatment. *Heliyon*. – 2024. – Vol. 10, No. 7. – P. e28942. DOI: 10.1016/j.heliyon.2024.e28942
 11. Zhou Q., Meng Y., Li D., Yao L., Le J., Liu Y., Sun Y., Zeng F., Chen X., Deng G. Ferroptosis in cancer: From molecular mechanisms to therapeutic strategies. *Signal Transduct Target Ther*. – 2024. – Vol. 9, No. 1. – P. 55. DOI: 10.1038/s41392-024-01769-5
 12. Sanarova E.V., Lantsova A.V., Nikolaeva L.L., Oborotova N.A., Borisova L.M. Ferroptosis inducers – erastin and analogues (review). *Drug development & registration*. – 2024. – Vol. 13, No. 2. – P. 77-83. DOI: 10.33380/2305-2066-2024-13-2-1786
 13. Sanarova E.V., Lantsova A.V., Nikolaeva L.L., Osipov V.N., Gusev D.V., Borisova L.M. Solubilization of 3-hydroxyquinazoline derivative with antitumor activity. *Russian Journal of Biotherapy*. – 2023. – Vol. 22, No. 4. – P. 60-67. DOI: 10.17650/1726-9784-2023-22-4-60-67
 14. Li Y., Liu M., Zheng Y., Wang Z., Zhao Y. Coenzyme-depleting nanocarriers for enhanced redox cancer therapy under hypoxia. *J Colloid Interface Sci*. – 2023. – Vol. 641. – P. 135-145. DOI: 10.1016/j.jcis.2023.03.057
 15. Mishchenko T., Balalaeva I., Gorokhova A., Vedunova M., Krysko D.V. Which cell death modality wins the contest for photodynamic therapy of cancer? *Cell Death Dis*. – 2022. – Vol. 13, No. 5. – P. 455. DOI: 10.1038/s41419-022-04851-4
 16. Turubanova V.D., Balalaeva I.V., Mishchenko T.A., Catanzaro E., Alzeibak R., Peskova N.N., Efimova I., Bachert C., Mitroshina E.V., Krysko O., Vedunova M.V., Krysko D.V. Immunogenic cell death induced by a new photodynamic therapy based on photosens and photodithazine. *J Immunother Cancer*. – 2019. – Vol. 7, No. 1. – P. 350. DOI: 10.1186/s40425-019-0826-3
 17. Kojima Y., Tanaka M., Sasaki M., Ozeki K., Shimura T., Kubota E., Kataoka H. Induction of ferroptosis by photodynamic therapy and enhancement of antitumor effect with ferroptosis inducers. *J Gastroenterol*. – 2024. – Vol. 59, No. 2. – P. 81-94. DOI: 10.1007/s00535-023-02054-y
 18. Chen Z., Wang Y., Li Z., Chen M., Li Y., Lu C., Lin Z., Zheng H., Chen L., Zhang Q. Improving ferroptosis-mediated immunotherapy for colorectal cancer through lysosome-targeted photodynamic therapy. *Mater Today Bio*. – 2025. – Vol. 31. – P. 101552. DOI: 10.1016/j.mtbio.2025.101552
 19. Meng X., Deng J., Liu F., Guo T., Liu M., Dai P., Fan A., Wang Z., Zhao Y. Triggered All-Active Metal Organic Framework: Ferroptosis Machinery Contributes to the Apoptotic Photodynamic Antitumor Therapy. *Nano Lett*. – 2019. – Vol. 19, No. 11. – P. 7866-7876. DOI: 10.1021/acs.nanolett.9b02904
 20. Yi M., Xiong B., Li Y., Guo W., Huang Y., Lu B. Manipulate tumor hypoxia for improved photodynamic therapy using nanomaterials. *Eur J Med Chem*. – 2023. – Vol. 247. – P. 115084. DOI: 10.1016/j.ejmech.2022.115084
 21. Moloudi K., Abrahamse H., George B.P. Nanotechnology-mediated photodynamic therapy: Focus on overcoming tumor hypoxia. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*. – 2024. – Vol. 16, No. 1. – P. e1937. DOI: 10.1002/wnan.1937
 22. Mishchenko T.A., Balalaeva I.V., Vedunova M.V., Krysko D.V. Ferroptosis and Photodynamic Therapy Synergism: Enhancing Anticancer Treatment. *Trends Cancer*. – 2021. – Vol. 7, No. 6. – P. 484-487. DOI: 10.1016/j.trecan.2021.01.013

23. Cheung E.C., Vousden K.H. The role of ROS in tumour development and progression. *Nat Rev Cancer*, 2022, Vol. 22(5), pp. 280-297. doi: 10.1038/s41568-021-00435-0
24. Cheung E.C., DeNicola G.M., Nixon C., Blyth K., Labuschagne C.F., Tuveson D.A., Vousden K.H. Dynamic ROS Control by TIGAR Regulates the Initiation and Progression of Pancreatic Cancer. *Cancer Cell*, 2020, Vol. 37(2), pp. 168-182.e4. doi: 10.1016/j.ccell.2019.12.012
25. Poprac P., Jomova K., Simunkova M., Kollar V., Rhodes C.J., Valko M. Targeting Free Radicals in Oxidative Stress-Related Human Diseases. *Trends Pharmacol Sci*, 2017, Vol. 38(7), pp. 592-607. doi: 10.1016/j.tips.2017.04.005
26. Rossi R., Dalle-Donne I., Milzani A., Giustarini D. Oxidized forms of glutathione in peripheral blood as biomarkers of oxidative stress. *Clin Chem*, 2006, Vol. 52(7), pp. 1406-1414. doi: 10.1373/clinchem.2006.067793
27. Cheng X., Xu H.D., Ran H.H., Liang G., Wu F.G. Glutathione-Depleting Nanomedicines for Synergistic Cancer Therapy. *ACS Nano*, 2021, Vol. 15(5), pp. 8039-8068. doi: 10.1021/acsnano.1c00498
28. Cui Q., Wang J.Q., Assaraf Y.G., Ren L., Gupta P., Wei L., Ashby C.R. Jr, Yang D.H., Chen Z.S. Modulating ROS to overcome multidrug resistance in cancer. *Drug Resist Updat*, 2018, Vol. 41, pp. 1-25. doi: 10.1016/j.drup.2018.11.001
29. Min H., Wang J., Qi Y., Zhang Y., Han X., Xu Y., Xu J., Li Y., Chen L., Cheng K., Liu G., Yang N., Li Y., Nie G. Biomimetic Metal-Organic Framework Nanoparticles for Cooperative Combination of Antiangiogenesis and Photodynamic Therapy for Enhanced Efficacy. *Adv Mater*, 2019, Vol. 31(15), pp. e1808200. doi: 10.1002/adma.201808200
30. Borisova L.M., Osipov V.N., Gusev D.V., Golubeva I.S., Kiseleva M.P., Vartanyan A.A. 3-Hydroxyquinazoline derivative, an analogue of erastin, induces ferroptosis in metastatic melanoma cells. *Russian Journal of Biotherapy*, 2021, Vol. 20(1), pp. 67-73. doi: 10.17650/1726-9784-2021-20-1-67-73
31. Borisova L.M., Osipov V.N., Golubeva I.S., Kiseleva M.P., Khochenkov D.A., Vartanyan A.A. 3-Hydroxyquinazoline derivatives, analogues of erastin, induced ferroptosis in breast cancer cells. *Advances in Molecular Oncology*, 2022, Vol. 9(1), pp. 48-56. doi: 10.17650/2313-805X-2022-9-1-48-56
32. Zhu L., Wang X., Tian T., Chen Y., Du W., Wei W., Zhao J., Guo Z., Wang X. A A-Ir(III)-phenylquinazolinone complex enhances ferroptosis by selectively inhibiting metallothionein-1. *Chem Sci*, 2024, Vol. 15(27), pp. 10499-10507. DOI: 10.1039/d4sc00422a
33. Shevchenko V.E., Nikiforova Z.N., Kushnir T.I., Kudryavtsev I.A., Mitrofanov A.A., Bekyashev A.Kh., Arnotskaya N.E.. Ferroptosis determinants – potential therapeutic targets glioblastoma stem cells. *Advances in Molecular Oncology*, 2022, Vol. 9(3), pp. 60-68. doi: 10.17650/2313-805X-2022-9-3-60-68
34. Ubellacker J.M., Tasdogan A., Ramesh V., Lymph protects metastasizing melanoma cells from ferroptosis. *Nature*, 2020, Vol. 585(7823), pp. 113-118. doi: 10.1038/s41586-020-2623-z
35. Basit F., van Oppen L.M., Schöckel L. Mitochondrial complex I inhibition triggers a mitophagy-dependent ROS increase leading to necroptosis and ferroptosis in melanoma cells. *Cell Death Dis*, 2017, Vol. 8(3), pp. e2716. doi: 10.1038/cddis.2017.133
36. Tsoi J., Robert L., Paraiso K., Galvan C., Sheu K.M., Lay J., Wong D.J.L., Atefi M., Shirazi R., Wang X., Braas D., Grasso C.S., Palaskas N., Ribas A., Graeber T.G.. Multi-stage Differentiation Defines Melanoma Subtypes with Differential Vulnerability to Drug-Induced Iron-Dependent Oxidative Stress. *Cancer Cell*, 2018, Vol. 33(5), pp. 890-904.e5. doi: 10.1016/j.ccell.2018.03.017
37. Guo W., Wu Z., Chen J., Guo S., You W., Wang S., Ma J., Wang H., Wang X., Wang H., Ma J., Yang Y., Tian Y., Shi Q., Gao T., Yi X., Li C. Nanoparticle delivery of miR-21-3p sensitizes melanoma to anti-PD-1 immunotherapy by promoting ferroptosis. *J Immunother Cancer*, 2022, Vol. 10(6), pp. e004381. doi: 10.1136/jitc-2021-004381
38. Tang F., Li S., Liu D., Chen J., Han C. Sorafenib sensitizes melanoma cells to vemurafenib through ferroptosis. *Transl Cancer Res*, 2020, Vol. 9(3), pp. 1584-1593. doi: 10.21037/tcr.2020.01.62
39. Turubanova V.D., Balalaeva I.V., Mishchenko T.A., Catanzaro E., Alzeibak R., Peskova N.N., Efimova I., Bachert C., Mitroshina E.V., Krysko O., Vedunova M.V., Krysko D.V. Immunogenic cell death induced by a new photodynamic therapy based on photosens and photodithazine. *J Immunother Cancer*, 2019, Vol. 7(1), pp. 350. doi: 10.1186/s40425-019-0826-3
40. Efimova I., Catanzaro E., Van der Meeren L., Turubanova V.D., Hammad H., Mishchenko T.A., Vedunova M.V., Fimognari C., Bachert C., Coppieters F., Lefever S., Skirtach A.G., Krysko O., Krysko D.V. Vaccination with early ferroptotic cancer cells induces
23. Cheung E.C., Vousden K.H. The role of ROS in tumour development and progression // *Nat Rev Cancer*. – 2022. – Vol. 22, No. 5. – P. 280-297. DOI: 10.1038/s41568-021-00435-0
24. Cheung E.C., DeNicola G.M., Nixon C., Blyth K., Labuschagne C.F., Tuveson D.A., Vousden K.H. Dynamic ROS Control by TIGAR Regulates the Initiation and Progression of Pancreatic Cancer // *Cancer Cell*. – 2020. – Vol. 37, No. 2. – P. 168-182.e4. DOI: 10.1016/j.ccell.2019.12.012
25. Poprac P., Jomova K., Simunkova M., Kollar V., Rhodes C.J., Valko M. Targeting Free Radicals in Oxidative Stress-Related Human Diseases // *Trends Pharmacol Sci*. – 2017. – Vol. 38, No. 7. – P. 592-607. DOI: 10.1016/j.tips.2017.04.005
26. Rossi R., Dalle-Donne I., Milzani A., Giustarini D. Oxidized forms of glutathione in peripheral blood as biomarkers of oxidative stress // *Clin Chem*. – 2006. – Vol. 52, No. 7. – P. 1406-1414. DOI: 10.1373/clinchem.2006.067793
27. Cheng X., Xu H.D., Ran H.H., Liang G., Wu F.G. Glutathione-Depleting Nanomedicines for Synergistic Cancer Therapy // *ACS Nano*. – 2021. – Vol. 15, No. 5. – P. 8039-8068. DOI: 10.1021/acsnano.1c00498
28. Cui Q., Wang J.Q., Assaraf Y.G., Ren L., Gupta P., Wei L., Ashby C.R. Jr, Yang D.H., Chen Z.S. Modulating ROS to overcome multidrug resistance in cancer // *Drug Resist Updat*. – 2018. – Vol. 41. – P. 1-25. DOI: 10.1016/j.drup.2018.11.001
29. Min H., Wang J., Qi Y., Zhang Y., Han X., Xu Y., Xu J., Li Y., Chen L., Cheng K., Liu G., Yang N., Li Y., Nie G. Biomimetic Metal-Organic Framework Nanoparticles for Cooperative Combination of Antiangiogenesis and Photodynamic Therapy for Enhanced Efficacy // *Adv Mater*. – 2019. – Vol. 31, No. 15. – P. e1808200. DOI: 10.1002/adma.201808200
30. Borisova L.M., Osipov V.N., Gusev D.V., Golubeva I.S., Kiseleva M.P., Vartanyan A.A. 3-Hydroxyquinazoline derivative, an analogue of erastin, induces ferroptosis in metastatic melanoma cells // *Russian Journal of Biotherapy*. – 2021. – Vol. 20, No. 1. – P. 67-73. DOI: 10.17650/1726-9784-2021-20-1-67-73
31. Borisova L.M., Osipov V.N., Golubeva I.S., Kiseleva M.P., Khochenkov D.A., Vartanyan A.A. 3-Hydroxyquinazoline derivatives, analogues of erastin, induced ferroptosis in breast cancer cells // *Advances in Molecular Oncology*. – 2022. – Vol. 9, No. 1. – P. 48-56. DOI: 10.17650/2313-805X-2022-9-1-48-56
32. Zhu L., Wang X., Tian T., Chen Y., Du W., Wei W., Zhao J., Guo Z., Wang X. A A-Ir(III)-phenylquinazolinone complex enhances ferroptosis by selectively inhibiting metallothionein-1 // *Chem Sci*. – 2024. – Vol. 15, No. 27. – P. 10499-10507. DOI: 10.1039/d4sc00422a
33. Shevchenko V.E., Nikiforova Z.N., Kushnir T.I., Kudryavtsev I.A., Mitrofanov A.A., Bekyashev A.Kh., Arnotskaya N.E.. Ferroptosis determinants – potential therapeutic targets glioblastoma stem cells // *Advances in Molecular Oncology*. – 2022. – Vol. 9, No. 3. – P. 60-68. DOI: 10.17650/2313-805X-2022-9-3-60-68
34. Ubellacker J.M., Tasdogan A., Ramesh V., Lymph protects metastasizing melanoma cells from ferroptosis // *Nature*. – 2020. – Vol. 585, No. 7823. – P. 113-118. DOI: 10.1038/s41586-020-2623-z
35. Basit F., van Oppen L.M., Schöckel L.. Mitochondrial complex I inhibition triggers a mitophagy-dependent ROS increase leading to necroptosis and ferroptosis in melanoma cells // *Cell Death Dis*. – 2017. – Vol. 8, No. 3. – P. e2716. DOI: 10.1038/cddis.2017.133
36. Tsoi J., Robert L., Paraiso K., Galvan C., Sheu K.M., Lay J., Wong D.J.L., Atefi M., Shirazi R., Wang X., Braas D., Grasso C.S., Palaskas N., Ribas A., Graeber T.G.. Multi-stage Differentiation Defines Melanoma Subtypes with Differential Vulnerability to Drug-Induced Iron-Dependent Oxidative Stress // *Cancer Cell*. – 2018. – Vol. 33, No. 5. – P. 890-904.e5. DOI: 10.1016/j.ccell.2018.03.017
37. Guo W., Wu Z., Chen J., Guo S., You W., Wang S., Ma J., Wang H., Wang X., Wang H., Ma J., Yang Y., Tian Y., Shi Q., Gao T., Yi X., Li C. Nanoparticle delivery of miR-21-3p sensitizes melanoma to anti-PD-1 immunotherapy by promoting ferroptosis // *J Immunother Cancer*. – 2022. – Vol. 10, No. 6. – P. e004381. DOI: 10.1136/jitc-2021-004381
38. Tang F., Li S., Liu D., Chen J., Han C. Sorafenib sensitizes melanoma cells to vemurafenib through ferroptosis // *Transl Cancer Res*. – 2020. – Vol. 9, No. 3. – P. 1584-1593. DOI: 10.21037/tcr.2020.01.62
39. Turubanova V.D., Balalaeva I.V., Mishchenko T.A., Catanzaro E., Alzeibak R., Peskova N.N., Efimova I., Bachert C., Mitroshina E.V., Krysko O., Vedunova M.V., Krysko D.V. Immunogenic cell death induced by a new photodynamic therapy based on photosens and photodithazine // *J Immunother Cancer*. – 2019. – Vol. 7, No. 1. – P. 350. DOI: 10.1186/s40425-019-0826-3
40. Efimova I., Catanzaro E., Van der Meeren L., Turubanova V.D., Hammad H., Mishchenko T.A., Vedunova M.V., Fimognari C., Bachert C., Coppieters F., Lefever S., Skirtach A.G., Krysko O., Krysko D.V. Vaccination with early ferroptotic cancer cells induces

