South Russian Journal of Cancer. 2025. Vol. 6, No. 3. P. 6-15
https://doi.org/10.37748/2686-9039-2025-6-3-1
https://elibrary.ru/pskoqy
ORIGINAL ARTICLE

Prognostic significance of AKT/mTOR signaling pathway components, transcription factors, and growth factors in the development of skin melanoma recurrence

V. A. Bogdanova[1], L. V. Spirina[1,2]✉, S. Yu. Chizhevskaya[2], I. V. Kovaleva[1,3], K. V. Nikulnikov[2], E. D. Merkulov[1]

[1] Siberian State Medical University, Tomsk, Russian Federation
[2] Cancer Research Institute – branch of the Federal State Budgetary Scientific Institution "Tomsk National Research Medical Center of the Russian Academy of Sciences", Tomsk, Russian Federation
[3] Voronezh State Medical University named after N. N. Burdenko, Voronezh, Russian Federation
✉ spirinalvl@mail.ru

ABSTRACT

Purpose of the study. Was to investigate the prognostic significance of the AKT/mTOR signaling pathway components, transcription, and growth factors in the development of skin melanoma recurrence.

Patients and methods. The study included 48 patients with a verified diagnosis of skin melanoma. All patients had a negative status for the $BRAF^{V600E}$ mutation.

The study material consisted of samples of tumor and unchanged skin located at a distance of at least 1 cm from the tumor border, obtained during surgical treatment, which were frozen and stored at a temperature of –80 °C after sampling. Gene expression was assessed by real-time PCR. The status of the BRAF gene was assessed using allele-specific PCR. Statistical processing was carried out using the Statistica 12.0 software package.

Results. Predicting the course of cancer is an important task in practical oncology. The unfavorable outcome of melanomas is largely due to the tendency to relapses and metastasis in both the short and long-term follow-up period. While analyzing the study results, we noted the association of AKT/mTOR signaling pathway components with the development of skin melanoma recurrence and disease progression. It was revealed that the expression level of AKT, c-RAF, GSK-3β and VEGFR2 is associated with an unfavorable outcome of the disease. A logistic regression model is presented that can accurately predict the risk of recurrence, taking into account the expression of protein kinase mTOR and the size of the tumor (T). The lack of significant indicators related to the biological features of the skin tumor does not allow to increase the effectiveness of treatment of such patients. Therefore, the attention of researchers is focused on finding optimal models for predicting the risk of an unfavorable outcome of the disease.

Conclusion. Molecular markers were identified that make it possible to predict an unfavorable outcome of the disease during the study. A logistic model based on the expression of the key mTOR kinase and the size of the T has been developed, which makes it possible to assess the risk of disease recurrence and change treatment tactics in a timely manner.

Keywords: AKT, c-RAF, GSK-3β, VEGFR2, mTOR, skin melanoma, prognosis

For citation: Bogdanova V. A., Spirina L. V., Chizhevskaya S. Yu., Kovaleva I. V., Nikulnikov K. V., Merkulov E. D. Prognostic significance of AKT/mTOR signaling pathway components, transcription factors, and growth factors in the development of skin melanoma recurrence. South Russian Journal of Cancer. 2025; 6(3): 6-15. https://doi.org/10.37748/2686-9039-2025-6-3-1, https://elibrary.ru/pskoqy

For correspondence: Liudmila V. Spirina – Dr. Sci. (Med.), Leading Researcher at the Laboratory of Tumor Biochemistry, Cancer Research Institute – branch of the Federal State Budgetary Scientific Institution "Tomsk National Research Medical Center of the Russian Academy of Sciences", Tomsk, Russian Federation; Head of the Department of Biochemistry and Molecular Biology with a course in clinical laboratory diagnostics, Siberian State Medical University, Tomsk, Russian Federation
Address: 5 Kooperativny str., Tomsk 634050, Russian Federation
Address: 2 Moskovsky trakt, Tomsk 634050, Russian Federation
E-mail: spirinalvl@mail.ru
ORCID: https://orcid.org/0000-0002-5269-736X
SPIN: 1336-8363, AuthorID: 441893
ResearcherID: A-7760-2012
Scopus Author ID: 36960462500

Compliance with ethical standards: the study is carried out in compliance with the ethical principles set forth in the World Medical Association Declaration of Helsinki, 1964, ed. 2013. The study was approved by the Committee on Biomedical Ethics at the Scientific Research Institute of Oncology, Tomsk Scientific Research Medical Center (extract from the protocol of meeting No. 5 dated 11/26/2023). Informed consent was obtained from all participants of the study

Funding: this work was not funded

Conflict of interest: the authors declare that there are no obvious and potential conflicts of interest associated with the publication of this article

The article was submitted 05.01.2025; approved after reviewing 07.04.2025; accepted for publication 05.08.2025

Южно-Российский онкологический журнал. 2025. Т. 6, № 3. С. 6-15
https://doi.org/10.37748/2686-9039-2025-6-3-1
https://elibrary.ru/pskoqy
3.1.6. Онкология, лучевая терапия
ОРИГИНАЛЬНАЯ СТАТЬЯ

Прогностическая значимость компонентов AKT/mTOR сигнального пути, транскрипционных и ростовых факторов в развитии рецидивов меланомы кожи

В. А. Богданова[1], Л. В. Спирина[1,2]✉, С. Ю. Чижевская[2], И. В. Ковалева[1,3], К. В. Никульников[2], Е. Д. Меркулов[1]

[1] ФГБОУ ВО «Сибирский государственный медицинский университет» Министерства здравоохранения Российской Федерации, г. Томск, Российская Федерация
[2] Научно-исследовательский институт онкологии – филиал ФГБНУ «Томский национальный исследовательский медицинский центр Российской академии наук», г. Томск, Российская Федерация
[3] ФГБОУ ВО «Воронежский государственный медицинский университет им Н. Н. Бурденко» Министерства здравоохранения Российской Федерации, г. Воронеж, Российская Федерация
✉ spirinalvl@mail.ru

РЕЗЮМЕ

Цель исследования. Исследование прогностической значимости компонентов сигнального пути AKT/mTOR, а также транскрипционных и ростовых факторов в развитии рецидивов меланомы кожи.
Пациенты и методы. В исследование были включены 48 пациентов с верифицированным диагнозом меланома кожи. Все пациенты имели отрицательный статус по мутации $BRAF^{V600E}$.
Материал исследования – образцы опухолевой и неизмененной кожи, находящиеся на расстоянии не менее 1 см от границы опухолей, полученные при проведении оперативного лечения, которые после забора замораживались и хранились при температуре –80 °С. Экспрессия генов оценивалась методом ПЦР в реальном времени. Статус гена *BRAF* оценивали с помощью аллель-специфичной ПЦР. Статистическая обработка проводилась с помощью пакета программ Statistica 12.0
Результаты. Прогнозирование течения онкологических заболеваний является важной задачей в практической онкологии. Неблагоприятный исход меланом во многом обусловлен склонностью к возникновению рецидивов и метастазов как в ближайшем, так и в отдаленном периоде наблюдения. При анализе результатов исследования нами отмечена ассоциация компонентов AKT/mTOR сигнального пути с развитием рецидивов меланом кожи и прогрессированием заболевания. Выявлено, что уровень экспрессии AKT, c-RAF, GSK-3β и VEGFR2 связан с неблагоприятным исходом заболевания. Представлена модель логистической регрессии, которая с высокой точностью может дать прогноз риска развития рецидивов заболевания с учетом экспрессии протеинкиназы mTOR и размера опухоли (T). Отсутствие значимых показателей, связанных с биологическими особенностями опухоли кожи, не позволяет повысить эффективность лечения таких больных. Поэтому внимание исследователей сосредоточено на поиске оптимальных моделей прогноза риска неблагоприятного исхода заболевания.
Заключение. В ходе исследования были идентифицированы молекулярные маркеры, которые позволяют прогнозировать неблагоприятный исход заболевания. Разработана логистическая модель, основанная на экспрессии ключевой киназы mTOR и T, которая позволяет оценить риск возникновения рецидивов заболевания и своевременно изменить тактику лечения.

Ключевые слова: AKT, c-RAF, GSK-3β, VEGFR2, mTOR, меланома кожи, прогноз

Для цитирования: Богданова В. А., Спирина Л. В., Чижевская С. Ю., Ковалева И. В., Никульников К. В., Меркулов Е. Д. Прогностическая значимость компонентов AKT/mTOR сигнального пути, транскрипционных и ростовых факторов в развитии рецидивов меланомы кожи. Южно-Российский онкологический журнал. 2025; 6(3): 6-15. https://doi.org/10.37748/2686-9039-2025-6-3-1, https://elibrary.ru/pskoqy

Для корреспонденции: Спирина Людмила Викторовна – д.м.н., ведущий научный сотрудник лаборатории биохимии опухолей, Научно-исследовательский институт онкологии – филиал ФГБНУ «Томский национальный исследовательский медицинский центр Российской академии наук», г. Томск, Российская Федерация; заведующий кафедрой биохимии и молекулярной биологии с курсом клинической лабораторной диагностики, ФГБОУ ВО «Сибирский государственный медицинский университет» Министерства здравоохранения Российской Федерации, г. Томск, Российская Федерация
Адрес: 634050, Российская Федерация, г. Томск, пер. Кооперативный, д. 5
Адрес: 634050, Российская Федерация, г.Томск, Московский тракт, д. 2
E-mail: spirinalvl@mail.ru
ORCID: https://orcid.org/0000-0002-5269-736X, SPIN: 1336-8363, AuthorID: 441893, ResearcherID: A-7760-2012, Scopus Author ID: 36960462500

Соблюдение этических стандартов: в работе соблюдались этические принципы, предъявляемые Хельсинкской декларацией Всемирной медицинской ассоциации (World Medical Association Declaration of Helsinki, 1964, ред. 2013). Исследование одобрено Комитетом по биомедицинской этике при Научно-исследовательском институте онкологии – филиал ФГБНУ «Томский национальный исследовательский медицинский центр Российской академии наук» (выписка из протокола заседания № 5 от 26.11.2023 г.). Информированное согласие получено от всех участников исследования

Финансирование: финансирование данной работы не проводилось

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

Статья поступила в редакцию 05.01.2025; одобрена после рецензирования 07.04.2025; принята к публикации 05.08.2025

BACKGROUND

Melanoma is the most dangerous skin malignancy, which is characterized by an aggressive course. According to a study based on GLOBOCAN data, the rate of increase in the incidence of melanoma will remain unchanged from 2020, which may lead to an increase in the incidence by about 1.5 times by 2040 [1]. In 2020, about 14 % of cases of melanoma among all malignant skin tumors were recorded in Russia. At the same time, it caused the death of 70 % of patients suffering from this group of diseases [2, 3].