- efficient antitumor immunity. *J Immunother Cancer*, 2020, Vol. 8(2), pp. e001369. doi: 10.1136/jitc-2020-001369
41. Liu Q., Han Q., Shi J., Wu Z., Ma L., Li Y., He H., Guo H. Ferroptosis promotes 5-aminolevulinic acid-based photodynamic therapy in cervical cancer. *Photodiagnosis Photodyn Ther*, 2025, Vol. 54, pp. 104726. doi: 10.1016/j.pdpdt.2025.104726
42. Chen J., Duan Z., Deng L., Li L., Li Q., Qu J., Li X., Liu R. Cell Membrane-Targeting Type I/II Photodynamic Therapy Combination with FSP1 Inhibition for Ferroptosis-Enhanced Photodynamic Immunotherapy. *Adv Healthc Mater*, 2024, Vol. 13(16), pp. e2304436. doi: 10.1002/adhm.202304436
43. Zou P., Lin R., Fang Z., Chen J., Guan H., Yin J., Chang Z., Xing L., Lang J., Xue X., Chen M. Implanted, Wireless, Self-Powered Photodynamic Therapeutic Tablet Synergizes with Ferroptosis Inducer for Effective Cancer Treatment. *Adv Sci (Weinh)*, 2023, Vol. 10(36), pp. e2302731. doi: 10.1002/advs.202302731
44. Xu Y., Yang L., Wang C., Sun W., Zheng Y., Ou B., Wu L., Shi L., Lin X., Chen W. Ferroptosis boosted oral cancer photodynamic therapy by carrier-free Sorafenib-Ce6 self-assembly nanoparticles. *J Control Release*, 2024, Vol. 366, pp. 798-811. doi: 10.1016/j.jconrel.2023.12.056
45. Zeping G., Shunzhe Z., Ken-Ichiro K., Chutong T. Recent progress in cancer therapy based on the combination of ferroptosis with photodynamic therapy. *Acta Materia Medica*, 2022, Vol. 1(4), pp. 411-426. doi: 10.15212/AMM-2022-0025
46. Liu Z.Y., Chen G., Wang X., Synergistic Photochemo Effects Based on Light-Activatable Dual Prodrug Nanoparticles for Effective Cancer Therapy. *Adv Healthc Mater*, 2023, Vol. 12(27), pp. e2301133. doi: 10.1002/adhm.202301133
47. Dong S., An S., Liu Q., Wang X., Hu Y., Jiang A. Study on the Synergistic Mechanism of Photodynamic Therapy Combined With Ferroptosis Inducer to Induce Ferroptosis in Cholangiocarcinoma. *Lasers Surg Med*, 2024, Vol. 56(10), pp. 845-853. doi: 10.1002/lsm.23857
48. Li J., Li J., Pu Y., Li S., Gao W., He B. PDT-Enhanced Ferroptosis by a Polymer Nanoparticle with pH-Activated Singlet Oxygen Generation and Superb Biocompatibility for Cancer Therapy. *Biomacromolecules*, 2021, Vol. 22(3), pp. 1167-1176. doi: 10.1021/acs.biomac.0c01679
49. Shi Z., Zheng J., Tang W., Multifunctional Nanomaterials for Ferroptotic Cancer Therapy. *Front Chem*, 2022, Vol. 10, pp. 868630. doi: 10.3389/fchem.2022.868630
50. Luo Y., Bai X.Y., Zhang L., Hu Q.Q., Zhang N., Cheng J.Z., Hou M.Z., Liu X.L. Ferroptosis in Cancer Therapy: Mechanisms, Small Molecule Inducers, and Novel Approaches. *Drug Des Devel Ther*, 2024, Vol. 18, pp. 2485-2529. doi: 10.2147/DDDT.S472178
51. Chen Q., Ma X., Xie L., Chen W., Xu Z., Song E., Zhu X., Song Y. Iron-based nanoparticles for MR imaging-guided ferroptosis in combination with photodynamic therapy to enhance cancer treatment. *Nanoscale*, 2021, Vol. 13(9), pp. 4855-4870. doi: 10.1039/d0nr08757b
52. Zhu T., Shi L., Yu C., Dong Y., Qiu F., Shen L., Qian Q., Zhou G., Zhu X. Ferroptosis Promotes Photodynamic Therapy: Supramolecular Photosensitizer-Inducer Nanodrug for Enhanced Cancer Treatment. *Theranostics*, 2019, Vol. 9(11), pp. 3293-3307. doi: 10.7150/thno.32867
53. Du J., Wan Z., Wang C., Lu F., Wei M., Wang D., Hao Q. Designer exosomes for targeted and efficient ferroptosis induction in cancer via chemo-photodynamic therapy. *Theranostics*, 2021, Vol. 11(17), pp. 8185-8196. doi: 10.7150/thno.59121
54. Sun X., Cao Z., Mao K., Wu C., Chen H., Wang J., Wang X., Cong X., Li Y., Meng X., Yang X., Yang Y.G., Sun T. Photodynamic therapy produces enhanced efficacy of antitumor immunotherapy by simultaneously inducing intratumoral release of sorafenib. *Biomaterials*, 2020, Vol. 240, pp. 119845. doi: 10.1016/j.biomaterials.2020.119845
55. Huang Y.P., Wang Y.X., Zhou H., Liu Z.T., Zhang Z.J., Xiong L., Zou H., Wen Y. Surufatinib combined with photodynamic therapy induces ferroptosis to inhibit cholangiocarcinoma *in vitro* and in tumor models. *Front Pharmacol*, 2024, Vol. 15, pp. 1288255. doi: 10.3389/fphar.2024.1288255
56. Li J., Li J., He J., He X., Chen D., Dong Z., Xiong L., Bai W., Li M., He Q. Neutrophil-mimetic hybrid liposome with ROS cascade amplification for synergistic ferroptosis-photodynamic therapy of breast cancer. *Nano Res*, 2024, Vol. 17(11), pp. 9932-9941. doi: 10.1007/s12274-024-6917-6
57. Guo Y., Qian R., Wei X., pH-Activated Nanoplatfrom Derived from M1 Macrophages' Exosomes for Photodynamic and Ferroptosis Synergistic Therapy to Augment Cancer Immunotherapy. *Biomater Res*, 2025, Vol. 29, pp. 0153. doi: 10.34133/bmr.0153
- efficient antitumor immunity // *J Immunother Cancer*. – 2020. – Vol. 8, No. 2. – P. e001369. DOI: 10.1136/jitc-2020-001369
41. Liu Q., Han Q., Shi J., Wu Z., Ma L., Li Y., He H., Guo H. Ferroptosis promotes 5-aminolevulinic acid-based photodynamic therapy in cervical cancer // *Photodiagnosis Photodyn Ther*. – 2025. – Vol. 54. – P. 104726. DOI: 10.1016/j.pdpdt.2025.104726
42. Chen J., Duan Z., Deng L., Li L., Li Q., Qu J., Li X., Liu R. Cell Membrane-Targeting Type I/II Photodynamic Therapy Combination with FSP1 Inhibition for Ferroptosis-Enhanced Photodynamic Immunotherapy // *Adv Healthc Mater*. – 2024. – Vol. 13, No. 16. – P. e2304436. DOI: 10.1002/adhm.202304436
43. Zou P., Lin R., Fang Z., Chen J., Guan H., Yin J., Chang Z., Xing L., Lang J., Xue X., Chen M. Implanted, Wireless, Self-Powered Photodynamic Therapeutic Tablet Synergizes with Ferroptosis Inducer for Effective Cancer Treatment // *Adv Sci (Weinh)*. – 2023. – Vol. 10, No. 36. – P. e2302731. DOI: 10.1002/advs.202302731
44. Xu Y., Yang L., Wang C., Sun W., Zheng Y., Ou B., Wu L., Shi L., Lin X., Chen W. Ferroptosis boosted oral cancer photodynamic therapy by carrier-free Sorafenib-Ce6 self-assembly nanoparticles // *J Control Release*. – 2024. – Vol. 366. – P. 798-811. DOI: 10.1016/j.jconrel.2023.12.056
45. Zeping G., Shunzhe Z., Ken-Ichiro K., Chutong T. Recent progress in cancer therapy based on the combination of ferroptosis with photodynamic therapy // *Acta Materia Medica*. – 2022. – Vol. 1, No. 4. – P. 411-426. DOI: 10.15212/AMM-2022-0025
46. Liu Z.Y., Chen G., Wang X., Synergistic Photochemo Effects Based on Light-Activatable Dual Prodrug Nanoparticles for Effective Cancer Therapy // *Adv Healthc Mater*. – 2023. – Vol. 12, No. 27. – P. e2301133. DOI: 10.1002/adhm.202301133
47. Dong S., An S., Liu Q., Wang X., Hu Y., Jiang A. Study on the Synergistic Mechanism of Photodynamic Therapy Combined With Ferroptosis Inducer to Induce Ferroptosis in Cholangiocarcinoma // *Lasers Surg Med*. – 2024. – Vol. 56, No. 10. – P. 845-853. DOI: 10.1002/lsm.23857
48. Li J., Li J., Pu Y., Li S., Gao W., He B. PDT-Enhanced Ferroptosis by a Polymer Nanoparticle with pH-Activated Singlet Oxygen Generation and Superb Biocompatibility for Cancer Therapy // *Biomacromolecules*. – 2021. – Vol. 22, No. 3. – P. 1167-1176. DOI: 10.1021/acs.biomac.0c01679
49. Shi Z., Zheng J., Tang W., Multifunctional Nanomaterials for Ferroptotic Cancer Therapy // *Front Chem*. – 2022. – Vol. 10. – P. 868630. DOI: 10.3389/fchem.2022.868630
50. Luo Y., Bai X.Y., Zhang L., Hu Q.Q., Zhang N., Cheng J.Z., Hou M.Z., Liu X.L. Ferroptosis in Cancer Therapy: Mechanisms, Small Molecule Inducers, and Novel Approaches // *Drug Des Devel Ther*. – 2024. – Vol. 18. – P. 2485-2529. DOI: 10.2147/DDDT.S472178
51. Chen Q., Ma X., Xie L., Chen W., Xu Z., Song E., Zhu X., Song Y. Iron-based nanoparticles for MR imaging-guided ferroptosis in combination with photodynamic therapy to enhance cancer treatment // *Nanoscale*. – 2021 / - Vol. 13, No. 9. – P. 4855-4870. DOI: 10.1039/d0nr08757b
52. Zhu T., Shi L., Yu C., Dong Y., Qiu F., Shen L., Qian Q., Zhou G., Zhu X. Ferroptosis Promotes Photodynamic Therapy: Supramolecular Photosensitizer-Inducer Nanodrug for Enhanced Cancer Treatment // *Theranostics*. – 2019. – Vol. 9, No. 11. – P. 3293-3307. DOI: 10.7150/thno.32867
53. Du J., Wan Z., Wang C., Lu F., Wei M., Wang D., Hao Q. Designer exosomes for targeted and efficient ferroptosis induction in cancer via chemo-photodynamic therapy // *Theranostics*. – 2021. – Vol. 11, No. 17. – P. 8185-8196. DOI: 10.7150/thno.59121
54. Sun X., Cao Z., Mao K., Wu C., Chen H., Wang J., Wang X., Cong X., Li Y., Meng X., Yang X., Yang Y.G., Sun T. Photodynamic therapy produces enhanced efficacy of antitumor immunotherapy by simultaneously inducing intratumoral release of sorafenib // *Biomaterials*. – 2020. – Vol. 240. – P. 119845. DOI: 10.1016/j.biomaterials.2020.119845
55. Huang Y.P., Wang Y.X., Zhou H., Liu Z.T., Zhang Z.J., Xiong L., Zou H., Wen Y. Surufatinib combined with photodynamic therapy induces ferroptosis to inhibit cholangiocarcinoma *in vitro* and in tumor models // *Front Pharmacol*. – 2024. – Vol. 15. – P. 1288255. DOI: 10.3389/fphar.2024.1288255
56. Li J., Li J., He J., He X., Chen D., Dong Z., Xiong L., Bai W., Li M., He Q. Neutrophil-mimetic hybrid liposome with ROS cascade amplification for synergistic ferroptosis-photodynamic therapy of breast cancer // *Nano Res*. – 2024. – Vol. 17, No. 11. – P. 9932-9941. DOI: 10.1007/s12274-024-6917-6
57. Guo Y., Qian R., Wei X., pH-Activated Nanoplatfrom Derived from M1 Macrophages' Exosomes for Photodynamic and Ferroptosis Synergistic Therapy to Augment Cancer Immunotherapy // *Biomater Res*. – 2025. – Vol. 29. – P. 0153. DOI: 10.34133/bmr.0153

POTENTIAL OF ENDOGENOUS PHOTSENSITIZERS IN PHOTODYNAMIC THERAPY OF MYCOBACTERIAL INFECTIONS

Shleeva M.O., Bagaeva D.I., Kasatkina S.I., Kaprelyants A.S., Savitsky A.P.

A.N. Bach Institute of Biochemistry, Federal Research Centre 'Fundamentals of Biotechnology' of the Russian Academy of Sciences, Moscow, Russia

Abstract

This review examines current approaches to antibacterial photodynamic therapy (aPDT) based on the use of endogenous photosensitizers (PS) – tetrapyrrole compounds that accumulate in bacterial cells upon transition to a dormant state or upon addition of the tetrapyrrole synthesis precursor (5-aminolevulinic acid) to the growth medium. The relevance of the problem is driven by the increasing drug resistance of *Mycobacterium tuberculosis* and the pathogen's ability to persist within the host, developing phenotypic tolerance to antibiotics. Particular attention is paid to the mechanisms of porphyrin accumulation and their methylated derivatives (including tetramethyl ester of coproporphyrin and Zn-porphyrins) in dormant mycobacterial cells. It is demonstrated that these compounds not only serve as markers of the dormant state but also act as effective endogenous PS, enabling selective pathogen inactivation upon irradiation with light of appropriate wavelengths. The review systematizes data on enzymatic targets of tetrapyrrole metabolism (siroheme synthase CysG, ferrochelatase HemH, coproporphyrinogen III oxidase HemN, and coproporphyrin methyltransferase CPmtA), whose activity modulation allows induction of fluorescent intermediate accumulation and enhancement of bacterial photosensitivity. Experimental results on photodynamic inactivation of both actively growing and persisting forms of mycobacteria *in vitro*, as well as inside infected macrophages, are discussed. The prospects of using endogenous PS to combat drug-resistant forms of pathogens, the potential for combining aPDT with existing anti-tuberculosis regimens, and the need for further investigation of porphyrin accumulation in *in vivo* models are considered. The review highlights the potential of aPDT as an alternative strategy aimed at eradicating persisting forms of mycobacteria, which is particularly important in the context of the global threat of multidrug-resistant tuberculosis.

Key words: mycobacterium, tuberculosis, mycobacteriosis, antibacterial photodynamic therapy (aPDT), porphyrin, flavin, photosensitizer.

Contacts: Shleeva M.O., e-mail: mshleeva@inbi.ras.ru

For citations: Shleeva M.O., Bagaeva D.I., Kasatkina S.I., Kaprelyants A.S., Savitsky A.P. Potential of endogenous photosensitizers in photodynamic therapy of mycobacterial infections, *Biomedical Photonics*, 2026, vol. 15, no. 3, pp. 39–56. doi: 10.24931/2413-9432-2026-15-3-39-56

ПОТЕНЦИАЛ ЭНДОГЕННЫХ ФОТОСЕНСИБИЛИЗАТОРОВ В АНТИБАКТЕРИАЛЬНОЙ ФОТОДИНАМИЧЕСКОЙ ТЕРАПИИ МИКОБАКТЕРИАЛЬНЫХ ИНФЕКЦИЙ

М.О. Шлеева, Д.И. Багаева, С.И. Касаткина, А.С. Капрельянц, А.П. Савицкий

Институт биохимии им. А.Н. Баха, Федеральный исследовательский центр «Фундаментальные основы биотехнологии» Российской академии наук, Москва, Россия

Резюме

В обзоре рассматриваются современные подходы к антибактериальной фотодинамической терапии (аФДТ), основанные на использовании эндогенных фотосенсибилизаторов (ФС) – тетрапиррольных соединений, накапливающихся в бактериальных клетках при переходе в покое состояние или при добавлении в среду выращивания предшественника синтеза тетрапирролов (5-аминолевулиновая кислота). Актуальность проблемы обусловлена ростом лекарственной устойчивости *Mycobacterium tuberculosis* и способностью патогена персистировать в организме хозяина, формируя фенотипическую толерантность к антибиотикам, что диктует необходимость альтернативных методов лечения. Особое внимание уделено механизмам накопления порфиринов и их метилированных производных (включая тетраметилловый эфир копропорфирина и Zn-порфирины) в различных моделях покоящихся клеток микобактерий. Экспериментально доказано, что эти соединения не только являются маркерами покоящегося состояния, но и служат эффективными эндогенными ФС, обеспечивающими селективную инактивацию патогена при облучении светом соответствующих длин волн. В работе систематизированы данные о ферментативных мишенях тетрапиррольного метаболизма

(сирогемсинтаза CysG, феррохелатаза HemH, копропорфириноген III оксидаза HemN, копропорфин метилтрансфераза CPmtA), модуляция активности которых позволяет индуцировать накопление флуоресцирующих интермедиатов и повышать фоточувствительность бактерий. Обсуждаются результаты экспериментальных исследований по фотодинамической инаktivации как активно растущих, так и персистирующих форм микобактерий *in vitro*, а также внутри инфицированных макрофагов. Рассмотрены перспективы использования эндогенных ФС для борьбы с лекарственно-устойчивыми формами патогенов, возможности комбинирования аФДТ с существующими противотуберкулёзными режимами лечения и необходимость дальнейшего изучения накопления порфиринов *in vivo*. Обзор подчёркивает потенциал аФДТ как альтернативной стратегии, направленной на эрадикацию персистирующих форм микобактерий, что особенно важно в условиях глобальной угрозы туберкулёза с множественной лекарственной устойчивостью.