Melanoma occurs due to a few genetic transformations, while ultraviolet radiation often serves as a mutagenic risk factor [4]. A deep understanding of the diversity of molecular signaling pathways characteristic of different types of melanomas makes it possible to describe these pathologies more accurately and provides tools for creating treatment methods based on the effects on the signals generated by these cascades [5]. Among the key molecular genetic indicators, a mutation of the *BRAF* gene can be identified, which occurs in 60–80 % of cases of all skin malignancies [6, 7].

Modern treatments for skin melanoma are becoming increasingly available, which improves survival rates, but there are no tools available to predict the recurrence of skin melanoma. To predict the recurrence of skin melanoma in the early stages of development, the use of clinical and histological data in machine learning technologies is being considered [8]. In addition, predicting an unfavorable outcome of the disease is possible by examining molecular markers in the homogenates of tumor tissue samples and the visually unchanged tissue adjacent to it [9]. Great importance is attached to the study of genetic factors that can predict the risk of developing an unfavorable outcome of the disease [10, 11]. In general, the rationale for the use of a number of signaling cascades associated with the molecular features of tumor growth is an urgent problem in fundamental oncology [12].

Previous studies have shown the role of microRNAs in the regulation of these markers [13, 14], reflecting the intensity of oncogenesis processes. Their association with the presence of signs of invasive growth and metastasis was noted. However, in general, there are practically no approaches that allow predicting the development of an unfavorable outcome of the disease with high accuracy.

Purpose of the study: was to study the prognostic role of components of the AKT/mTOR signaling pathway, as well as transcription and growth factors in predicting skin melanoma recurrence.

PATIENTS AND METHODS

The study involved 48 patients with a confirmed diagnosis of skin melanoma. The age of the study participants ranged from 45 to 72 years, and the stages of the disease ranged from I to IV. All patients underwent surgical treatment, which included extensive excision of the skin tumor with a sentinel lymph node biopsy.

T1a N0M0 stage of the disease was detected in 10 patients (21 %), T1b N0M0 – in 12 (25 %), T2b N0M0 – in 2 (4 %), T3a N0M0 – in 11 (23 %), T3b N0M0 – in 2 (4 %), T4a N1M0 – in 9 (19 %), T4b N1M0 – in 2 (4 %).

Tumor ulceration was absent in 23 (47 %) patients, and the presence of tumor ulceration was noted in 25 (53 %) patients. The level of Clark invasion of the 1st degree was not recorded, the 2nd degree was noted in 17 (35 %); the 3rd degree – in 17 (35 %); the 4th degree – in 3 (6.5 %); the 5th degree – in 11 (23.5 %).

The Breslow tumor thickness of less than 0.75 mm was recorded in 6 (12 %), from 0.75 mm to 1.5 mm was observed in 20 (41 %), from 1.51 to 3.0 mm – in 2 (6 %), from 3.01 mm to 4.0 mm – in 6 (12 %), from 4.01 mm and more than 14 (29 %).

It is important to note that all patients lacked the $BRAF^{V600E}$ mutation. The study was approved by the Ethics Committee of the Scientific Research Institute of Oncology, a branch of the Tomsk National Research Medical Center of the Russian Academy of Sciences. 6 patients with melanocytic nevus were selected as a control group. All procedures involving patients were conducted in accordance with the Helsinki Declaration on Human Rights (1964). Before starting the study, all participants signed an informed consent. The material for the study was samples of tumor and healthy skin obtained during surgical treatment. The samples were taken at a distance of at least 1 cm from the tumor border and frozen for subsequent storage at a temperature of –80 °C.

Isolation of RNA. RNA was isolated using the RNeasy mini-Kit containing DNAase I (Qiagen, Germany). To estimate the amount of isolated RNA, the concentration and purity of the isolated RNA were evaluated on a NanoDrop-2000 spectrophotometer (Thermo Scientific, USA). The RNA concentration ranged from 80 to 250 ng/ml, A260/A280 = 1.95–2.05; A260/A230 = 1.90–2.31. RNA integrity was assessed using capillary electrophoresis on a TapeStation device (Agilent Technologies, USA) and an R6K ScreenTape kit (Agilent Technologies, USA). The RIN was 5.6–7.8.