Ключевые слова: микобактерия, туберкулез, микобактериоз, антибактериальная фотодинамическая терапия (аФДТ), порфирин, флаavin, фотосенсибилизатор.

Контакты: Шлеева М.О., e-mail: mshleeva@inbi.ras.ru

Для цитирования: Шлеева М.О., Багаева Д.И., Касаткина С.И., Капрельянц А.С., Савицкий А.П. Потенциал эндогенных фотосенсибилизаторов в фотодинамической терапии микобактериальных инфекций // Biomedical Photonics. – 2026. – Т. 15, № 3. – С. 39–56. doi: 10.24931/2413–9432–2026–15–3–39–56

Introduction

Tuberculosis remains one of the leading causes of mortality from infectious diseases worldwide. According to the World Health Organization, a record number of tuberculosis cases was registered in 2023, which once again placed it first among causes of mortality from infectious diseases, surpassing COVID-19 indicators [1]. Of particular concern is the increase in the number of cases of multidrug-resistant and extensively drug-resistant *Mycobacterium tuberculosis*, the causative agent of tuberculosis. The situation is complicated by the ability of mycobacteria to transition into a dormant (resting) state [2,3], in which they become practically insensitive to the action of standard anti-tuberculosis drugs, leading to the need for prolonged (at least 6 months), toxic, and often ineffective therapy [4]. Latent tuberculosis is estimated to affect about a quarter of the world's population and represents a reservoir for infection reactivation, making the problem of eradicating persisting forms of the pathogen one of the most urgent tasks of modern phthisiology [5,6].

The difficulty of combating latent tuberculosis is due to the unique adaptive mechanisms of *M. tuberculosis*, which allow the bacteria to survive under stress conditions that occur when the pathogen enters macrophage cells, including hypoxia, acidification, oxidative and nitrosative stress, and nutrient deficiency [7,8]. An important consequence of these mechanisms is the acquisition of phenotypic tolerance to antibiotics—the ability to survive without multiplication in the presence of antimicrobial drugs without any genetic changes. This feature creates serious obstacles to treatment and requires the development of fundamentally new therapeutic strategies aimed specifically at eradicating persisting forms of the pathogen.

In recent years, considerable research interest as an alternative to antibiotics has been attracted by the potential use of antibacterial photodynamic therapy (aPDT), a method based on the generation of reactive oxygen species under the action of light, leading to nonspecific inactivation of the pathogen [9,10].

Unlike traditional antibiotics, whose action is directed at specific molecular targets, aPDT causes multiple damage to cellular structures (proteins, lipids, nucleic acids), which practically excludes the possibility of resistance development [11,12]. The implementation of this method requires the use of photosensitizers (PS), which can be exogenous (added to cell cultures or introduced into the host organism) or endogenous. The use of endogenous PS has an important advantage over exogenous ones: it does not require the separate administration of systemically acting drugs possessing varying degrees of toxicity (phototoxicity) and, accordingly, solving problems associated with their delivery, biodistribution, and potential impact on cells of the macroorganism. Furthermore, endogenous tetrapyrroles accumulate in bacterial cells in response to specific physiological signals (for example, the transition to a dormant state), which allows the natural metabolic processes of the pathogen to be used to induce its photosensitivity. Unlike exogenous PS, which require the development of targeted delivery strategies, endogenous sensitizers are synthesized directly in pathogen cells using the intracellular pool of precursors, which is particularly promising for the eradication of intracellular and persisting forms of pathogens.

Endogenous sensitizers for aPDT include fluorescent compounds such as tetrapyrrole compounds (porphyrins and their derivatives), as well as flavins and their derivatives, for example, riboflavin and flavin mononucleotide. In this regard, the phenomenon of tetrapyrrole compound accumulation in mycobacterial

cells under special physiological conditions associated with the formation of dormant bacterial forms is of particular importance [13,14].

This review summarizes current understanding of the mechanisms of endogenous tetrapyrrole accumulation in mycobacteria, discusses enzymatic targets for modulating their metabolism to induce photosensitivity, and analyzes the results of experimental studies on photodynamic inactivation (PDI) of both dormant and actively growing forms of mycobacteria and other bacteria *in vitro*. The prospects and limitations of using endogenous PS to combat drug-resistant and persisting forms of pathogens are considered, as well as the possibilities of combining aPDT with existing anti-tuberculosis treatment regimens.

Accumulation of endogenous PS (porphyrins) during the transition of mycobacteria to a dormant state

The main mechanism for increasing the concentration of tetrapyrroles inside the bacterial cell is associated with the transition of mycobacteria to a dormant state characterized by the absence of multiplication, low metabolic activity, and high resistance to antibacterial drugs. The ability of *Mycobacterium tuberculosis* to transition into a state of metabolic dormancy (dormant state) is a key mechanism ensuring prolonged persistence of infection in the host organism (latent tuberculosis) and is the main reason for the failures of modern anti-tuberculosis therapy [5]. The diversity of approaches to modeling this state *in vitro* and *in vivo* reflects the complexity of the dormancy phenomenon, and existing models can be divided into two large groups: *in vitro* models and *in vivo* models, each of which has its advantages and limitations [8].

Among *in vitro* models, the most well-known is the classical Wayne hypoxia model, based on the gradual depletion of oxygen in hermetically sealed flasks. When the concentration of dissolved oxygen decreases, mycobacterial cells (*M. tuberculosis* and *M. smegmatis*) sequentially pass through two stages of a non-replicating state (NRP-1 and NRP-2). A key feature is that while rapid oxygen depletion leads to cell death, a gradual decrease in its concentration induces a transition into a metabolically low-active, non-replicating, reversible state without loss of the ability of such cells to multiply when plated on solid media, accompanied by expression of the DosR regulon, accumulation of triacylglycerols, and acquisition of phenotypic resistance to isoniazid [5]. Other *in vitro* models include starvation of essential nutrients, in which bacteria in physiological solution in the absence of carbon and nitrogen sources stop replication and become highly resistant to antibiotics, as well as the

oxidative or nitrosative stress model with the addition of nitric oxide or reactive oxygen species (ROS), imitating the microbicidal mechanisms of macrophages [8]. The most physiological among *in vitro* systems is considered to be the Deba multifactorial stress model, which involves keeping cells under conditions of low oxygen concentration (5%), elevated CO₂ level (10%), acidic pH (5.0), and nutrient deficiency, leading to the formation of a heterogeneous population with three subpopulations: actively dividing, transitional, and truly dormant cells [8].

A special place among *in vitro* systems is occupied by the gradual acidification model, since a decrease in the pH of the macrophage phagosome is one of the key stress factors that mycobacteria encounter inside the host [7]. In contrast to traditional experiments with rapid acidification, a model was proposed in which *M. tuberculosis* is cultivated for a long time in the stationary phase, with the pH of the medium gradually decreasing from 8.5 to 4.7 solely due to the metabolic activity of the bacteria [15]. This results in the formation of morphologically distinct ovoid cells characterized by thickening of the cell wall, low metabolic activity, increased resistance to antibiotics and heating, and loss of the ability to form colonies on solid media, which is a common property of mycobacteria in the dormant state [15]. A key fact revealed in the study of this model was the dependence of reactivation of long-persisting cells on resuscitation-promoting factors (Rpf) of protein nature secreted by active bacterial cells. While at early stages of acidification, ovoid cells are capable of spontaneous recovery, cells stored for a long time require the addition of supernatant from active *M. tuberculosis* or exogenous recombinant Rpf for successful reactivation [15].

Modeling of the transition of mycobacteria to a dormant state *in vivo* is carried out primarily using the Cornell mouse model, where combined antibiotic therapy after the establishment of infection creates a state of latency, and after cessation of treatment, reactivation is observed, allowing the study of mechanisms of exit from the dormant state [16]. In chronic models in mice and guinea pigs, granulomas are formed with the preservation of persisting bacteria, and an important feature is the loss of acid-fastness in a significant portion of the population, which is associated with cell wall remodeling [17].

An alternative approach to modeling the dormant state *in vivo* involves experiments on infecting mice with dormant forms of *M. tuberculosis* obtained *in vitro* under conditions of prolonged acid stress. In these experiments, mice were intratracheally administered dormant mycobacteria characterized by loss of the ability to grow on standard solid media (non-culturability phenotype). A key result was the discovery of a direct

dependence of infection outcome on the depth of the bacterial dormant state. Dormant mycobacteria that retained the ability to spontaneously reactivate in liquid nutrient medium *in vitro* (early dormancy stage) were able to resume growth and multiplication after administration to mice [6].

In contrast, mycobacteria in a deep dormant state that required the addition of exogenous resuscitation-promoting factors Rpf for their reactivation *in vitro* completely lost the ability to multiply even in the organism of genetically susceptible mice. In this case, the bacteria did not cause active disease until the mice reached old age, remaining in tissues in a metabolically inactive, non-culturable state for many months. This picture reproduced the key features of latent tuberculosis in humans, where infection can remain subclinical for decades, and reactivation often occurs upon weakening of immunity, for example, against the background of aging, immunosuppressive therapy, or HIV infection [6].

The role of porphyrins in mycobacterial metabolism and their connection with the dormant state

The first indications of pigment accumulation in dormant mycobacterial cells were obtained in the work of A.F. Cunningham et al. [18]. In this study, the authors observed the appearance of pigmentation in old cultures of *M. tuberculosis* and *M. bovis* BCG maintained under anaerobic conditions inducing the transition of bacteria to a non-replicating state. However, the nature of the accumulating substance remained unclear; it was only suggested that this pigment does not belong to carotenoids. Subsequently, similar results were obtained when growing *M. smegmatis* under hypoxic conditions [19]. In this work, M. Berney and G.M. Cook also observed cell coloration, but the authors associated this phenomenon with an increased content of cytochromes without further identification of the pigment [19]. Thus, in both works, the nature of the fluorescent compounds accumulating during the transition of mycobacteria to the dormant state was not established, and only subsequent studies showed that these pigments apparently represent porphyrin derivatives [14,20]. Thus, later, in our studies it was found that the transition of *M. tuberculosis* and *M. smegmatis* to a dormant state under conditions of gradual acidification of the external environment is accompanied by the accumulation of a bright fluorescent pigment. Using spectroscopy and mass spectrometry, this pigment was identified as a mixture of coproporphyrin and uroporphyrin, as well as a coproporphyrin derivative unusual for biological systems—its tetramethyl ester or tetramethylcoproporphyrin (TMC) [14,20]. Unlike water-soluble free coproporphyrin, TMC is localized in the

hydrophobic structures of the mycobacterial cell wall, which indicates its association with lipid components of the membrane. Indeed, the incorporation of TMC into the lipid bilayer was confirmed experimentally, and it was shown that this compound is capable of changing the physical properties of the membrane, in particular reducing its fluidity [13]. Thus, it can be assumed that it was precisely tetrapyrrole compounds, primarily TMC and its derivatives, that were the pigments that were detected but not identified in the early works of A.F. Cunningham et al. [18] and M. Berney and G.M. Cook [19].

Porphyrins and their metal complexes are among the most fundamental biomolecules, performing diverse functions in cells—from electron and oxygen transfer to catalysis of redox reactions and protection from stress. In bacteria, these tetrapyrrole compounds serve as prosthetic groups of such vital proteins as respiratory chain cytochromes, catalases, peroxidases, and cytochrome P-450 [21]. Accordingly, heme biosynthesis is an essential process for most aerobic and facultatively anaerobic microorganisms.

Heme biosynthesis in most bacteria occurs via the classical pathway leading to the formation of protoporphyrin IX. However, in gram-positive bacteria of the phyla *Firmicutes* (with low GC (guanine-cytosine) content) and *Actinobacteria* (with high GC content), to which mycobacteria belong, an alternative, more ancient pathway has been discovered that does not use protoporphyrin IX as an intermediate [22]. This coproporphyrin-dependent pathway includes three key enzymes: HemY, HemH, and HemQ. In this pathway, coproporphyrinogen III is oxidized to coproporphyrin III, then iron is inserted into coproporphyrin III to form Fe-coproporphyrin (coproheme), which is then decarboxylated by the enzyme coproheme decarboxylase to form protoheme. Coproheme decarboxylase (ChdC, also known as HemQ) catalyzes the final step of this pathway, using hydrogen peroxide for the sequential oxidative decarboxylation of propionate side chains [22]. The *M. tuberculosis* genome contains genes encoding the corresponding enzymes: *hemY* and *hemH* (ferrochelatase). It is important to note that the *M. tuberculosis* enzyme HemH (also known as HemZ or CpfC), which catalyzes the final step of heme biosynthesis, is capable of inserting not only iron but also other divalent cations, including Zn^{2+} , Co^{2+} , and Cu^{2+} , into the porphyrin ring (BRENDA data EC 4.99.1.9) [22]. This indicates a relatively broad substrate specificity of the enzyme with respect to metal ions. Taken together, these data suggest that the formation of Zn-coproporphyrin, which accumulates in dormant mycobacteria [14], may be the result of HemH activity or of spontaneous Zn^{2+} insertion under favorable redox conditions.

For a long time, the enzyme responsible for coproporphyrin methylation remained unknown. Quite recently, the methyltransferase CPmtA (MSMEG_0614 in *M. smegmatis*) was identified, which catalyzes the S-adenosyl-L-methionine-dependent conversion of coproporphyrin to TMC through sequential methylation of all four carboxyl groups [23]. Fig. 1 presents a scheme of tetrapyrrole metabolism in mycobacteria, including the synthesis of siroheme, heme, and TMC. Overexpression of CPmtA in both *M. smegmatis* and *M. tuberculosis* leads to an increase in the intracellular level of TMC and a decrease in the respiratory activity of the bacteria, which directly links this enzymatic pathway to the transition into the dormant state. Using structural-dynamic modeling, a unique "carousel" mechanism was revealed, in which coproporphyrin is sequentially reoriented in the active site of the enzyme, allowing its carboxyl groups to be methylated one after another without release of intermediate products. This mechanism provides an approximately 9-fold increase in conversion efficiency. Mutational analysis based on computational predictions confirmed the proposed catalytic mechanism [23].

The accumulation of TMC in the membranes of dormant cells has a significant effect on their physical properties. Fluorescence anisotropy studies of the BODIPY FL C16 probe at the single-cell level showed that anisotropy increases from 0.05 in vegetative cells to 0.22 in dormant *M. smegmatis* cells. Similarly, an increase in TMC concentration in vegetative cells

grown in the presence of 5-aminolevulinic acid (ALA, a precursor in porphyrin synthesis) led to an analogous increase in anisotropy. Since anisotropy is inversely proportional to membrane fluidity, these data indicate a significant decrease in the fluidity of the hydrophobic phase (lipids) of membranes upon TMC accumulation [13]. Importantly, these changes in membrane fluidity are accompanied by inhibition of respiratory chain activity. It is assumed that the decrease in membrane fluidity leads to inhibition of respiratory chain activity, although direct interaction of TMC with membrane-bound enzymes is also not excluded. Notably, during the first 8 h of reactivation of dormant cells, the porphyrin content and anisotropy return to levels characteristic of vegetative bacteria [13].

An important addition to the picture of porphyrin metabolism during mycobacterial dormancy is the finding that a significant fraction of coproporphyrin exists in the form of a zinc complex. Analysis of spectra from single living cells confirmed that this porphyrin is predominantly present as Zn-coproporphyrin [14]. This observation was confirmed by spectral analysis of extracts from dormant *M. tuberculosis* cells. In extracts of dormant cells grown in the presence of ALA, Zn-porphyrin is predominantly detected with a small admixture of free porphyrin [14]. The formation of Zn-coproporphyrin is probably catalyzed by the same enzyme HemH (ferrochelatase), which, as mentioned above, is capable of inserting various metals into the porphyrin ring. Although the specific activity of HemH

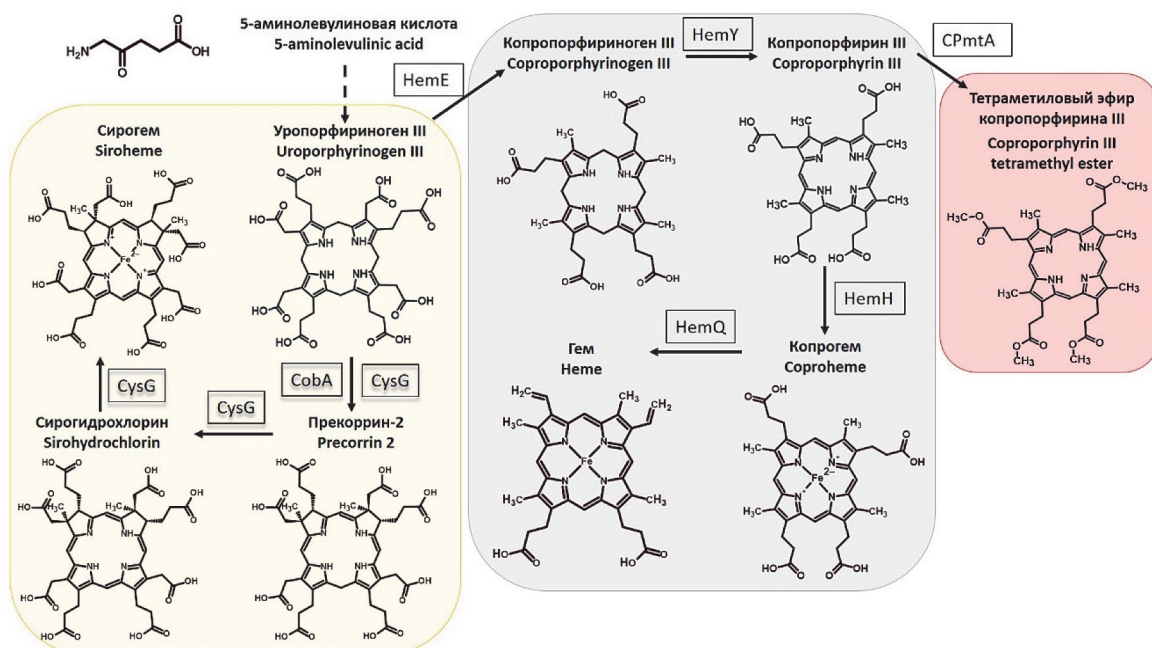


Рис. 1. Схема метаболизма тетрапирролов у микобактерий, включающая синтез сирогема, гема и ТМК. Пунктирная стрелка обозначает несколько последовательных ферментативных реакций, не показанных на схеме.