Real-time quantitative reverse transcription PCR. The level of gene expression was assessed using quantitative real-time reverse transcriptase PCR (RT-qPCR) using the SYBR Green dye on an iCycler amplifier (Bio-Rad, USA; CCP "Medical Genetics"). To obtain cDNA on an RNA template, a reverse transcription reaction was performed using the OT m-MuLV-RH kit (Biolabmix, Russia) with random hexanucleotide primers according to the kit instructions. PCR was performed in three replicas in a volume of 25 µl containing 12.5 µl of HS-qPCR SYBR Blue BioMaster (Biolabmix, Russia), 300 nM of direct and reverse primers and 50 ng of cDNA:
CAIX: F 5'-GTTGCTGTCTCGCTTGGAA-3',
R 5'-CAGGGTGTCAGAGAGGGTGT-3';
HIF-1α: F 5'- CAAGAACCTACTGCTAATGCCA-3',
R 5'- TTTGGTGAGGCTGTCCGA-3';
EPAS1: F 5'- TGGAGTATGAAGAGCAAGCCT-3',
R 5'-GGGAACCTGCTCTTGCTGT-3';
NFKB1: F 5'-CGTGTAAACCAAAGCCCTAAA-3',
R 5'-AACCAAGAAAGGAAGCCAAGT-3';
RELA: F 5'-GGAGCACAGATACCACCAAGA-3',
R 5'-GGGTTGTTGTTGGTCTGGAT-3';
VEGFA: F 5'-AGGGCAGAATCATCACGAA-3',
R 5'-TCTTGCTCTATCTTTCTTTGGTCT-3';
KDR: F 5'-AACACAGCAGGAATCAGTCA-3',
R 5'-GTGGTGTCTGTGTCATCGGA-3';
4E-BP1: F 5'- CAGCCCTTTCTCCCTCACT-3',
R 5'- TTCCCAAGCACATCAACCT-3';
AKT1: F 5'- CGAGGACGCCAAGGAGA-3',
R 5'- GTCATCTTGGTCAGGTGGTGT-3';
C-RAF: F 5'- TGGTGTGTCCTGCTCCCT-3',
R 5'- ACTGCCTGCTACCTTACTTCCT-3';
GSK-3β: F 5'- AGACAAGGACGGCAGCAA-3',
R 5'- TGGAGTAGAAGAAATAACGCAAT-3';
70S kinase alpha:
F 5'- CAGCACAGCAAATCCTCAGA-3',
R 5'- ACACATCTCCCTCTCCACCTT-3';
mTOR: F 5'- CCAAAGGCAACAAGCGAT-3',
R 5'- TTCACCAAACCGTCTCCAA-3';
PDK1: F 5'- TCACCAGGACAGCCAATACA-3',
R 5'- CTCCTCGGTCACTCATCTTCA-3';
VHL: F 5´ – GGCAGGCGAATCTCTTGA-3´,
R 5´-CTATTTCCTTTACTCAGCACCATT-3´;
PD-L2: F 5'-GTTCCACATACCTCAAGTCCAA-3',
R 5'-ATAGCACTGTTCACTTCCCTCTT-3';
PD-L1: F 5'-AGGGAGAATGATGGATGTGAA-3',
R 5'-ATCATTCACAACCACACTCACAT-3';
PD-1–1: F 5'-CTGGGCGGTGCTACAACT-3',
R 5'-CTTCTGCCCTTCTCTCTGTCA-3';
AMPK: F 5'- AAGATGTCCATTGGATGCACT-3',
R 5'- TGAGGTGTTGAGGAACCAGAT-3';
LC3B: F 5'- CCCAAACCGCAGACACAT-3',
R 5'-ATCCCACCAGCCAGCAC-3';
GAPDH: F 5'- GGAAGTCAGGTGGAGCGA-3',
R 5'-GCAACAATATCCACTTTACCAGA-3'.

The two-step amplification program included: preliminary denaturation of the reaction mixture at 94 °C, 10 minutes – 1 cycle; denaturation at 94 °C, 10 seconds and annealing/elongation at 60 °C, 20 seconds – 40 cycles. The primers were selected using the Vector NTI Advance 11.5 program and the NCBI database (https://www.ncbi.nlm.nih.gov/nuccore).

The "housekeeping" gene of the GAPDH enzyme (glyceraldehyde-3-phosphate dehydrogenase) was used as a reference gene, and the expression level of each target gene was normalized relative to GAPDH expression. Quantitative analysis of expression was performed using 2ΔΔCt in relation to the constitutively expressed referee gene of the GAPDH enzyme.

Statistical analysis

Statistical processing of the results was carried out using the Statistica 12.0 software package. Normality was checked using the Kolmogorov-Smirnov criterion. The results of the determination of gene expression are presented as Me (Q1; Q3). The significance of the differences in independent parameters was assessed by the Mann-Whitney criterion. The differences were considered significant at $p < 0.05$. When comparing the differences in more than two study groups, nonparametric analysis of variance (Kruskal-Wallis test) was used.

STUDY RESULTS

Predicting cancer treatment outcomes is one of the key tasks in oncology. An unfavorable prognosis for melanoma is associated with a high probability of recurrence and metastasis. As part of this study, we analyzed the accuracy of molecular markers of skin melanoma in predicting its development. To do this, we used the log-rank criterion and Kaplan-Meyer survival curves. As a result of the study, it was found that the expression of markers such as AKT, c-RAF, GSK-3β and VEGFR2 are of the greatest importance for predicting disease-free survival (Table 1).

Table 1. One-factor analysis of prognostic parameters in patients with skin melanoma using a log rank test

Parameter	Expression, RU/ml	Progression-free survival, *p*
4EBP1, RU/ml	< 1.00 > 1.00	0.57770
AKT, RU/ml	< 1.0 > 1.0	0.00548
c-RAF	< 0.5 > 0.5	0.04345
GSK-3β	< 0.85 > 0.85	0.04229
70S 6 kinase	< 0.03 > 0.03	0.99828
mTOR, RU/ml	< 0.97 > 0.97	0.11658
PDK1, RU/ml	< 1.53 > 1.53	0.47026
PTEN, RU/ml	< 0.01 > 0.01	0.68012
NF-kB p65, RU/ml	< 0.20 > 0.20	0.18567
NF-kB p50, RU/ml	< 0.05 > 0.05	0.36003
VEGFR2, RU/ml	< 1.00 > 1.00	0.00156
VEGF, RU/ml	< 1.00 > 1.00	0.36003
CAIX, RU/ml	< 0.13 > 0.13	0.51732
HIF-1, RU/ml	< 0.26 > 0.26	0.18567
HIF-2, RU/ml	< 0.81 > 0.81	0.92342
VHL, RU/ml	< 0.14 > 0.14	0.81110
PD-1, RU/ml	< 1.15 > 1.15	0.82655
PD-L1, RU/ml	< 0.60 > 0.60	0.22916
PD-L2, RU/ml	< 1.34 > 1.34	0.88087
AMPK, RU/ml	< 0.00 > 0.00	0.48182
LC3B expression, RU/ml	< 0.50 > 0.50	0.29877