Fig. 1. Scheme of tetrapyrrole metabolism in mycobacteria, including the biosynthesis of siroheme, heme, and TMC. Dashed arrow indicates several consecutive enzymatic reactions that are not shown in the scheme.

toward Zn^{2+} is not indicated in the literature, the ability of this enzyme to effectively use other divalent cations suggests that Zn^{2+} is also a substrate. Alternatively, zinc insertion may occur spontaneously, without enzymatic catalysis, provided there is a sufficient concentration of zinc ions and a suitable redox environment.

The accumulation of Zn-porphyrins in dormant mycobacteria may have a dual significance. On the one hand, complex formation with zinc substantially changes the photoluminescent properties of porphyrins (shift of emission maxima and appearance of phosphorescence in the red region of the spectrum with a significant quantum yield of triplets at room temperature), which promotes ROS generation upon photoexcitation. This is directly relevant to PDI, since the efficiency of photoinactivation depends on the spectral characteristics of the accumulated PS [14]. On the other hand, competition between Zn^{2+} and Fe^{2+} for binding to coproporphyrin apparently reflects fundamental changes in metal homeostasis during the transition to the dormant state.

The accumulation of precursor porphyrins during the transition to the dormant state or stationary phase is apparently not the result of a single-point inhibition in the porphyrin metabolism pathway but rather a combination of: (a) uneven activation of genes of the early stage of the pathway without an adequate increase in ferrochelatase activity, (b) possible displacement of iron by other metals with the formation of inhibitory metalloporphyrins, and (c) a general decrease in the availability of free iron in the cell in a state of low metabolic activity [14,24]. Moreover, the process itself appears to be physiologically regulated and reversible. This dual toxicity (of both precursor porphyrins and the final product, heme) explains why the tetrapyrrole biosynthesis pathway is tightly regulated at every step [25,26]. At the same time, the accumulation of tetramethyl derivatives of porphyrins is evidently a directed adaptive mechanism during the transition of cells to dormancy using a unique enzyme [23].

The biological role of the accumulation of methylated porphyrins and their zinc complexes during the transition of mycobacteria to the dormant state remains a subject of active discussion. Several hypotheses have been proposed. First, given the antioxidant properties of porphyrins, as well as their structuring effect on the lipid phase of membranes, it can be assumed that their accumulation contributes to the survival of dormant bacteria under stress conditions, including the aggressive environment of the macrophage. Second, TMC, by incorporating into the lipid bilayer, leads to inhibition of the respiratory chain and, as a consequence, to suppression of energy-dependent reactions, which may be part of the mechanism of transition to a hypometabolic state.

Finally, a significant amount of porphyrins in dormant cells may serve as a depot meeting the cell's needs at early stages of reactivation, when enzymatic processes have not yet been restored [27].

To date, it remains incompletely clear whether tetrapyrroles accumulate in pathogen cells *in vivo* in the absence of exogenous stimuli. However, the fact that mycobacteria, upon entering macrophages, are able to accumulate fluorescent compounds (obviously of tetrapyrrole nature), which enables their photoinactivation, supports this assumption [14]. It was shown that when active *M. tuberculosis* cells were co-cultured with lung macrophages in the presence of ALA for several days, the bacteria accumulated porphyrins, as evidenced by red fluorescence recorded inside macrophages [14]. These data indicate that the intracellular environment of the macrophage can create conditions favorable for tetrapyrrole accumulation, at least when their synthesis is stimulated by a precursor, and open prospects for using this phenomenon in therapy [14]. However, systematic studies using spectroscopy and mass spectrometry on tissue sections of infected animals, including in the Cornell model or in chronic granulomatous models, have not been conducted to date.

Thus, porphyrin metabolism in mycobacteria reveals unique features, including the use of a coproporphyrin-dependent pathway of heme biosynthesis, the ability to form methylated coproporphyrin derivatives through the "carousel" mechanism, and the accumulation of various forms of porphyrins, including zinc-porphyrins, during the transition to the dormant state. These processes are not only of fundamental importance for understanding the pathogen's adaptation to stress but also define molecular targets for therapeutic intervention, including PDI using endogenous PS.

Enzymes of tetrapyrrole metabolism as targets for inducing the accumulation of fluorescent intermediates in bacteria

While the natural accumulation of tetrapyrroles during the transition of mycobacteria to the dormant state represents an endogenous adaptive mechanism, targeted modulation of the activity of key enzymes in the heme biosynthesis pathway opens fundamentally new possibilities for creating artificial photosensitivity in bacteria. Such an approach, based on inducing the accumulation of fluorescent intermediates, has several important advantages: (1) it does not require the addition of exogenous PS, which solves the problems of toxicity and targeted delivery; (2) it can be selective for bacterial cells, since many enzymes of the tetrapyrrole pathway are absent in mammals; (3) it is potentially active against both actively growing and persisting forms. This section examines key enzymatic targets

whose activity modulation leads to the accumulation of fluorescent and photosensitive intermediates, and analyzes strategies for their use in aPDT.

A promising target for this approach is siroheme synthase (CysG), a unique multidomain enzyme absent in mammals that plays a central role in tetrapyrrole metabolism [28]. CysG catalyzes three sequential reactions converting uroporphyrinogen III into siroheme, the prosthetic group of nitrite and sulfite reductases required for sulfur and nitrogen metabolism [29]. CysG is a homodimeric protein in which dimerization creates a series of active centers enabling three enzymatic functions: two S-adenosyl-L-methionine-dependent methylations, NAD⁺-dependent dehydrogenation, and the ferrochelatase reaction of iron insertion [30]. This architectural organization allows CysG to switch the metabolic flux between two key branches of the tetrapyrrole pathway: heme biosynthesis and cobalamin (vitamin B₁₂) biosynthesis [29].

An important aspect of the physiological role of CysG is its regulation in response to stress conditions characteristic of the transition of mycobacteria to the dormant state.

Direct evidence of siroheme synthase induction during starvation was obtained in a proteomic study by J. Albrethsen et al. In this work, the authors compared the proteomic profiles of culture filtrates of *M. tuberculosis* H37Rv grown in Sauton's medium (logarithmic phase) and after 6 weeks of cultivation under starvation conditions in phosphate-buffered saline. The analysis revealed that among the proteins during starvation, the category "porphyrin biosynthetic process" was statistically significantly enriched. This category included four proteins: CysG (siroheme synthase), HemC (prophobilinogen deaminase), HemZ (ferrochelatase), and Rv1314c (probable cobalamin adenosyltransferase) [31].

Similarly, in the model of *M. tuberculosis* transition to the dormant state under gradual acidification of the external environment, an increase in the expression of the Rv0511 gene encoding siroheme synthase in *M. tuberculosis* was detected [14].

It was demonstrated that overexpression of the MSMEG_2618 gene encoding siroheme synthase in *Mycobacterium smegmatis*, as well as heterologous expression of this gene in *Escherichia coli*, lead to dramatic changes in tetrapyrrole metabolism and the accumulation of fluorescent intermediates [28]. Already at the colony level, the transformants visually differed by gray coloration in visible light and bright pink fluorescence under ultraviolet illumination (365 nm), whereas control strains carrying empty vectors did not exhibit such properties [28]. Spectral analysis of *M. smegmatis* cells overexpressing MSMEG_2618 revealed characteristic emission peaks at 605 nm (presumably

corresponding to precorrin-2), 625 nm (porphyrins, including uroporphyrin and coproporphyrin), and 650 nm (presumably sirohydrochlorin) [28]. The addition of ALA to the culture medium led to a significant enhancement of the porphyrin peak; however, the fluorescence intensity of precorrin did not increase, which was interpreted as evidence of regulation of biosynthesis at the level of precorrin-2 conversion to sirohydrochlorin upon *cysG* overexpression [28]. In *E. coli* cells expressing MSMEG_2618, the spectral profile differed by the absence of the porphyrin peak at 625 nm, which probably reflects species-specific differences in the organization of the tetrapyrrole pathway and the activity of coupled enzymes in gram-negative bacteria [28].

The most important result of this work from the point of view of practical application is the finding that the accumulation of fluorescent tetrapyrroles in strains overexpressing *cysG* makes the bacteria highly sensitive to PDI [28]. Upon irradiation with monochromatic light at a wavelength of 565 nm, corresponding to the porphyrin absorption band, the survival of *M. smegmatis* overexpressing *cysG* was only 4.5%, and that of *E. coli* was 6%, compared to the control, where survival reached 100% [28]. Irradiation with 640 nm light, absorbed by chlorins, was less effective for *M. smegmatis* (90% survival) but unexpectedly led to a more pronounced effect on *E. coli* (1% survival) [28]. This contrast may indicate the accumulation of different types of tetrapyrroles in the two bacterial species upon overexpression of the same enzyme, which opens possibilities for selective action on different groups of pathogens by selecting the irradiation wavelength.

Structural-functional studies of CysG conducted on *Salmonella typhimurium* showed that the enzyme is a phosphoprotein, and phosphorylation at a conserved serine residue plays a regulatory role by inhibiting dehydrogenase activity and thereby modulating the metabolic flux between the siroheme and cobalamin pathways [29]. This observation is directly relevant to the development of strategies for targeted modulation of CysG activity: inhibition of dehydrogenase or ferrochelatase activity (while preserving methyltransferase activity) should lead to the accumulation of fluorescent intermediates, precorrin-2 and sirohydrochlorin, which possess photosensitizing properties. The search for selective inhibitors of the dehydrogenase and ferrochelatase domains of CysG that do not affect other host enzymes is a promising area of research.

It is known from the literature that blockade of ferrochelatase in mammals (for example, by N-methylprotoporphyrin inhibitors) causes the accumulation of protoporphyrin IX and is successfully used to enhance ALA-induced photodynamic therapy

of tumors [32]. Analogous experiments in yeast showed that deletion of the siroheme synthase gene leads to significant accumulation of fluorescent tetrapyrroles [33]. In bacteria, such inhibitors have not yet been described; however, the emergence of highly selective low-molecular-weight compounds acting on CysG could become a breakthrough in the field of aPDT.

Thus, CysG represents not only an interesting object for studying the regulation of tetrapyrrole metabolism but also a promising target for the development of new antibacterial strategies based on inducing the accumulation of endogenous PS [28]. Unlike exogenous PS, which require separate administration and often possess toxicity, activation of endogenous tetrapyrroles through modulation of CysG activity can provide selectivity of action on bacterial cells, since this enzyme is absent in humans. Further research should be aimed at the search for selective inhibitors of the dehydrogenase or ferrochelatase domains of CysG, which would allow control over the accumulation of photosensitive intermediates and create the basis for a new class of antibacterial drugs active against both actively growing and persisting forms of mycobacteria.

In addition to siroheme synthase, the tetrapyrrole pathway of heme biosynthesis contains several other enzymes whose activity modulation leads to the accumulation of fluorescent porphyrin compounds.

One of the most studied examples is ferrochelatase (HemH), the enzyme catalyzing the final step of heme biosynthesis: the insertion of a divalent iron ion into protoporphyrin IX. A defect in this enzyme leads to the accumulation of protoporphyrin IX, which is known to be an endogenous PS [34]. In *E. coli*, *hemH* mutants demonstrate an approximately 100-fold increase in sensitivity to blue and white light compared to the wild type, which is due to the accumulation of protoporphyrin IX [34]. Upon irradiation, excited protoporphyrin IX generates ROS that damage DNA, lipids, and proteins. Moreover, it was shown that the level of apurinic/aprimidinic sites in the DNA of *hemH* mutants is significantly elevated, and additional defects in the repair of apurinic sites (for example, mutations in the *xth*, *nfo*, and *nth* genes) enhance photosensitization 50-fold [34].

Another important target is coproporphyrinogen III oxidase, which is represented in bacteria by two isoforms: oxygen-dependent HemF (in aerobes) and oxygen-independent HemN (in anaerobes and facultative bacteria) [35,36]. HemN belongs to the Radical SAM superfamily and contains one or two S-adenosylmethionine cofactors and a [4Fe-4S] cluster [36]. The presence of such a cluster makes HemN vulnerable to oxidative stress and toxic metals. It has been shown that copper ions inhibit HemN,

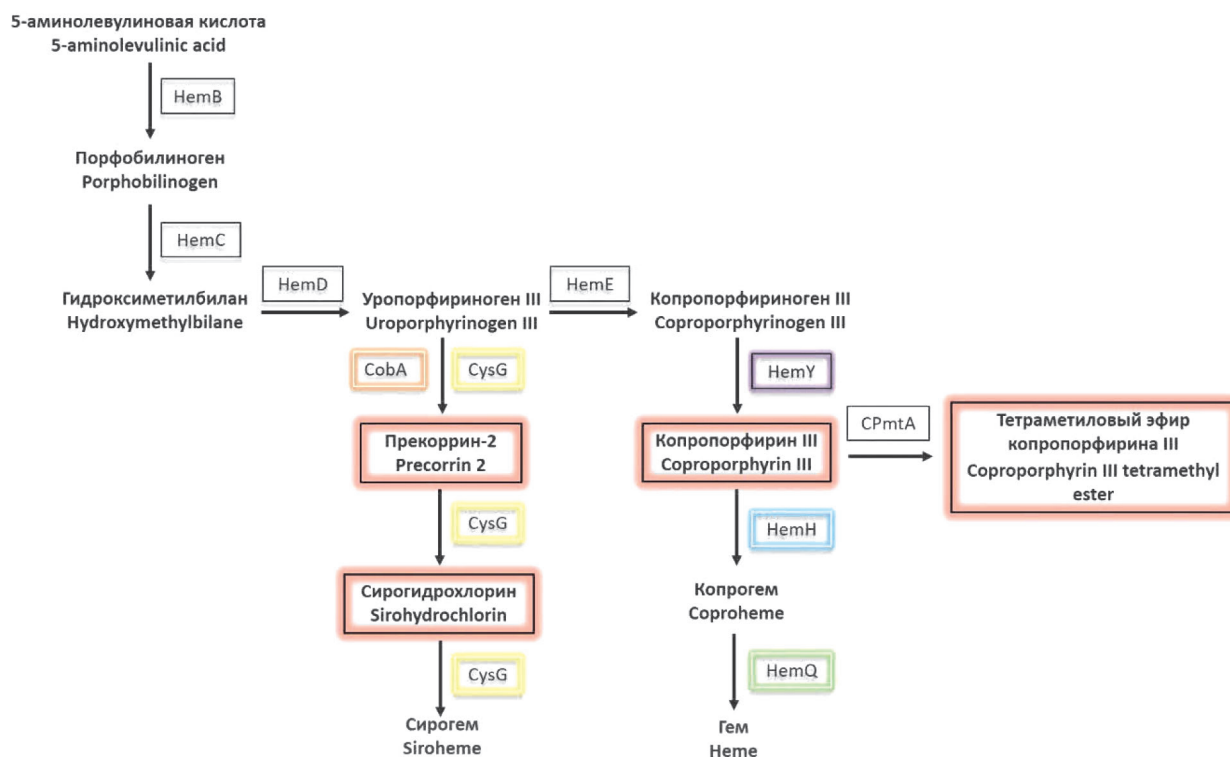


Рис. 2. Пути биосинтеза сирогема и гема у микобактерий. Ферменты, модуляция активности которых приводит к накоплению флуоресцирующих интермедиатов, а также соответствующие интермедиаты выделены цветными рамками.

Fig. 2. Biosynthetic pathways of siroheme and heme in mycobacteria. Enzymes whose activity modulation results in the accumulation of fluorescent intermediates, as well as the corresponding intermediates, are highlighted in colored frames.

leading to the accumulation of coproporphyrin III (coproporphyrinogen III is oxidized to coproporphyrin III), which is easily detected by its characteristic fluorescence [36]. Under oxidative stress conditions induced by hydrogen peroxide, *E. coli* induces the oxygen-dependent isoform HemF (a manganese-containing protein), which replaces the iron-sulfur HemN, whose activity is impaired [35]. This represents an example of metabolic adaptation in which the replacement of one enzyme by another prevents the accumulation of toxic and fluorescent intermediates.

It is important to note that gram-positive bacteria, including mycobacteria, possess an additional component of the tetrapyrrole pathway—HemQ—which is absent in gram-negative bacteria and eukaryotes [37]. HemQ was identified as a protein required for heme biosynthesis in *Actinobacteria* and *Firmicutes*. In the genomes of these organisms, the hemQ gene is typically located adjacent to the *hemY* or *hemH* genes [37]. Interestingly, HemY and HemH from gram-positive bacteria are not able to complement the corresponding *E. coli* mutants when expressed separately; however, upon co-expression of all three components (HemY, HemH, and HemQ), functional complementation is achieved [37]. Since HemQ is essential for heme biosynthesis in mycobacteria and is absent in humans, this protein represents a promising target for the development of new anti-tuberculosis drugs.