In a study aimed at determining the prognostic value of the expression of transcription and growth factors, as well as components of the AKT/mTOR signaling cascade for assessing progression-free survival in patients with skin melanoma, it was found that the best results are achieved when the expression level of AKT genes is above 1.0 (Fig. 1); c-RAF is below 0.5 (Fig. 2); GSK-3β is below 0.85 (Fig. 3) and VEGFR2 is above 1.0 (Fig. 4) cont. units.

Based on the results of clinical and morphological studies, as well as the expression of certain molecular markers, a model has been developed that makes it possible to predict the recurrence of skin melanoma and an unfavorable outcome of the disease.

The method consists in evaluating the expression of the serine-threonine protein kinase mTOR and taking into account the size of the tumor (T). These data are used to calculate the risk of skin melanoma progression (P). If *p* is less than 0.8, then a high risk of disease progression is predicted, and if *p* is greater than or equal to 0.8, then a low risk of progression is predicted. The method is based on the analysis of laboratory and clinical research results, as well as on the calculation of a logistic regression model. The following

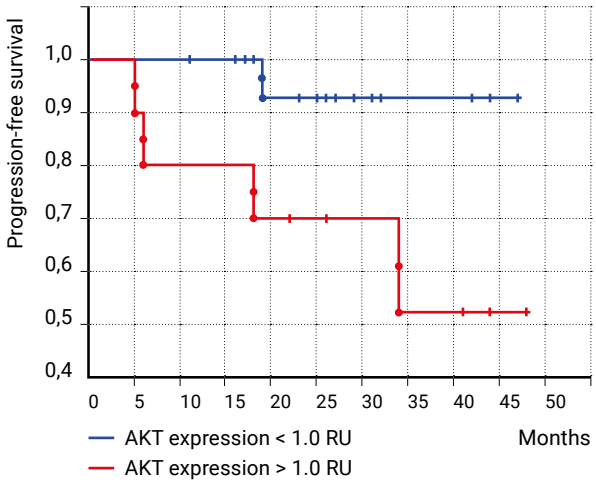

Fig. 1. Progression-free survival in patients with skin melanomas depending on AKT expression

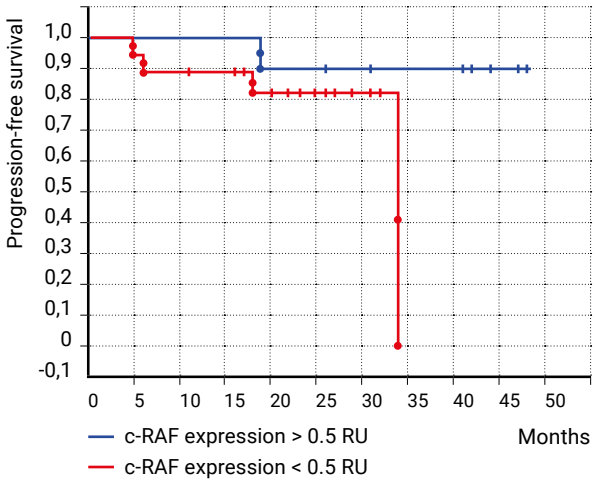

Fig. 2. Progression-free survival in patients with skin melanomas depending on c-RAF expression

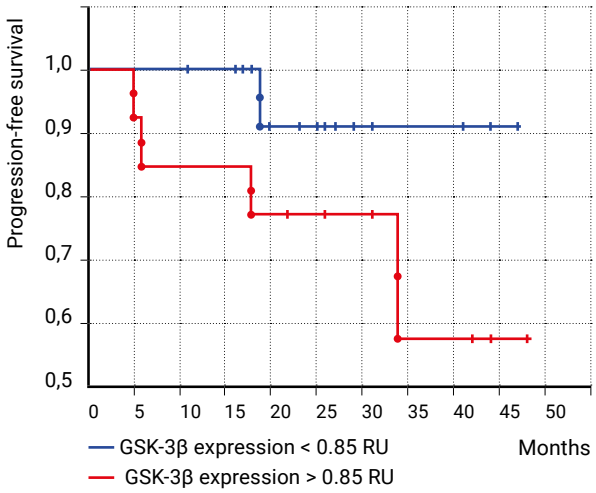

Fig. 3. Progression-free survival in patients with skin melanomas depending on the expression of GSK-3β

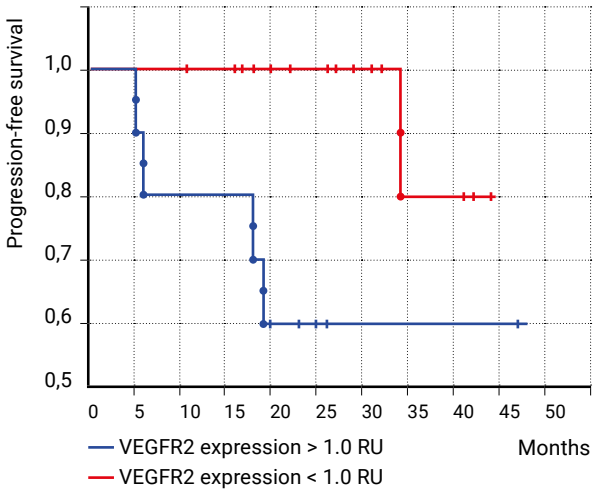

Fig. 4. Progression-free survival in patients with skin melanomas depending on VEGFR2 expression

parameters were included in the model: T and mTOR expression. A polynomial logistic regression was used to build the model. The analysis was performed using Statistica 12.0 software. Based on the optimal set of parameters with a significance level of $p < 0.05$, the regression function (F) was determined to calculate the risk of skin melanoma progression in patients.