In contrast to mutations that inactivate enzymes and cause PS accumulation, overexpression of certain enzymes of the tetrapyrrole pathway can also be used to create fluorescent phenotypes. The most striking example is uroporphyrinogen III methyltransferase (CobA) from *Propionibacterium freudenreichii* [38,39]. Overexpression of *cobA* in *E. coli*, yeast, and even mammalian cells leads to the accumulation of fluorescent precorrins, making the cells bright red under ultraviolet illumination [38,39]. Similarly, overexpression of a truncated variant of *cysG* (*cysGA*) from *E. coli*, encoding only the methyltransferase domain, leads to an analogous effect [38]. These observations formed the basis for the use of CobA and CysGA as reporters of gene expression that do not require the addition of an exogenous substrate. Notably, the red fluorescence generated by these systems has an advantage over green fluorescent protein (GFP), since the level of autofluorescence of cells and nutrient media is lower in the red region of the spectrum [39].

Thus, the spectrum of tetrapyrrole metabolism enzymes whose activity modulation leads to the accumulation of fluorescent intermediates is quite broad. It includes ferrochelatase (HemH), oxygen-independent coproporphyrinogen III oxidase (HemN), oxygen-dependent coproporphyrinogen III oxidase

(HemY), uroporphyrinogen III methyltransferases (CobA and CysGA), and the auxiliary protein HemQ in gram-positive bacteria. Fig. 2 presents the metabolic pathways involving these enzymes in mycobacteria. Each of these enzymes represents a potential target for targeted inhibition to induce the accumulation of endogenous PS, which can be used for PDI of bacteria. Given that many of these enzymes are absent in humans (for example, HemQ and HemN), such an approach can provide selectivity of antibacterial action, which is especially important for the treatment of infections caused by pathogens with multidrug resistance.

Photodynamic inactivation of mycobacteria and other bacteria in vitro using endogenous tetrapyrroles

PDI, which forms the basis of aPDT, is a method of destroying microorganisms based on the combined application of a PS and light of an appropriate wavelength. In the presence of oxygen, the PS generates ROS that nonspecifically damage cellular structures, causing the death of the pathogen regardless of its drug resistance [9,10,40]. A key advantage of PDI over traditional antibiotics is that its action does not depend on the presence of specific targets, and it is therefore effective against both antibiotic-resistant and metabolically low-active persisting forms of bacteria [9]. Of particular interest is the use of endogenous PS, mainly porphyrins and their derivatives, which accumulate in bacterial cells either in response to stress influences or as a result of the addition of an exogenous precursor (ALA) of their biosynthesis [9,40].

The first experimental confirmations of the effectiveness of PDI using endogenous porphyrins were obtained on a model of dormant cells of *Mycobacterium smegmatis*, a fast-growing relative of *M. tuberculosis* that accumulates endogenous porphyrins (see above) [20,41]. Irradiation of such cells with light at wavelengths of 395 nm and 575 nm, which correspond to the main absorption maxima of porphyrins, led to effective inactivation of dormant bacteria already within 15–30 min, whereas actively growing cells that do not accumulate porphyrins remained resistant to light exposure [41,42]. This result was of fundamental importance, since it demonstrated for the first time the possibility of selective killing of dormant mycobacteria using their own metabolites without the addition of exogenous PS.

Subsequent studies were aimed at enhancing the accumulation of endogenous porphyrins and increasing the efficiency of PDI. Increasing the concentration of magnesium ions (up to 25 mM) and zinc ions (up to 62 μ M) in the cultivation medium of *M. smegmatis* significantly increases the production of endogenous porphyrins in dormant mycobacteria [43].

In the absorption spectra of extracts from such cells, an additional shoulder appeared in the long-wavelength region of the Soret band, which was interpreted as the formation of Zn-porphyrin. Fluorescence analysis confirmed the presence of a mixture of free porphyrin (maxima at 620 and 685 nm) and Zn-porphyrin (maximum at 580 nm) [43]. Cells grown at elevated metal concentrations demonstrated substantially higher photosensitivity, especially after phagocytosis by macrophages, which brings the experimental conditions closer to the situation inside the host organism [43].

The most impressive results were obtained on the pathogen itself, *M. tuberculosis*. Dormant forms of *M. tuberculosis* obtained in the acidification model accumulate free porphyrins and their Zn complexes, especially when ALA is added to the cultivation medium. Transcriptomic analysis revealed activation of eight genes involved in tetrapyrrole metabolism during the transition of *M. tuberculosis* to the dormant state, which explains the observed porphyrin accumulation [14]. The viability of dormant *M. tuberculosis* decreased by more than 99.99% upon irradiation with 565 nm light (300 J/cm² for 30 min), a wavelength corresponding to the absorption bands of Zn-porphyrin and coproporphyrin [14]. Interestingly, actively growing mycobacteria *in vitro* did not exhibit photosensitivity. However, after infecting macrophages and persisting inside them for several days in the presence of ALA, active *M. tuberculosis* cells also acquired pronounced sensitivity to light (viability reduction of ~99.99%) [14]. This result has important practical significance, since it shows that even actively growing bacteria can become targets of PDI after entering phagocytes in the presence of a porphyrin precursor.

Analysis of the literature allows two main groups of bacteria to be distinguished for which the effectiveness of PDI based on endogenous porphyrins has been demonstrated. The first group comprises bacteria that naturally produce porphyrins during normal metabolism. The most well-known and clinically applied example is *Propionibacterium acnes* (*Cutibacterium acnes*), a bacterium that plays a key role in the pathogenesis of acne. These microorganisms naturally produce significant amounts of porphyrins, predominantly coproporphyrin, in the stationary growth phase. In the classical work of H. Ashkenazi et al., it was shown that the porphyrins produced by *P. acnes* were identified as coproporphyrin based on HPLC data, and the addition of ALA enhanced intracellular porphyrin synthesis [44]. The viability of 24-hour cultures decreased by less than two orders of magnitude upon a single irradiation dose of 75 J/cm², and the best effect was achieved with 2–3-fold irradiation at 24-hour intervals [44]. This phenomenon formed the basis of the blue light therapy

of acne (415 nm) widely used today: *P. acnes* synthesizes protoporphyrin and coproporphyrin as part of normal metabolism, and these endogenous porphyrins achieve optimal photoexcitation upon irradiation with 407–420 nm light [45]. Blue light provides selective destruction of porphyrin-producing bacteria without significant damage to surrounding tissues, making this method safe and effective for the treatment of acne.

Natural porphyrin producers also include oral periodontopathogens: *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* [46,47]. It has been shown that *P. gingivalis* cells in liquid medium are effectively inactivated by 460 nm LED irradiation, achieving a reduction in CFU count of approximately 4 orders of magnitude at a dose of 100 J/cm² [47]. Early work by N.S. Soukos et al. showed that bacteria (*Prevotella intermedia*, *P. nigrescens*, *P. melaninogenica*, and *Porphyromonas gingivalis*) colonizing the oral cavity and forming black pigment contain large amounts of endogenous porphyrins (predominantly protoporphyrin IX) and are killed by visible light both in pure cultures and in dental plaque samples [48]. In *A. actinomycetemcomitans*, both porphyrins and flavins have been detected as endogenous PS [46].

The second group comprises bacteria that become photosensitive only after artificial stimulation of porphyrin synthesis by exogenous ALA. F. Gábor et al. showed that treatment with ALA followed by irradiation with white light effectively inactivates *E. coli* cells (gram-negative bacteria) but has no noticeable effect on *Enterococcus hirae* (gram-positive cocci) [49]. Upon the addition of ALA (50 µg/ml), significant accumulation of porphyrins inside cells of both species was observed. In *S. aureus*, uroporphyrin accumulated intracellularly with coproporphyrin released into the medium; in *E. coli*, uroporphyrin and protoporphyrin accumulated inside cells with coproporphyrin and protoporphyrin released outward [49]. The most effective photoinactivation of these species was achieved with blue light (400–450 nm) at a dose of about 50 J/cm²; *S. aureus* proved more sensitive than *E. coli* [49].

Among other bacteria sensitized by ALA, *Helicobacter pylori* should be noted, which is the causal agent of gastric ulcer and gastritis development and which, as it turned out, accumulates significant amounts of coproporphyrin and protoporphyrin IX both in cells and in the growth medium *in vitro*. This feature made it possible to perform PDI of such cells with a reduction in viable cell concentration of more than 5 orders of magnitude after illumination with low doses of visible light (blue/violet light being most effective) [50]. The degree of killing increased with culture age and was higher than that of *P. acnes* [50]. This work formed the basis of clinical trials of intragastric phototherapy of *H. pylori* with 408 nm violet light [51].

Moreover, *H. pylori* cells proved sensitive to PDI also as part of biofilms, which is important for the prospects of applying this method *in vivo* [52]. In this regard, attempts were made to eradicate *H. pylori* in patients with peptic ulcer disease using endoscopic techniques with illumination of certain areas of the gastric mucosa with blue light (405 nm). Before and after PDI, titers of viable bacteria in biopsy samples were assessed. The obtained reduction in the number of *H. pylori* colonies per gram of tissue between treated and control areas averaged 91% (1–2 orders of magnitude), which is substantially inferior to the bactericidal effect observed *in vitro* on pure cultures. Nevertheless, given the localization of the pathogen in the submucosal layer, this result should be regarded as encouraging and promising for further research [53]. In a subsequent work using an endoscopic balloon allowing wide-field illumination at 408 nm, it was found that the most sensitive zone for *H. pylori* eradication is the antral region of the stomach, while the least sensitive is the gastric fundus. This may be related to unequal light penetration into different regions or to differences in porphyrin content in the pathogen in different parts of the stomach [51].

For *Pseudomonas aeruginosa*, it was shown that incubation with ALA led to enhanced accumulation of protoporphyrin; however, the main part of ALA was converted into non-fluorescent and photodynamically inactive porphyrinogens [54]. Protoporphyrin IX endogenously produced by *P. aeruginosa* has five absorption bands (405, 505, 542, 580, and 631 nm) that can be used for PDI [55]. The authors demonstrated that excitation with a blue LED at 418 nm is most effective, which is explained by the maximum overlap of the source emission spectrum with the absorption band of protoporphyrin IX. These data confirm the fundamental possibility of using endogenous protoporphyrin IX for PDI of pathogens and indicate the need to select optimal irradiation wavelengths for each specific microorganism.

For *Salmonella enterica*, it was shown that an increasing amount of endogenously produced porphyrins does not guarantee a proportional decrease in cell survival; moreover, the presence of endogenous porphyrins released into the medium causes secondary sensitization of cells and produces a noticeably stronger photoinactivating effect [56]. For *Haemophilus parainfluenzae*, it was shown that upon incubation with increasing amounts of ALA, survival decreased after irradiation with 630 nm light, whereas no effect was observed without the addition of ALA [57]. The formation of dormant cells of *Corynebacterium jeikeium* upon acidification of the medium in the stationary phase, followed by stimulation of endogenous porphyrin synthesis, is accompanied by an increase in

sensitivity to PDI [58], which confirms the universality of the phenomenon of porphyrin accumulation during the transition to the dormant state in actinobacteria. For *Corynebacterium diphtheriae*, a predominance of coproporphyrin III and a positive correlation of porphyrin production with biofilm formation were found [59], which opens prospects for the use of PDI in combating biofilm infections.

The potential of endogenous porphyrins produced by *Corynebacterium diphtheriae* as PS for PDI was studied. Qualitative analysis revealed the presence of porphyrins in all studied strains, with a predominance of coproporphyrin III. The addition of ALA enhanced porphyrin production, which was confirmed by an increase in fluorescence intensity. Furthermore, analysis using high-performance liquid chromatography with diode-array and mass spectrometric detection confirmed the presence of coproporphyrin III and protoporphyrin IX in all strains; the addition of ALA did not change the porphyrin profile. Quantitative analysis showed that strain-dependent levels of coproporphyrin III significantly increased upon the addition of ALA. In addition, biofilm formation positively correlated with porphyrin production, suggesting their role in biofilm formation [59]. Photoinactivation experiments showed that strains responded differently to light irradiation, and the addition of ALA reduced the time required for a significant reduction in CFU/ml. Moreover, survival in biofilms exceeded that of planktonic cells, highlighting the complexity posed by biofilm structure with respect to PDI efficiency. Despite the observed variability in response, all strains demonstrated reduced viability after light irradiation, confirming the potential of endogenous porphyrins for application in antimicrobial photoinactivation [59]. In a recent review, A. Zdubek and I. Maliszewska provide numerous examples of successful application of ALA-induced PDI for killing a wide range of pathogens, including bacteria, fungi, yeasts, and parasites [40]. Clinical applications of this technique include the treatment of ulcers and disinfection of dental root canals [40].

An important direction of research is the elucidation of the molecular targets of PDI. A proteomic profiling analysis of dormant *M. smegmatis* cells before and after irradiation was performed using SDS-electrophoresis and MALDI-TOF mass spectrometry. It was found that already after 5 min of irradiation, 45.2% of individual proteins disappear from the separating gel, and after 15 min, 63.9% [43]. Among the light-sensitive proteins were enzymes of the glycolytic pathway, the tricarboxylic acid cycle, the pentose phosphate pathway, oxidative phosphorylation, and energy production. Proteins involved in protection against oxygen stress and protein aggregation were also identified [43]. PDI causes inhibition of respiratory

chain activity and destroys enzymes necessary for protein and nucleic acid synthesis, processes critically important for the reactivation of dormant bacterial cells and their transition to an actively growing state [14,43].

Other endogenous PS of bacteria: flavins, NADH, and lumichrome

Along with porphyrins, other cellular components can also act as endogenous PS. Understanding the involvement of these compounds is important for explaining species-specific differences in bacterial sensitivity to PDI and for optimizing aPDT protocols [60].

The most studied alternative PS are riboflavin (vitamin B₂) derivatives, including flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). Flavins have characteristic absorption bands in the blue region of the spectrum, predominantly around 450 nm, and play a key role in cellular metabolism as cofactors of redox enzymes [60]. According to current understanding, antimicrobial blue light (aBL, 400–470 nm) activates endogenous flavins, which generate ROS causing oxidative damage to membranes, proteins, and metabolic pathways [61]. This mechanism does not require the addition of exogenous PS, making aBL an attractive approach for combating infections [60,61]. Thus, in the work of V.Y. Plavskii et al., it was shown that both porphyrins and flavins are acceptors of optical radiation in the blue region of the spectrum, determining the photoinactivation of microbial cells [62]. The authors demonstrated that flavins make a significant contribution to the photodynamic effect upon irradiation in the 400–450 nm range, especially in bacteria with a low content of endogenous porphyrins [62]. This confirms that flavins are important endogenous PS whose action is not limited to porphyrin-producing species.

However, studies on enterococci have shown that the picture of endogenous PS may be more complex. Studies on *Enterococcus moraviensis*, which are considered not to contain free porphyrins, showed that irradiation with 405 nm light nevertheless effectively inactivates these bacteria, and significantly more strongly than 450 nm light [63]. Fluorescence analysis revealed the absence of free porphyrins but the presence of spectra of NADH and lumichrome, a product of flavin photodegradation [63]. The authors suggest that it is lumichrome or NADH that may be responsible for photoinactivation at 405 nm in porphyrin-deficient bacteria [63]. Since NADH and flavins are present in all microorganisms, their photoproducts may participate in photoinactivation processes in other bacterial species as well [63].

This finding is important because it expands the range of potential endogenous targets for PDI and explains why some porphyrin-deficient bacteria

nevertheless exhibit photosensitivity. In a recent review devoted to the mechanisms and determinants of bacterial sensitivity to antimicrobial blue light, it is concluded that, in addition to porphyrins, flavins and bacterial pigments can act as PS [60]. The diversity of endogenous chromophores explains the species-dependent sensitivity of bacteria to light exposure and emphasizes the need for a comprehensive approach to assessing the photosensitizing potential of microorganisms.

It should be noted that the use of endogenous PS to combat bacterial infections remains a subject of active research. Unlike exogenous PS used in clinical practice, which require separate consideration and may possess toxicity, endogenous compounds (porphyrins, flavins, NADH, lumichrome) are initially present in cells, which opens possibilities for selective action. Further research should be aimed at a detailed study of the photophysical properties of these compounds, their intracellular localization and concentration in various physiological states of bacteria, and the optimization of irradiation regimens to achieve maximum inactivation efficiency with minimal damage to host tissues.

Limitations and unresolved issues

Despite the significant potential of aPDT using endogenous PS, the proposed approach has a number of fundamental limitations that must be taken into account when assessing its clinical applicability.

The main obstacle to the application of aPDT in pulmonary tuberculosis is the limited depth of light penetration into biological tissues. For optimal light penetration into biological tissues, it is necessary to use wavelengths in the so-called “optical transparency window” (or therapeutic window), which lies in the range from ~600 to ~1300 nm. In this range, absorption by endogenous tissue chromophores (hemoglobin, melanin, water) is minimal, ensuring maximum depth of light penetration. However, the main absorption maxima of porphyrins (primarily the Soret band) lie in the blue-violet region of the spectrum (~400–420 nm), and they also have less intense Q-bands in the green and red regions (~500–650 nm). Flavins also absorb predominantly in the blue region (~450 nm). Radiation in the blue-violet range penetrates lung tissue extremely poorly, making direct irradiation of deeply located foci practically impossible without invasive methods. For visible light, especially in the blue and green regions of the spectrum (405–565 nm), in which endogenous porphyrins and flavins predominantly absorb, the depth of penetration into tissue is only from a few hundred micrometers to several millimeters.

The lungs represent a heterogeneous and complex environment. Airways, alveoli, blood vessels, and fibrous

granuloma tissue create unique challenges for light delivery. Deeply located granulomas characteristic of tuberculosis may be at a considerable distance from the lung surface. Effective action on the pathogen localized in them requires the use of invasive techniques, such as bronchoscopy with fiber-optic probes. The latter approach may be applicable for localized foci but presents significant difficulties for disseminated processes.

One possible solution is the development of two-photon or multiphoton excitation approaches, as well as the use of combination with optical tissue clearing methods [10].

In the case of systemic ALA administration to induce endogenous PS, it will be absorbed not only by bacterial cells but also by cells of the macroorganism, which are also capable of synthesizing protoporphyrin IX from ALA. Although protoporphyrin IX is normally rapidly converted to heme by ferrochelatase, in some cases (tumor cells) its excessive accumulation in host cells is observed. In the context of aPDT, this can lead to phototoxicity of healthy tissues upon light irradiation. Furthermore, macrophages infected with mycobacteria and accumulating porphyrins inside phagosomes may also undergo oxidative damage upon irradiation [14]. Potential side effects include phototoxicity of surrounding epithelial and endothelial cells of the lungs, as well as induction of an inflammatory response mediated by ROS. To minimize risks, optimization of irradiation regimens (duration, intensity, wavelength) is necessary, as well as the development of strategies for selective delivery of light to infected areas using endoscopic or interventional control [10].

Thus, a key aspect is the differential accumulation of endogenous PS in bacterial cells compared to host cells. It has been shown that mycobacteria accumulate porphyrins (including TMC and Zn-porphyrins) in the dormant state or during persistence inside macrophages in the presence of ALA [14,20]. This indicates potential selectivity. However, more in-depth studies are needed to accurately assess the levels of accumulation and clearance of PS in various types of host and pathogen cells *in vivo*, as well as to identify the mechanisms ensuring this selectivity.

To date, the vast majority of experimental data on aPDT using endogenous PS have been obtained on isolated bacterial cultures *in vitro* or on macrophage cell lines [14, 41, 43]. Systematic studies in mouse models of granulomatous tuberculosis (Cornell model, chronic granulomatous models) assessing the accumulation of endogenous porphyrins in granulomas *in vivo*, as well as the efficiency of photoinactivation of persisting forms of *M. tuberculosis* under conditions approximating clinical ones, are practically absent. In the available studies on mice with granulomatous lesions caused

by *M. bovis* BCG, exogenous PS were used, which does not allow extrapolation of these results to the endogenous approach. Studies are also needed on the pharmacokinetics and tissue distribution of ALA and endogenous porphyrins upon systemic or inhalation administration, as well as on the long-term effects of aPDT on the structural and functional state of lung tissue. To minimize systemic phototoxicity, it may be necessary to develop local methods of ALA delivery (for example, inhalation forms for pulmonary tuberculosis) or PS obtained through modulation of endogenous synthesis that do not possess the ability to significantly accumulate in healthy tissues of the macroorganism. In addition, precise selection of light dose and irradiation time plays a decisive role in achieving a therapeutic effect while minimizing side effects.

As discussed in the section devoted to enzymatic targets, a promising approach for inducing the accumulation of fluorescent intermediates is the modulation of the activity of enzymes such as siroheme synthase CysG, ferrochelatase HemH, coproporphyrinogen III oxidase HemN, and methyltransferase CPmtA. However, to date there are no selective low-molecular-weight inhibitors of these enzymes suitable for clinical use. The development of such compounds, active against bacterial enzymes and not affecting homologous host enzymes, is a complex task requiring further structural and functional studies [23, 28].

Thus, despite the promising results of preclinical studies, the translation of aPDT using endogenous PS into clinical practice requires solving fundamental problems related to light delivery to deep foci of infection, minimizing phototoxicity to host tissues, obtaining convincing evidence of efficacy in adequate animal models of granulomatous tuberculosis, and developing selective pharmacological modulators of tetrapyrrole metabolism.

Conclusion

Endogenous PS are represented in many bacterial species, including mycobacteria, mainly in the form of tetrapyrroles, as well as in the form of flavins, NADH, and lumichrome. The accumulation of such compounds inside the cell in sufficient quantities allows bacteria to be inactivated using aPDT, which has been demonstrated for both gram-positive and gram-negative species. The most advanced research in this area is associated with mycobacteria capable of accumulating porphyrins either under special physiological conditions or upon the addition of the tetrapyrrole synthesis precursor ALA to the growth medium. PDT using endogenous tetrapyrroles and other compounds represents a promising strategy for combating bacterial infections such as tuberculosis,

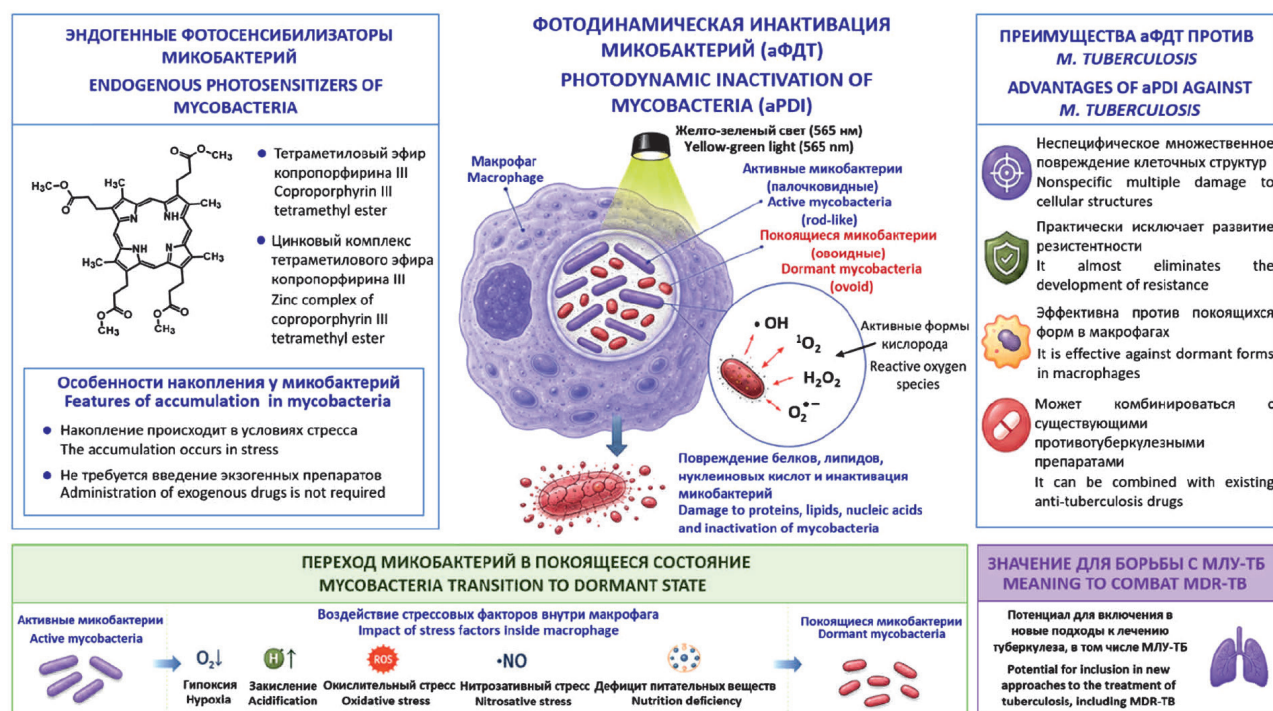


Рис. 3. Механизм действия и потенциальные преимущества аФДТ в отношении *Mycobacterium tuberculosis*, включая покоящиеся формы и штаммы с множественной лекарственной устойчивостью (МЛУ).

Fig. 3. Mechanism of action and potential advantages of aPDT against *Mycobacterium tuberculosis*, including dormant forms and multidrug-resistant (MDR) strains.

especially those caused by multidrug-resistant and persisting forms of pathogens (Fig. 3).

The main advantages of the approach are: (1) the absence of the need for exogenous PS, which solves the problems of toxicity and the need for targeted delivery; (2) selectivity of action, since porphyrin accumulation occurs predominantly in bacterial cells in response to stress; (3) a multiple mechanism of action (damage to proteins, lipids, nucleic acids), excluding the development of resistance. Further research should be aimed at optimizing irradiation regimens for deeply located infections, as well as at studying the possibility of combining PDI with traditional anti-tuberculosis drugs for the eradication of both active and latent forms of *M. tuberculosis*.

The work was supported by the Russian Science Foundation grant 25-45-10015 for the chapters "Accumulation of endogenous PS (porphyrins) during the transition of mycobacteria to a dormant state", "Enzymes of tetrapyrrole metabolism as targets for inducing the accumulation of fluorescent intermediates in bacteria", and "Photodynamic inactivation of mycobacteria and other bacteria in vitro using endogenous tetrapyrroles"; and by the State Assignment of the Federal Research Centre "Fundamentals of Biotechnology" of the Russian Academy of Sciences, A.N. Bach Institute of Biochemistry for the chapters "The role of porphyrins in mycobacterial metabolism and their connection with the dormant state" and "Other endogenous photosensitizers of bacteria: flavins, NADH, and lumichrome".

REFERENCES

- Global tuberculosis report 2024. Geneva: World Health Organization, 2024. Licence: CC BY-NC-SA 3.0 IGO.
- Shleeva M.O., Kudykina Y.K., Vostroknutova G.N., Suzina N.E., Mulyukin A.L., Kaprelyants A.S. Dormant ovoid cells of *Mycobacterium tuberculosis* are formed in response to gradual external acidification. *Tuberculosis*, 2011, vol. 91, pp. 146-154. doi:10.1016/j.tube.2010.12.006.
- Kaprelyants A., Salina E., Makarov V. How to kill dormant *Mycobacterium tuberculosis*. *Int. J. Mycobacteriology*, 2018, vol. 7, pp. 399-400. doi:10.4103/ijmy.ijmy.
- Zhang Y. Persistent and dormant tubercle bacilli and latent tuberculosis. *Front. Biosci.*, 2004, vol. 9, pp. 1136-1156. doi:10.2741/1291.

ЛИТЕРАТУРА

- Global tuberculosis report 2024. Geneva: World Health Organization. – 2024. Licence: CC BY-NC-SA 3.0 IGO.
- Shleeva M.O., Kudykina Y.K., Vostroknutova G.N., Suzina N.E., Mulyukin A.L., Kaprelyants A.S. Dormant ovoid cells of *Mycobacterium tuberculosis* are formed in response to gradual external acidification // *Tuberculosis*. – 2011. – Vol. 91. – P. 146-154. doi:10.1016/j.tube.2010.12.006.
- Kaprelyants A., Salina E., Makarov V. How to kill dormant *Mycobacterium tuberculosis* // *Int. J. Mycobacteriology*. – 2018. – Vol. 7. – P. 399-400. doi:10.4103/ijmy.ijmy.
- Zhang Y. Persistent and dormant tubercle bacilli and latent tuberculosis // *Front. Biosci.* – 2004. – Vol. 9. – P. 1136-1156. doi: 10.2741/1291.

5. Lipworth S., Hammond R.J.H., Baron V.O., Hu Y., Coates A., Gillespie S.H. Defining dormancy in mycobacterial disease. *Tuberculosis*, 2016, vol. 99, pp. 131-142. doi:10.1016/j.tube.2016.05.006.
6. Kondratieva T., Shleeva M., Kapina M., Rubakova E., Linge I., Dyatlov A., Kondratieva E., Kaprelyants A., Apt A. Prolonged infection triggered by dormant *Mycobacterium tuberculosis*: Immune and inflammatory responses in lungs of genetically susceptible and resistant mice. *PLoS One*, 2020, vol. 15, pp. e0239668. doi:10.1371/journal.pone.0239668.
7. Welin A., Raffetseder J., Eklund D., Stendahl O., Lerm M. Importance of phagosomal functionality for growth restriction of *Mycobacterium tuberculosis* in primary human macrophages. *J. Innate Immun.*, 2011, vol. 3, pp. 508-518. doi:10.1159/000325297.
8. Alnir A.M. Dormancy models for *Mycobacterium tuberculosis*: A minireview. *Brazilian J. Microbiol.*, 2015, vol. 46, pp. 641-647. doi:10.1590/S1517-838246320140507.
9. Shleeva M., Savitsky A., Kaprelyants A. Photoinactivation of mycobacteria to combat infection diseases: Current state and perspectives. *Appl. Microbiol. Biotechnol.*, 2021, vol. 105, pp. 4099-4109. doi:10.1007/s00253-021-11349-0.
10. Shleeva M.O., Demina G.R., Savitsky A.P. A systematic overview of strategies for photosensitizer and light delivery in antibacterial photodynamic therapy for lung infections. *Adv. Drug Deliv. Rev.*, 2024, vol. 215, pp. 115472. doi:10.1016/j.addr.2024.115472.
11. Tanu R., Chaudhary A.A., Prakash G., Yasmeen N., Ali M.A.M., Raza N., Sharma P.K., Kumar A., Yadav T., Kumar V. Exploring the potential of photodynamic therapy in overcoming multidrug resistance: Mechanisms synergies and clinical advancements in infectious diseases. *Front. Cell Infect. Microbiol.*, 2025, vol. 15, pp. 1624036. doi:10.3389/fcimb.2025.1624036.
12. Bonnardot M., Montier T., Rapacka-Zdonzyk A., Gall T. Le Shedding light on direct and indirect effects of antimicrobial photodynamic therapy: A review update. *Biomed. Pharmacother.*, 2026, vol. 200, pp. 119396. doi:10.1016/j.biopha.2026.119396.
13. Gligonov I.A., Bagaeva D.I., Demina G.R., Vostroknutova G.N., Vorozhtsov D.S., Kaprelyants A.S., Savitsky A.P., Shleeva M.O. The accumulation of methylated porphyrins in dormant cells of *Mycobacterium smegmatis* is accompanied by a decrease in membrane fluidity and an impede of the functioning of the respiratory chain. *Biochim. Biophys. Acta - Biomembr.*, 2024, vol. 1866, pp. 184270. doi:10.1016/j.bbamem.2024.184270.
14. Shleeva M.O., Linge I.A., Gligonov I.A., Vostroknutova G.N., Shashin D.M., Tsedilin A.M., Apt A.S., Kaprelyants A.S., Savitsky A.P. Acquiring of photosensitivity by *Mycobacterium tuberculosis* in vitro and inside infected macrophages is associated with accumulation of endogenous Zn-porphyrins. *Sci. Rep.*, 2024, vol. 14, pp. 846. doi:10.1038/s41598-024-51227-z.
15. Shleeva M.O., Kudykina Y.K., Vostroknutova G.N., Suzina N.E., Mulyukin A.L., Kaprelyants A.S. Dormant ovoid cells of *Mycobacterium tuberculosis* are formed in response to gradual external acidification. *Tuberculosis*, 2011, vol. 91, pp. 146-154. doi:10.1016/j.tube.2010.12.006.
16. Khan M.Z., Nandicoori V.K. Deletion of *pknG* abates reactivation of latent *Mycobacterium tuberculosis* in mice. *Antimicrob. Agents Chemother.*, 2021, vol. 65, pp. e02095-20. doi:10.1128/AAC.02095-20.
17. Vilch  ze C., Kremer L. Acid-fast positive and acid-fast negative *Mycobacterium tuberculosis*: The Koch paradox. *Microbiol. Spectr.*, 2017, vol. 5, pp. TBTB2-0003-2015. doi:10.1128/microbiolspec.
18. Cunningham A.F., Ashton P.R., Spreadbury C.L., Lammass D.A., Craddock R., Wharton C.W., Wheeler P.R. Tubercle bacilli generate a novel cell wall-associated pigment after long-term anaerobic culture. *FEMS Microbiol. Lett.*, 2004, vol. 235, pp. 191-198. doi:10.1016/j.femsle.2004.04.036.
19. Berney M., Cook G.M. Unique flexibility in energy metabolism allows mycobacteria to combat starvation and hypoxia. *PLoS One*, 2010, vol. 5, pp. e0008614. doi:10.1371/journal.pone.0008614.
20. Nikitushkin V.D., Shleeva M.O., Zinin A.I., Trutneva K.A., Ostrovsky D.N., Kaprelyants A.S. The main pigment of the dormant *Mycobacterium smegmatis* is porphyrin. *FEMS Microbiol. Lett.*, 2016, vol. 363, pp. 1-8. doi:10.1093/femsle/fnw206.
21. Thunell S. Porphyrins, porphyrin metabolism and porphyrias. I. Update. *Scand. J. Clin. Lab. Invest.*, 2000, vol. 60, pp. 509-540. doi:10.1080/003655100448310.
5. Lipworth S., Hammond R.J.H., Baron V.O., Hu Y., Coates A., Gillespie S.H. Defining dormancy in mycobacterial disease. *Tuberculosis*. – 2016. – Vol. 99. – P. 131-142. doi:10.1016/j.tube.2016.05.006.
6. Kondratieva T., Shleeva M., Kapina M., Rubakova E., Linge I., Dyatlov A., Kondratieva E., Kaprelyants A., Apt A. Prolonged infection triggered by dormant *Mycobacterium tuberculosis*: Immune and inflammatory responses in lungs of genetically susceptible and resistant mice. *PLoS One*. – 2020. – Vol. 15. – P. e0239668. doi:10.1371/journal.pone.0239668.
7. Welin A., Raffetseder J., Eklund D., Stendahl O., Lerm M. Importance of phagosomal functionality for growth restriction of *Mycobacterium tuberculosis* in primary human macrophages. *J. Innate Immun.* – 2011. – Vol. 3. – P. 508-518. doi:10.1159/000325297.
8. Alnir A.M. Dormancy models for *Mycobacterium tuberculosis*: A minireview. *Brazilian J. Microbiol.* – 2015. – Vol. 46. – P. 641-647. doi:10.1590/S1517-838246320140507.
9. Shleeva M., Savitsky A., Kaprelyants A. Photoinactivation of mycobacteria to combat infection diseases: Current state and perspectives. *Appl. Microbiol. Biotechnol.* – 2021. – Vol. 105. – P. 4099-4109. doi:10.1007/s00253-021-11349-0.
10. Shleeva M.O., Demina G.R., Savitsky A.P. A Systematic overview of strategies for photosensitizer and light delivery in antibacterial photodynamic therapy for lung infections. *Adv. Drug Deliv. Rev.* – 2024. – Vol. 215. – P. 115472. doi:10.1016/j.addr.2024.115472.
11. Tanu R., Chaudhary A.A., Prakash G., Yasmeen N., Ali M.A.M., Raza N., Sharma P.K., Kumar A., Yadav T., Kumar V. Exploring the potential of photodynamic therapy in overcoming multidrug resistance: Mechanisms synergies and clinical advancements in infectious diseases. *Front. Cell Infect. Microbiol.* – 2025. – Vol. 15. – P. 1624036. doi:10.3389/fcimb.2025.1624036.
12. Bonnardot M., Montier T., Rapacka-Zdonzyk A., Gall T. Le Shedding light on direct and indirect effects of antimicrobial photodynamic therapy: A review update. *Biomed. Pharmacother.* – 2026. – Vol. 200. – P. 119396. doi:10.1016/j.biopha.2026.119396.
13. Gligonov I.A., Bagaeva D.I., Demina G.R., Vostroknutova G.N., Vorozhtsov D.S., Kaprelyants A.S., Savitsky A.P., Shleeva M.O. The accumulation of methylated porphyrins in dormant cells of *Mycobacterium smegmatis* is accompanied by a decrease in membrane fluidity and an impede of the functioning of the respiratory chain. *Biochim. Biophys. Acta - Biomembr.* – 2024. – Vol. 1866. – P. 184270. doi:10.1016/j.bbamem.2024.184270.
14. Shleeva M.O., Linge I.A., Gligonov I.A., Vostroknutova G.N., Shashin D.M., Tsedilin A.M., Apt A.S., Kaprelyants A.S., Savitsky A.P. Acquiring of photosensitivity by *Mycobacterium tuberculosis* in vitro and inside infected macrophages is associated with accumulation of endogenous Zn-porphyrins. *Sci. Rep.* – 2024. – Vol. 14. – P. 846. doi:10.1038/s41598-024-51227-z.
15. Shleeva M.O., Kudykina Y.K., Vostroknutova G.N., Suzina N.E., Mulyukin A.L., Kaprelyants A.S. Dormant ovoid cells of *Mycobacterium tuberculosis* are formed in response to gradual external acidification. *Tuberculosis*. – 2011. – Vol. 91. – P. 146-154. doi:10.1016/j.tube.2010.12.006.
16. Khan M.Z., Nandicoori V.K. Deletion of *pknG* abates reactivation of latent *Mycobacterium tuberculosis* in mice. *Antimicrob. Agents Chemother.* – 2021. – Vol. 65. – P. e02095-20. doi:10.1128/AAC.02095-20.
17. Vilch  ze C., Kremer L. Acid-fast positive and acid-fast negative *Mycobacterium tuberculosis*: The Koch paradox. *Microbiol. Spectr.* – 2017. – Vol. 5. – P. TBTB2-0003-2015. doi:10.1128/microbiolspec.
18. Cunningham A.F., Ashton P.R., Spreadbury C.L., Lammass D.A., Craddock R., Wharton C.W., Wheeler P.R. Tubercle bacilli generate a novel cell wall-associated pigment after long-term anaerobic culture. *FEMS Microbiol. Lett.* – 2004. – Vol. 235. – P. 191-198. doi:10.1016/j.femsle.2004.04.036.
19. Berney M., Cook G.M. Unique flexibility in energy metabolism allows mycobacteria to combat starvation and hypoxia. *PLoS One*. – 2010. – Vol. 5. – P. e8614. doi:10.1371/journal.pone.0008614.
20. Nikitushkin V.D., Shleeva M.O., Zinin A.I., Trutneva K.A., Ostrovsky D.N., Kaprelyants A.S. The main pigment of the dormant *Mycobacterium smegmatis* is porphyrin. *FEMS Microbiol. Lett.* – 2016. – Vol. 363. – P. 1-8. doi:10.1093/femsle/fnw206.
21. Thunell S. Porphyrins, porphyrin metabolism and porphyrias. I. Update. *Scand. J. Clin. Lab. Invest.* – 2000. – Vol. 60. – P. 509-540. doi:10.1080/003655100448310.