In patients, the following informative indicators with a significance level of $p < 0.05$ were included in the regression function: T, expression of serine threonine protein kinase mTOR. At the next stage, the value of the F is determined by the formula:

$F(x_1, x_2) = 4.551 + b_1x_1 + b_2x_2$,

where the coefficients of the regression function are: $b_1 = -1.693$, $b_2 = 0.508$; x_1 – T; x_2 – expression of serine threonine protein kinase mTOR.

Subsequently, using the value of the regression function and the base of the natural logarithm (e), a mathematical model was developed in the form of a formula for determining the risk of progression in patients.

General view of the mathematical model:

$$P = \frac{1}{1 + e^{-s}}$$

where P is the probability of melanoma progression, *e* (base of the natural logarithm) = 2.718, S is the regression function.

The effectiveness of the mathematical model was determined using ROC analysis (Receiver Operating Characteristic). The sensitivity of the model in patients with skin melanomas was 86.7 %, and the specificity was 90 %. To assess the quality of the constructed mathematical model, the characteristic of the area under the ROC curve, AUC (Area Under Curve), was used, the results are presented in Table 2.

For the variable or variables of the test result: Predicted probability there is at least one relationship between the positive current state group and the negative current state group. Statistics may be distorted.

The AUC parameter value for this model was 0.933, which makes it possible to characterize it as excellent according to the generally accepted criteria presented in Table 3.

The requirement of maximum overall sensitivity and specificity of the model was chosen as the optimal criterion for dividing the results into two categories.

optimal cut-off value = max|Se + Sp|,

Where Se is the sensitivity of the model, and Sp is the specificity of the model.

The threshold value was determined based on the results of calculations of the coordinates of the ROC curve (Table 4).

Table 2. Area under the curve

Validation result variables: Predicted probability				
Area	Standard error *	Asymptotic val.**	Asymptotic 95 % confidence interval	
			Lower border	Upper border
0.933	0.040	0.000	0.855	1.000

Note: * – according to a nonparametric assumption; ** – null hypothesis: = actual area = 0.5

Table 3. Characteristics of the logistic model

AUC parameter value	Quality of the model
0.9 – 1.0	great
0.8 – 0.89	very good
0.7 – 0.79	good
0.6 – 0.69	average
0.5 – 0.59	unsatisfactory

The maximum total value of sensitivity and specificity was 1.867, which corresponds to the cut-off threshold of 0.803. The results of constructing the ROC curve for this model are shown in Figure 5.

DISCUSSION

The study revealed a link between the components of the AKT/mTOR signaling pathway and the recurrence of skin melanoma, as well as the progression of the disease. It was found that the expression level of proteins such as AKT, c-RAF, GSK-3β and VEGFR2 in tumor tissue correlates with the intensity of oncogenesis processes and reflects key features of the biological behavior of the tumor. These results were confirmed in previous studies, which also indicated the involvement of these molecular markers in the processes of oncogenesis [11–14]. The presented data indicate activation of the processes of proliferation of transformed cells, invasive growth and neoangiogenesis.

Obviously, the lack of significant indicators related to the biological features of the skin tumor does not allow to increase the effectiveness of treatment of such patients. In this regard, the attention of researchers is focused on finding optimal models for predicting the risk of an adverse outcome of the disease. The study presents a logistic regression

Table 4. Coordinates of the curve

True if it is greater than or equal[a]	Sensitivity (Se)	1 – Specificity	Specificity (Sp)	Se + Sp
0.0000000	1.000	1.000	0.000	1.000
0.1420958	1.000	0.800	0.200	1.200
0.1553039	0.933	0.800	0.200	1.133
0.1675397	0.933	0.600	0.400	1.333
0.3685236	0.933	0.400	0.600	1.533
0.5837076	0.933	0.200	0.800	1.733
0.6749142	0.867	0.200	0.800	1.667
0.8027119	0.867	0.000	1.000	1.867
0.8809557	0.800	0.000	1.000	1.800
0.9220825	0.733	0.000	1.000	1.733
0.9456728	0.667	0.000	1.000	1.667
0.9460033	0.600	0.000	1.000	1.600
0.955173	0.533	0.000	1.000	1.533
0.9653566	0.467	0.000	1.000	1.467
0.9691635	0.400	0.000	1.000	1.400
0.977768	0.333	0.000	1.000	1.333
0.9877487	0.267	0.000	1.000	1.267
0.994264	0.200	0.000	1.000	1.200
0.9975519	0.133	0.000	1.000	1.133
0.9984705	0.067	0.000	1.000	1.067
1.0000000	0.000	0.000	1.000	1.000

Note: The lowest threshold value corresponds to the minimum observed test value minus 1, and the highest threshold value corresponds to the maximum observed test value plus 1. All other threshold values are the averages of two consecutive ordered observed test values.

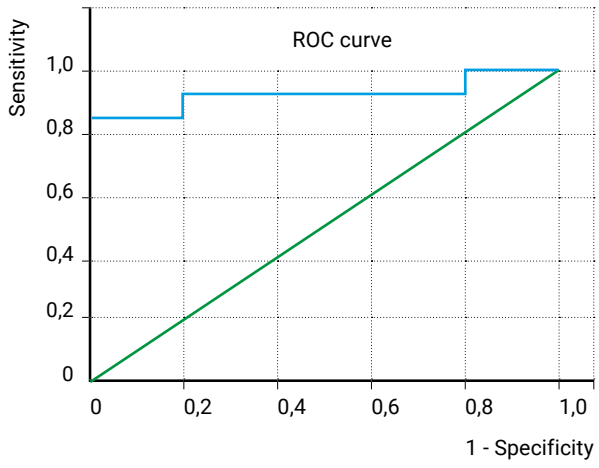

Fig. 5. ROC curve for the model determining the probability of progression of skin melanomas

model that can accurately predict the risk of disease recurrence, taking into account the expression of protein kinase mTOR and T. The value of this marker has been substantiated in many studies and mediates the effectiveness of treatment [15]. It is worth noting that the $BRAF^{V600E}$ mutation was not found in these patients, which indicates the presence of other molecular features in the mechanisms of tumor growth. At the same time, mTOR expression is essential, which is probably due to its involvement in the mechanisms of formation of resistance to treatment and, in particular, in the progression of the disease [16, 17].

CONCLUSION

As a result of the study, molecular markers were identified that can predict the unfavorable course of the disease associated with tumor recurrence. A logistic model was proposed that takes into account the expression of the key kinase mTOR and T, which allows predicting the risk of disease recurrence. The data obtained indicate the prospects of this model as a tool for predicting the progression of the disease.

References

1. Arnold M, Singh D, Laversanne M, Vignat J, Vaccarella S, Meheus F, et al. Global Burden of Cutaneous Melanoma in 2020 and Projections to 2040. JAMA Dermatol. 2022 May 1;158(5):495–503. https://doi.org/10.1001/jamadermatol.2022.0160
2. Rabbie R, Ferguson P, Molina-Aguilar C, Adams DJ, Robles-Espinoza CD. Melanoma subtypes: genomic profiles, prognostic molecular markers and therapeutic possibilities. J Pathol. 2019 Apr;247(5):539–551. https://doi.org/10.1002/path.5213
3. Tehranian C, Fankhauser L, Harter PN, Ratcliffe CDH, Zeiner PS, Messmer JM, et al. The PI3K/Akt/mTOR pathway as a preventive target in melanoma brain metastasis. Neuro Oncol. 2022 Feb 1;24(2):213–225. https://doi.org/10.1093/neuonc/noab159
4. Ito T, Hashimoto H, Kaku-Ito Y, Tanaka Y, Nakahara T. Nail Apparatus Melanoma: Current Management and Future Perspectives. J Clin Med. 2023 Mar 12;12(6):2203. https://doi.org/10.3390/jcm12062203
5. Manzano JL, Martin-Liberal J, Fernández-Morales LA, Benítez G, Medina Martínez J, Quindós M, et al. Adjuvant dabrafenib and trametinib for patients with resected BRAF -mutated melanoma: DESCRIBE-AD real-world retrospective observational study. Melanoma Res. 2023 Oct 1;33(5):388–397. https://doi.org/10.1097/CMR.0000000000000888
6. Johnson DB, Menzies AM, Zimmer L, Eroglu Z, Ye F, Zhao S, et al. Acquired BRAF inhibitor resistance: A multicenter meta-analysis of the spectrum and frequencies, clinical behaviour, and phenotypic associations of resistance mechanisms. Eur J Cancer. 2015 Dec;51(18):2792–2799. https://doi.org/10.1016/j.ejca.2015.08.022
7. Schadendorf D, van Akkooi ACJ, Berking C, Griewank KG, Gutzmer R, Hauschild A, et al. Melanoma. Lancet. 2018 Sep 15;392(10151):971–984. https://doi.org/10.1016/S0140-6736(18)31559-9
8. Wan G, Nguyen N, Liu F, DeSimone MS, Leung BW, Rajeh A, et al. Prediction of early-stage melanoma recurrence using clinical and histopathologic features. NPJ Precis Oncol. 2022 Oct 31;6(1):79. https://doi.org/10.1038/s41698-022-00321-4
9. Patent of the Russian Federation RU 2643761 C1. Kit OI, Zlatnik EYu, Kochuev SS, Demidova AA, Novikova IA, Zakora GI, et al. A method for calculating the prognosis of early progression of skin melanoma. Registered: 08/10/2017. Published: 02/05/2018. (In Russ.). EDN: DQCTYR
10. Ferreira AMC, Carron J, Gomez GVB, Vazquez V de L, Serrano SV, Lourenço GJ, et al. Association of Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4) Genetic Variants with Risk and Outcome of Cutaneous Melanoma. Int J Mol Sci. 2024 Nov 17;25(22):12327. https://doi.org/10.3390/ijms252212327
11. Zhang Y, Xie A, Wang D, Deng W. Novel Prognostic Markers for Skin Cutaneous Melanoma. Clin Cosmet Investig Dermatol. 2024;17:2615–2625. https://doi.org/10.2147/CCID.S486679