22. Dailey H.A., Gerdes S., Dailey T.A., Burch J.S., Phillips J.D. Noncanonical coproporphyrin-dependent bacterial heme biosynthesis pathway that does not use protoporphyrin. *Proc. Natl. Acad. Sci. USA*, 2015, vol. 112, pp. 2210-2215. doi:10.1073/pnas.1416285112.
23. Bagaeva D.I., Demina G.R., Panina I.S., Krylov N.A., Khrenova M.G., Agaphonov M.O., Savitsky A.P., Efremov R.G., Kaprelyants A.S., Shleeva M.O. Methyltransferase CPmtA drives dormancy-associated synthesis of coproporphyrin III tetramethyl ether via a "carousel" mechanism in *Mycobacterium smegmatis*. *Int. J. Biol. Macromol.*, 2026, vol. 358, pp. 151714. doi:10.1016/j.ijbiomac.2026.151714.
24. Hamza I., Chauhan S., Hassett R., O'Brian M.R. The bacterial Irr protein is required for coordination of heme biosynthesis with iron availability. *J. Biol. Chem.*, 1998, vol. 273, pp. 21669-21674. doi:10.1074/jbc.273.34.21669.
25. Wang M., Wang Y., Wang M., Liu M., Cheng A. Heme acquisition and tolerance in gram-positive model bacteria: An orchestrated balance. *Heliyon*, 2023, vol. 9, pp. e18233. doi:10.1016/j.heliyon.2023.e18233.
26. Wilks A., Egoshi R. Heme trafficking and the importance of handling nature's most versatile cofactor. *Chem. Rev.*, 2025, vol. 125, pp. 11358-11378. doi:10.1021/acs.chemrev.5c00535.
27. Nikitushkin V.D., Trenkamp S., Demina G.R., Shleeva M.O., Kaprelyants A.S. Metabolic profiling of dormant *Mycobacterium smegmatis* cells' reactivation reveals a gradual assembly of metabolic processes. *Metabolomics*, 2020, vol. 16, pp. 1-14. doi:10.1007/s11306-020-1645-8.
28. Касаткина С.И., Багаева Д.И., Демина Г.Р., Вострокнутова Г.Н., Савицкий А.П., Шлеева М.О. Повышенная экспрессия сирогемсинтазы нарушает метаболизм тетрапирролов и вызывает фотодинамическую чувствительность у *Escherichia coli* и *Mycobacterium smegmatis*. *Биотехнология*, 2026, vol. 42, pp. 24-33. doi:10.56304/S0234275826010035.
29. Stroupe M.E., Leech H.K., Daniels D.S., Warren M.J., Getzoff E.D. CysG structure reveals tetrapyrrole-binding features and novel regulation of siroheme biosynthesis. *Nat. Struct. Biol.*, 2003, vol. 10, pp. 1064-1073. doi:10.1038/nsb1007.
30. Pennington J.M., Kemp M., McGarry L., Chen Y., Stroupe M.E. Siroheme synthase orients substrates for dehydrogenase and chelatase activities in a common active site. *Nat. Commun.*, 2020, vol. 11, pp. 864. doi:10.1038/s41467-020-14722-1.
31. Albrethsen J., Agner J., Piersma S.R., Højrup P., Pham T.V., Weldingh K., Jimenez C.R., Andersen P., Rosenkrands I. Proteomic profiling of *Mycobacterium tuberculosis* identifies nutrient-starvation-responsive toxin-antitoxin systems. *Mol. Cell Proteomics*, 2013, vol. 12, pp. 1180-1191. doi:10.1074/mcp.M112.018846.
32. Fukuhara H., Inoue K., Kurabayashi A., Furihata M., Fujita H., Utsumi K., Sasaki J., Shuin T. The inhibition of ferrochelatase enhances 5-aminolevulinic acid-based photodynamic action for prostate cancer. *Photodiagnosis Photodyn. Ther.*, 2013, vol. 10, pp. 399-409. doi:10.1016/j.pdpdt.2013.03.003.
33. Karginov A.V., Alexandrov A.I., Kushnirov V.V., Agaphonov M.O. Perturbations in the heme and siroheme biosynthesis pathways causing accumulation of fluorescent free base porphyrins and auxotrophy in *Ogataea* yeasts. *J. Fungi*, 2021, vol. 7, pp. 884. doi:10.3390/jof7100884.
34. Sikora (née Wójcik) A., Grzesiuk E., Zbiec R., Janion C. Lethality of visible light for *Escherichia coli* hemH1 mutants influence of defects in DNA repair. *DNA Repair (Amst.)*, 2003, vol. 2, pp. 61-71. doi:10.1016/s1568-7864(02)00186-6.
35. Mancini S., Imlay J.A. The induction of two biosynthetic enzymes helps *Escherichia coli* sustain heme synthesis and activate catalase during hydrogen peroxide stress. *Mol. Microbiol.*, 2015, vol. 96, pp. 744-763. doi:10.1111/mmi.12967.
36. Djoko K.Y., McEwan A.G. Antimicrobial action of copper is amplified via inhibition of heme biosynthesis. *ACS Chem. Biol.*, 2013, vol. 8, pp. 2217-2223. doi:10.1021/cb4002443.
37. Dailey T.A., Boynton T.O., Albetel A., Gerdes S., Johnson M.K., Dailey H.A. Discovery and characterization of HemQ. *J. Biol. Chem.*, 2010, vol. 285, pp. 25978-25986. doi:10.1074/jbc.M110.142604.
38. Roessner C.A. Use of cobA and cysGA as red fluorescent indicators. *Methods Mol. Biol.*, 2002, vol. 183, pp. 19-30. doi:10.1385/1-59259-280-5:019.
22. Dailey H.A., Gerdes S., Dailey T.A., Burch J.S., Phillips J.D. Noncanonical coproporphyrin-dependent bacterial heme biosynthesis pathway that does not use protoporphyrin. *Proc. Natl. Acad. Sci. U.S.A.* – 2015. – Vol. 112. – P. 2210-2215. doi:10.1073/pnas.1416285112.
23. Bagaeva D.I., Demina G.R., Panina I.S., Krylov N.A., Khrenova M.G., Agaphonov M.O., Savitsky A.P., Efremov R.G., Kaprelyants A.S., Shleeva M.O. Methyltransferase CPmtA drives dormancy-associated synthesis of coproporphyrin III tetramethyl ether via a "carousel" mechanism in *Mycobacterium smegmatis*. *Int. J. Biol. Macromol.* – 2026. – Vol. 358. – P. 151714. doi:10.1016/j.ijbiomac.2026.151714.
24. Hamza I., Chauhan S., Hassett R., O'Brian M.R. The bacterial Irr protein is required for coordination of heme biosynthesis with iron availability. *J. Biol. Chem.* – 1998. – Vol. 273. – P. 21669-21674. doi:10.1074/jbc.273.34.21669.
25. Wang M., Wang Y., Wang M., Liu M., Cheng A. Heme acquisition and tolerance in gram-positive model bacteria: An orchestrated balance. *Heliyon*. – 2023. – Vol. 9. – P. e18233. doi:10.1016/j.heliyon.2023.e18233.
26. Wilks A., Egoshi R. Heme trafficking and the importance of handling nature's most versatile cofactor. *Chem. Rev.* – 2025. – Vol. 125. – P. 11358-11378. doi:10.1021/acs.chemrev.5c00535.
27. Nikitushkin V.D., Trenkamp S., Demina G.R., Shleeva M.O., Kaprelyants A.S. Metabolic profiling of dormant *Mycobacterium smegmatis* cells' reactivation reveals a gradual assembly of metabolic processes. *Metabolomics*. – 2020. – Vol. 16. – P. 1-14. doi:10.1007/s11306-020-1645-8.
28. Касаткина С.И., Багаева Д.И., Демина Г.Р., Вострокнутова Г.Н., Савицкий А.П., Шлеева М.О. Повышенная экспрессия сирогемсинтазы нарушает метаболизм тетрапирролов и вызывает фотодинамическую чувствительность у *Escherichia coli* и *Mycobacterium smegmatis*. *Биотехнология*. – 2026. – Vol. 42. – P. 24-33. doi:10.56304/S0234275826010035.
29. Stroupe M.E., Leech H.K., Daniels D.S., Warren M.J., Getzoff E.D. CysG structure reveals tetrapyrrole-binding features and novel regulation of siroheme biosynthesis. *Nat. Struct. Biol.* – 2003. – Vol. 10. – P. 1064-1073. doi:10.1038/nsb1007.
30. Pennington J.M., Kemp M., McGarry L., Chen Y., Stroupe M.E. Siroheme synthase orients substrates for dehydrogenase and chelatase activities in a common active site. *Nat. Commun.* – 2020. – Vol. 11. – P. 864. doi:10.1038/s41467-020-14722-1.
31. Albrethsen J., Agner J., Piersma S.R., Højrup P., Pham T.V., Weldingh K., Jimenez C.R., Andersen P., Rosenkrands I. Proteomic profiling of *Mycobacterium tuberculosis* identifies nutrient-starvation-responsive toxin-antitoxin systems. *Mol. Cell Proteomics*. – 2013. – Vol. 12. – P. 1180-1191. doi:10.1074/mcp.M112.018846.
32. Fukuhara H., Inoue K., Kurabayashi A., Furihata M., Fujita H., Utsumi K., Sasaki J., Shuin T. The inhibition of ferrochelatase enhances 5-aminolevulinic acid-based photodynamic action for prostate cancer. *Photodiagnosis Photodyn. Ther.* – 2013. – Vol. 10. – P. 399-409. doi:10.1016/j.pdpdt.2013.03.003.
33. Karginov A. V., Alexandrov A.I., Kushnirov V. V., Agaphonov M.O. Perturbations in the heme and siroheme biosynthesis pathways causing accumulation of fluorescent free base porphyrins and auxotrophy in *Ogataea* yeasts. *J. Fungi*. – 2021. – Vol. 7. – P. 884. doi:10.3390/jof7100884.
34. Sikora (née Wójcik) A., Grzesiuk E., Zbiec R., Janion C. Lethality of visible light for *Escherichia coli* hemH1 mutants influence of defects in DNA repair. *DNA Repair (Amst.)*. – 2003. – Vol. 2. – P. 61-71. doi:10.1016/s1568-7864(02)00186-6.
35. Mancini S., Imlay J.A. The induction of two biosynthetic enzymes helps *Escherichia coli* sustain heme synthesis and activate catalase during hydrogen peroxide stress. *Mol. Microbiol.* – 2015. – Vol. 96. – P. 744-763. doi:10.1111/mmi.12967.
36. Djoko K.Y., McEwan A.G. Antimicrobial action of copper is amplified via inhibition of heme biosynthesis. *ACS Chem. Biol.* – 2013. – Vol. 8. – P. 2217-2223. doi:10.1021/cb4002443.
37. Dailey T.A., Boynton T.O., Albetel A., Gerdes S., Johnson M.K., Dailey H.A. Discovery and characterization of HemQ. *J. Biol. Chem.* – 2010. – Vol. 285. – P. 25978-25986. doi:10.1074/jbc.M110.142604.
38. Roessner C.A. Use of cobA and cysGA as red fluorescent indicators. *Methods Mol. Biol.* – 2002. – Vol. 183. – P. 19-30. doi:10.1385/1-59259-280-5:019.