12. Luo WF, Song DM, Shen T, He YB, Du HY, Si MJ, et al. Exploring new mechanisms in cancer molecular pathways and pathogenic cell transformation: PIP4K2A as a prognostic marker and therapeutic target in cutaneous malignant melanoma. Discov Oncol. 2024 Nov 23;15(1):697. https://doi.org/10.1007/s12672-024-01555-3
13. Nikulnikov KV, Bogdanova VA, Spirina LV, Chizhevskaya SYu, Kondakova IV, Choynzonov EL, et al. Transcriptional, growth factors, components of the AKT/mTOR signaling pathway, receptors and ligands of programmed cell death expression in melanoma. Advances in Molecular Oncology. 2024;11(1):46-54. (In Russ.). https://doi.org/10.17650/2313-805X-2024-11-1-46-54, EDN: JEOVHB
14. Abd-Allah GM, Ismail A, El-Mahdy HA, Elsakka EGE, El-Husseiny AA, Abdelmaksoud NM, et al. miRNAs as potential game-changers in melanoma: A comprehensive review. Pathol Res Pract. 2023 Apr;244:154424. https://doi.org/10.1016/j.prp.2023.154424
15. Jiang Y, Hu X, Wang Z, Zhang Q, Chen D, Zhao P. RPTOR mutation: a novel predictor of efficacious immunotherapy in melanoma. Invest New Drugs. 2024 Feb;42(1):60–69. https://doi.org/10.1007/s10637-023-01413-z
16. Indini A, Fiorilla I, Ponzone L, Calautti E, Audrito V. NAD/NAMPT and mTOR Pathways in Melanoma: Drivers of Drug Resistance and Prospective Therapeutic Targets. Int J Mol Sci. 2022 Sep 1;23(17):9985. https://doi.org/10.3390/ijms23179985
17. Spirina LV, Usynin YA, Yurmazov ZA, Slonimskaya EM, Kolegova ES, Kondakova IV. [Transcription factors NF-kB, HIF-1, HIF-2, growth factor VEGF, VEGFR2 and carboanhydrase IX mRNA and protein level in the development of kidney cancer metastasis]. Mol Biol (Mosk). 2017;51(2):372–377. https://doi.org/10.7868/S0026898417020197

Information about authors:

Veronika A. Bogdanova – Laboratory researcher, Assistant at the Biochemistry and Molecular Biology with a course in clinical laboratory diagnostics, Siberian State Medical University, Tomsk, Russian Federation
ORCID: https://orcid.org/0009-0003-8473-4182, SPIN: 8903-4911, AuthorID: 1250988

Liudmila V. Spirina ✉ – Dr. Sci. (Med.), Leading Researcher at the Laboratory of Tumor Biochemistry, Cancer Research Institute – branch of the Federal State Budgetary Scientific Institution "Tomsk National Research Medical Center of the Russian Academy of Sciences", Tomsk, Russian Federation; Head of the Department of Biochemistry and Molecular Biology with a course in clinical laboratory diagnostics, Siberian State Medical University, Tomsk, Russian Federation
ORCID: https://orcid.org/0000-0002-5269-736X, SPIN: 1336-8363, AuthorID: 441893, ResearcherID: A-7760-2012, Scopus Author ID: 36960462500

Svetlana Yu. Chizhevskaya – MD, Dr. (Med) Sci., oncologist of the highest category, Leading Researcher at the Department of Head and Neck Tumors, Cancer Research Institute – branch of the Federal State Budgetary Scientific Institution "Tomsk National Research Medical Center of the Russian Academy of Sciences", Tomsk, Russian Federation
ORCID: https://orcid.org/0000-0003-2974-4778, SPIN: 9561-3382, AuthorID: 560288, ResearcherID: C-8960-2012, Scopus Author ID: 23033628800

Irina V. Kovaleva – Cand. Sci. (Med.), Assistant at the Biochemistry and Molecular Biology with a course in clinical laboratory diagnostics, Siberian State Medical University, Tomsk, Russian Federation; Assistant Professor of the Department of Clinical and Laboratory Diagnostics, Voronezh State Medical University named after N. N. Burdenko, Voronezh, Russian Federation
ORCID: https://orcid.org/0000-0003-2964-9041, SPIN: 9737-7086, AuthorID: 1076520, ResearcherID: HLQ-0894-2023, Scopus Author ID: 57215197522

Konstantin V. Nikulnikov – MD, oncologist at the Department of Head and Neck Tumors, Cancer Research Institute – branch of the Federal State Budgetary Scientific Institution "Tomsk National Research Medical Center of the Russian Academy of Sciences", Tomsk, Russian Federation
ORCID: https://orcid.org/0009-0004-7211-7686, SPIN: 5258-7989, AuthorID: 1191435, ResearcherID: LXB-3679-2024

Evgeny D. Merkulov – Laboratory assistant at the Biochemistry and Molecular Biology with a course in clinical laboratory diagnostics, Siberian State Medical University, Tomsk, Russian Federation
ORCID: https://orcid.org/0000-0002-7082-9389, SPIN: 8716-0505, AuthorID: 1274379, ResearcherID: GPK-8198-2022

Contribution of the authors:

Bogdanova V. A. – writing the draft; final conclusions, participation in development of curricula and their implementation, follow-up revision of the text, final conclusion;
Spirina L. V. – scientific management, research concept, methodology development;
Chizhevskaya S. Yu. – participation in development of curricula and their implementation;
Kovaleva I. V. – participation in development of curricula and their implementation;
Nikulnikov K. V. – participation in development of curricula and their implementation;
Merkulov E. D. – statistical data processing.