39. Wildt S., Deuschle U. *cobA* a red fluorescent transcriptional reporter for *Escherichia coli*, yeast and mammalian cells. *Nat. Biotechnol.*, 1999, vol. 17, pp. 1175-1178. doi:10.1038/70713.
40. Zdubek A., Maliszewska I. On the possibility of using 5-aminolevulinic acid in the light-induced destruction of microorganisms. *Int. J. Mol. Sci.*, 2024, vol. 25, pp. 3590. doi:10.3390/ijms25073590.
41. Shleeva M.O., Savitsky A.P., Nikitushkin V.D., Soloviyev I.D., Kazachkina N.I., Perevarov V.V., Kaprelyants A.S. Photoinactivation of dormant *Mycobacterium smegmatis* due to its endogenous porphyrins. *Appl. Microbiol. Biotechnol.*, 2019, vol. 103, pp. 9687-9695. doi:10.1007/s00253-019-10197-3.
42. Shleeva M.O., Savitsky A.P., Nikitushkin V.D., Soloviev I.D., Trutneva K.A. Effect of photodynamic inactivation against dormant forms and active growing cells of *Mycobacterium smegmatis*. *Appl. Biochem. Microbiol.*, 2020, vol. 56, pp. 242-249. doi:10.1134/S000368382003014X.
43. Shashin D.M., Demina G.R., Linge I.A., Vostroknutova G.N., Kaprelyants A.S., Savitsky A.P., Shleeva M.O. The effect of antimicrobial photodynamic inactivation on the protein profile of dormant *Mycobacterium smegmatis* containing endogenous porphyrins. *Int. J. Mol. Sci.*, 2023, vol. 24, pp. 13968. doi:10.3390/ijms241813968.
44. Ashkenazi H., Malik Z., Harth Y., Nitzan Y. Eradication of *Propionibacterium acnes* by its endogenic porphyrins after illumination with high intensity blue light. *FEMS Immunol. Med. Microbiol.*, 2003, vol. 35, pp. 17-24. doi:10.1016/S0928-8244(02)00423-6.
45. Bhargava S., Listopadzki T., Diletti S., Crane J.K., Duquin T.R., Boyle K.K. Effect of blue light and photosensitizers on *Cutibacterium acnes* on shoulder periprosthetic joint infection isolates. *J. Bone Jt. Infect.*, 2020, vol. 5, pp. 187-197. doi:10.7150/jbji.46199.
46. Fyrestam J., Bjurshammar N., Paulsson E., Mansouri N., Johannsen A., Östman C. Influence of culture conditions on porphyrin production in *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*. *Photodiagnosis Photodyn. Ther.*, 2017, vol. 17, pp. 115-123. doi:10.1016/j.pdpdt.2016.11.001.
47. Yoshida A., Sasaki H., Toyama T., Araki M., Fujioka J., Tsukiyama K., Hamada N., Yoshino F. Antimicrobial effect of blue light using *Porphyromonas gingivalis* pigment. *Sci. Rep.*, 2017, vol. 7, pp. 5225. doi:10.1038/s41598-017-05706-1.
48. Soukos N.S., Som S., Abernethy A.D., Ruggiero K., Dunham J., Lee C., Doukas A.G., Goodson J.M. Phototargeting oral black-pigmented bacteria. *Antimicrob. Agents Chemother.*, 2005, vol. 49, pp. 1391-1396. doi:10.1128/AAC.49.4.1391.
49. Gábor F., Szocs K., Maillard P., Csik G. Photobiological activity of exogenous and endogenous porphyrin derivatives in *Escherichia coli* and *Enterococcus hirae* cells. *Radiat. Environ. Biophys.*, 2001, vol. 40, pp. 145-151. doi:10.1007/s004110100092.
50. Hamblin M.R., Viveiros J., Yang C., Ahmadi A., Ganz R.A., Tolkoff M.J. *Helicobacter pylori* accumulates photoactive porphyrins and is killed by visible light. *Antimicrob. Agents Chemother.*, 2005, vol. 49, pp. 2822-2827. doi:10.1128/AAC.49.7.2822-2827.2005.
51. Lembo A.J., Ganz R.A., Sheth S., Cave D., Kelly C., Levin P., Kazlas P.T., Baldwin P.C. 3rd, Lindmark W.R., Mcgrath J.R., Hamblin M.R. Treatment of *Helicobacter pylori* infection with intra-gastric violet light phototherapy: A pilot clinical trial. *Lasers Surg. Med.*, 2009, vol. 41, pp. 337-344. doi:10.1002/lsm.20770.
52. Battisti A., Morici P., Signore G., Ghetti F., Sgarbossa A. Compositional analysis of endogenous porphyrins from *Helicobacter pylori*. *Biophys. Chem.*, 2017, vol. 229, pp. 25-30. doi:10.1016/j.bpc.2017.06.006.
53. Ganz R.A., Viveiros J., Ahmad A., Ahmadi A., Khalil A., Tolkoff M.J., Nishioka N.S., Hamblin M.R. *Helicobacter pylori* in patients can be killed by visible light. *Lasers Surg. Med.*, 2005, vol. 36, pp. 260-265. doi:10.1002/lsm.20161.
54. Sailer R., Strauss W.S.L., Kiinig K., Riick A., Steiner R. Correlation between porphyrin biosynthesis and photodynamic inactivation of *Pseudomonas aeruginosa* after incubation with 5-aminolevulinic acid. *J. Photochem. Photobiol. B: Biol.*, 1997, vol. 39, pp. 236-242. doi:10.1016/S1011-1344(96)00019-X.
55. Bilal M., Anjum R., Imtiaz S., Saleem M. Exploiting endogenous protoporphyrin IX for photodynamic inactivation of *Pseudomonas*
39. Wildt S., Deuschle U. *cobA* a red fluorescent transcriptional reporter for *Escherichia coli*, yeast and mammalian cells // *Nat. Biotechnol.* – 1999. – Vol. 17. – P. 1175-1178. doi:10.1038/70713.
40. Zdubek A., Maliszewska I. On the possibility of using 5-aminolevulinic acid in the light-induced destruction of microorganisms // *Int. J. Mol. Sci.* – 2024. – Vol. 25. – P. 3590. doi:10.3390/ijms25073590.
41. Shleeva M.O., Savitsky A.P., Nikitushkin V.D., Soloviyev I.D., Kazachkina N.I., Perevarov V.V., Kaprelyants A.S. Photoinactivation of dormant *Mycobacterium smegmatis* due to its endogenous porphyrins // *Appl. Microbiol. Biotechnol.* – 2019. – Vol. 103. – P. 9687-9695. doi:10.1007/s00253-019-10197-3.
42. Shleeva M.O., Savitsky A.P., Nikitushkin V.D., Soloviev I.D., Trutneva K.A. Effect of photodynamic inactivation against dormant forms and active growing cells of *Mycobacterium smegmatis* // *Appl. Biochem. Microbiol.* – 2020. – Vol. 56. – P. 242-249. doi:10.1134/S000368382003014X.
43. Shashin D.M., Demina G.R., Linge I.A., Vostroknutova G.N., Kaprelyants A.S., Savitsky A.P., Shleeva M.O. The effect of antimicrobial photodynamic inactivation on the protein profile of dormant *Mycobacterium smegmatis* containing endogenous porphyrins // *Int. J. Mol. Sci.* – 2023. – Vol. 24. – P. 13968. doi:10.3390/ijms241813968.
44. Ashkenazi H., Malik Z., Harth Y., Nitzan Y. Eradication of *Propionibacterium acnes* by its endogenic porphyrins after illumination with high intensity blue light FEMS Immunol. Med. Microbiol. – 2003. – Vol. 35. – P. 17-24. doi:10.1016/S0928-8244(02)00423-6.
45. Bhargava S., Listopadzki T., Diletti S., Crane J.K., Duquin T.R., Boyle K.K. Effect of blue light and photosensitizers on *Cutibacterium acnes* on shoulder periprosthetic joint infection isolates // *J. Bone Jt. Infect.* – 2020. – Vol. 5. – P. 187-197. doi:10.7150/jbji.46199.
46. Fyrestam J., Bjurshammar N., Paulsson E., Mansouri N., Johannsen A., Östman C. Influence of culture conditions on porphyrin production in *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* // *Photodiagnosis Photodyn. Ther.* – 2017. – Vol. 17. – P. 115-123. doi:10.1016/j.pdpdt.2016.11.001.
47. Yoshida A., Sasaki H., Toyama T., Araki M., Fujioka J., Tsukiyama K., Hamada N., Yoshino F. Antimicrobial effect of blue light using *Porphyromonas gingivalis* pigment // *Sci. Rep.* – 2017. – Vol. 7. – P. 5225. doi:10.1038/s41598-017-05706-1.
48. Soukos N.S., Som S., Abernethy A.D., Ruggiero K., Dunham J., Lee C., Doukas A.G., Goodson J.M. Phototargeting oral black-pigmented bacteria // *Antimicrob. Agents Chemother.* – 2005. – Vol. 49. – P. 1391-1396. doi:10.1128/AAC.49.4.1391.
49. Gábor F., Szocs K., Maillard P., Csik G. Photobiological activity of exogenous and endogenous porphyrin derivatives in *Escherichia coli* and *Enterococcus hirae* cells // *Radiat. Environ. Biophys.* – 2001. – Vol. 40. – P. 145-151. doi:10.1007/s004110100092.
50. Hamblin M.R., Viveiros J., Yang C., Ahmadi A., Ganz R.A., Tolkoff M.J. *Helicobacter pylori* accumulates photoactive porphyrins and is killed by visible light // *Antimicrob. Agents Chemother.* – 2005. – Vol. 49. – P. 2822-2827. doi:10.1128/AAC.49.7.2822-2827.2005.
51. Lembo A.J., Ganz R.A., Sheth S., Cave D., Kelly C., Levin P., Kazlas P.T., Baldwin P.C. 3rd, Lindmark W.R., Mcgrath J.R., Hamblin M.R. Treatment of *Helicobacter pylori* infection with intra-gastric violet light phototherapy: A pilot clinical trial // *Lasers Surg. Med.* – 2009. – Vol. 41. – P. 337-344. doi:10.1002/lsm.20770.
52. Battisti A., Morici P., Signore G., Ghetti F., Sgarbossa A. Compositional analysis of endogenous porphyrins from *Helicobacter pylori* // *Biophys. Chem.* – 2017. – Vol. 229. – P. 25-30. doi:10.1016/j.bpc.2017.06.006.
53. Ganz R.A., Viveiros J., Ahmad A., Ahmadi A., Khalil A., Tolkoff M.J., Nishioka N.S., Hamblin M.R. *Helicobacter pylori* in patients can be killed by visible light // *Lasers Surg. Med.* – 2005. – Vol. 36. – P. 260-265. doi:10.1002/lsm.20161.
54. Sailer R., Strauss W.S.L., Kiinig K., Riick A., Steiner R. Correlation between porphyrin biosynthesis and photodynamic inactivation of *Pseudomonas aeruginosa* after incubation with 5-aminolevulinic acid // *J. Photochem. Photobiol. B: Biol.* – 1997. – Vol. 39. – P. 236-242. doi:10.1016/S1011-1344(96)00019-X.
55. Bilal M., Anjum R., Imtiaz S., Saleem M. Exploiting endogenous protoporphyrin IX for photodynamic inactivation of *Pseudomonas*

- aeruginosa*. *J. Fluoresc.*, 2026, vol. 36, pp. 2993-3004. doi:10.1007/s10895-026-04747-y.
56. Polmickaite-Smirnova E., Buchovec I., Bagdonas S., Suziedelienė E., Ramanavicius A., Anusevicius Z. Photoinactivation of *Salmonella enterica* exposed to 5-aminolevulinic acid: Impact of sensitization conditions and irradiation time. *J. Photochem. Photobiol. B: Biol.*, 2022, vol. 231, pp. 112446. doi:10.1016/j.jphotobiol.2022.112446.
 57. van der Meulen F.W., Ibrahim K., Sterenborg H.J., Alphen L.V., Maikoe A., Dankert J. Photodynamic destruction of *Haemophilus parainfluenzae* by endogenously produced porphyrins. *J. Photochem. Photobiol. B: Biol.*, 1997, vol. 40, pp. 204-208. doi:10.1016/S1011-1344(97)00057-2.
 58. Shleeva M., Savitsky A., Kaprelyants A. *Corynebacterium jeikeium* dormant cell formation and photodynamic inactivation. *Front. Microbiol.*, 2020, vol. 11, pp. 605899. doi:10.3389/fmicb.2020.605899.
 59. Alves G.B., Marques C., Nunes E., Oliveira L. De, Sanches L., Mattos-Guaraldi A.L. De Photodynamic inactivation mediated by endogenous porphyrins of *Corynebacterium diphtheriae* in planktonic and biofilm forms. *ACS Omega*, 2025, vol. 10, pp. 9177-9186. doi:10.1021/acsomega.4c09308.
 60. Kruszevska-Naczek B., Burzynska N., Goik D., Grinholc M., Nakonieczna J., Pawlik N., Pieranski M.K., Wozniak-Pawlikowska A., Rapacka-Zdonczyk A., Dai T. Mechanisms and determinants of bacterial susceptibility to antimicrobial blue light: From chromophores to transcriptomes. *Drug Resist. Updat.*, 2026, vol. 86, pp. 101391. doi:10.1016/j.drup.2026.101391.
 61. Seo H., Dai T. Unveiling the microbial bullseyes: Microbial target of antimicrobial blue light. In: Hamblin M.R. (eds) Handbook of Antimicrobial Photoinactivation. *Springer Cham*, 2026, pp. 1-21. doi:10.1007/978-3-031-55858-0_114-1.
 62. Plavskii V.Y., Mikulich A.V., Tretyakova A.I., Leusenka I.A., Plavskaya L.G., Kazyuchits O.A., Dobysh I.I., Krasnenkova T.P. Porphyrins and flavins as endogenous acceptors of optical radiation of blue spectral region determining photoinactivation of microbial cells. *J. Photochem. Photobiol. B: Biol.*, 2018, vol. 183, pp. 172-183. doi:10.1016/j.jphotobiol.2018.04.021.
 63. Hessling M., Wenzel U., Meurle T., Spellerberg B., A K.H. Photoinactivation results of *Enterococcus moraviensis* with blue and violet light suggest the involvement of an unconsidered photosensitizer. *Biochem. Biophys. Res. Commun.*, 2020, vol. 533, pp. 813-817. doi:10.1016/j.bbrc.2020.09.091.
 - aeruginosa* // *J. Fluoresc.* – 2026. – Vol. 36. – P. 2993-3004. doi:10.1007/s10895-026-04747-y.
 56. Polmickaite-Smirnova E., Buchovec I., Bagdonas S., Suziedelienė E., Ramanavicius A., Anusevicius Z. Photoinactivation of *Salmonella enterica* exposed to 5-aminolevulinic acid: Impact of sensitization conditions and irradiation time // *J. Photochem. Photobiol. B: Biol.* – 2022. – Vol. 231. – P. 112446. doi:10.1016/j.jphotobiol.2022.112446.
 57. van der Meulen F.W., Ibrahim K., Sterenborg H.J., Alphen L. V, Maikoe A., Dankert J. Photodynamic destruction of *Haemophilus parainfluenzae* by endogenously produced porphyrins // *J. Photochem. Photobiol. B: Biol.* – 1997. – Vol. 40. – P. 204-208. doi:10.1016/S1011-1344(97)00057-2.
 58. Shleeva M., Savitsky A., Kaprelyants A. *Corynebacterium jeikeium* dormant cell formation and photodynamic inactivation // *Front. Microbiol.* – 2020. – Vol. 11. – P. 605899. doi:10.3389/fmicb.2020.605899.
 59. Alves G.B., Marques C., Nunes E., Oliveira L. De, Sanches L., Mattos-guaraldi A.L. De Photodynamic inactivation mediated by endogenous porphyrins of *Corynebacterium diphtheriae* in planktonic and biofilm forms // *ACS Omega*. – 2025. – Vol. 10. – P. 9177-9186. doi:10.1021/acsomega.4c09308.
 60. Kruszevska-Naczek B., Burzynska N., Goik D., Grinholc M., Nakonieczna J., Pawlik N., Pieranski M.K., Wozniak-Pawlikowska A., Rapacka-Zdonczyk A., Dai T. Mechanisms and determinants of bacterial susceptibility to antimicrobial blue light: From chromophores to transcriptomes // *Drug Resist. Updat.* – 2026. – Vol. 86. – P. 101391. doi:10.1016/j.drup.2026.101391.
 61. Seo H., Dai T. Unveiling the Microbial Bullseyes: Microbial Target of Antimicrobial Blue Light. In: Hamblin M.R. (eds) Handbook of Antimicrobial Photoinactivation // *Springer, Cham.* – 2026. – P. 1-21. doi:10.1007/978-3-031-55858-0_114-1.
 62. Plavskii V.Y., Mikulich A. V, Tretyakova A.I., Leusenka I.A., Plavskaya L.G., Kazyuchits O.A., Dobysh I.I., Krasnenkova T.P. Porphyrins and flavins as endogenous acceptors of optical radiation of blue spectral region determining photoinactivation of microbial cells // *J. Photochem. Photobiol. B: Biol.* – 2018. – Vol. 183. – P. 172-183. doi:10.1016/j.jphotobiol.2018.04.021.
 63. Hessling M., Wenzel U., Meurle T., Spellerberg B., A K.H. Photoinactivation results of *Enterococcus moraviensis* with blue and violet light suggest the involvement of an unconsidered photosensitizer // *Biochem. Biophys. Res. Commun.* – 2020. – Vol. 533. – P. 813-817. doi:10.1016/j.bbrc.2020.09.091.

ФОТОСЕНСИБИЛИЗАТОР ХЛОРИНОВОГО РЯДА ДЛЯ ФОТОДИНАМИЧЕСКОЙ ТЕРАПИИ



«ФОТОДИТАЗИН®» концентрат для приготовления раствора для инфузий — лекарственное средство РУ ЛП-№(007290)-(РГ-RU) от 17.10.2024 г.

«ФОТОДИТАЗИН®» применяется для флуоресцентной диагностики и фотодинамической терапии онкологических заболеваний различных нозологических форм, а также патологий неонкологического характера в следующих областях медицины:

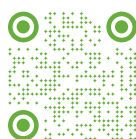
- | | | |
|------------------------|--------------------|-----------------------------|
| ✓ нейрохирургия | ✓ урология | ✓ дерматология |
| ✓ гинекология | ✓ стоматология | ✓ комбустиология |
| ✓ торакальная хирургия | ✓ гнойная хирургия | ✓ травматология и ортопедия |

Фотодинамическая терапия внесена в клинические рекомендации МЗ РФ.

Фотодинамическая терапия включена в перечень видов высокотехнологичной медицинской помощи Программы государственных гарантий бесплатного оказания гражданам медицинской помощи, утверждаемой Правительством РФ.

ООО «ВЕТА-ГРАНД»

123056, г. Москва, ул. Красина, д. 27, стр. 2
Тел.: +7 (929) 971-44-46, +7 (499) 250-40-00
E-mail: veta-grand@mail.ru



fotoditazin.com
фотодитазин.рф