УДК 616.345-006.6:575.117:616.155.33
https://doi.org/10.20538/1682-0363-2024-4-55-63

Phenotypic profile of blood monocytes and tumor-associated macrophages in relation to the expression of galectins 1 and 3 in colorectal cancer

Kurnosenko A.V.[1, 2], Reingardt G.V.[1, 2], Poletika V.S.[1], Kolobovnikova Yu.V.[1], Chumakova S.P.[1], Urazova O.I.[1], Grishchenko M.Yu.[1, 2], Churina E.G.[1], Gamirova K.A.[1, 2]

[1] *Siberian State Medical University*
2, Moscow Trakt, Tomsk, 634050, Russian Federation

[2] *Tomsk Regional Cancer Center*
115, Lenina Av., Tomsk, 634050, Russian Federation

ABSTRACT

Aim. To identify the features of the subpopulation composition of blood monocytes and tumor macrophages in relation to the plasma concentration and intratumoral expression of galectins 1 and 3 in patients with colorectal cancer.

Materials and methods. A total of 23 patients with colorectal cancer (ICD C18-20) were examined – 5 men and 18 women (average age 63.8 ± 9.4 years). The control group consisted of healthy volunteers; the comparison group encompassed age- and sex-matched patients with colon adenomas. The study materials included whole blood and tumor biopsies. The concentration of galectins 1 and 3 in the blood was determined by enzyme-linked immunosorbent assay, the content of tumor galectin-1^+ and galectin-3^+ cells – by immunohistochemistry. Subpopulations of blood monocytes were evaluated by flow cytometry; the macrophage immunophenotypes M1 ($CD68^+CD80^+$) and M2d ($CD68^+CD206^+$) in tumor tissues were determined using immunofluorescence staining. Statistical processing of the research results was performed by the Jamovi 2.3.21 software package for Windows.

Results. In patients with colorectal cancer (CRC), a positive relationship was identified between high plasma concentrations of galectins 1 and 3 and an imbalance of blood monocytes manifested by a decrease in the relative count of classical $CD14^{++}CD16^-$ monocytes and, conversely, an increase in the number of non-classical $CD14^+CD16^{++}$ and intermediate $CD14^+CD16^-$ cells. The relative numbers of M1 ($CD68^+CD80^+$) and M2d ($CD68^+CD206^+$) macrophages in CRC tissue samples turned out to be comparable and did not depend on the level of galectins 1 and 3 in the blood and tumor. In patients with colon adenomas, the M2d subpopulation of tumor-associated macrophages was predominant ($p = 0.031$).

Conclusion. In patients with CRC, galectins 1 and 3 have a modulating effect on the ratio of non-classical $CD14^+CD16^{++}$, intermediate $CD14^+CD16^-$, and classical $CD14^{++}CD16^-$ monocytes in the blood and do not affect the M1/M2d expression profile of tumor-associated macrophages.

Keywords: galectins, monocytes, macrophages, colorectal cancer, immunophenotype

Conflict of interest. The authors declare the absence of obvious or potential conflicts of interest related to the publication of this article.

Source of financing. The authors state that they received no funding for the study.

Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the Ethics Committee at Siberian State Medical University (Protocol No. 8881 of 29.11.2021).

For citation: Kurnosenko A.V., Reingardt G.V., Poletika V.S., Kolobovnikova Yu.V., Chumakova S.P., Urazova O.I., Grishchenko M.Yu., Churina E.G., Gamirova K.A. Phenotypic profile of blood monocytes and tumor-

✉ *Kurnosenko Anna V.,* kurnosenko.av@ssmu.ru

associated macrophages in relation to the expression of galectins 1 and 3 in colorectal cancer. *Bulletin of Siberian Medicine.* 2024;23(4):55–63. https://doi.org/10.20538/1682-0363-2024-4-55-63.

Фенотипический профиль моноцитов крови и опухоль-ассоциированных макрофагов во взаимосвязи с экспрессией галектинов 1 и 3 при раке толстой кишки

Курносенко А.В.[1,2], Рейнгардт Г.В.[1,2], Полетика В.С.[1], Колобовникова Ю.В.[1], Чумакова С.П.[1], Уразова О.И.[1], Грищенко М.Ю.[1,2], Чурина Е.Г.[1], Гамирова К.А.[1,2]

[1] *Сибирский государственный медицинский университет (СибГМУ)*
Россия, 634055, г. Томск, Московский тракт, 2

[2] *Томский областной онкологический диспансер (ТООД)*
Россия, 634040, г. Томск, пр. Ленина, 115

РЕЗЮМЕ

Цель. Выявить особенности субпопуляционного состава моноцитов крови и макрофагов опухоли во взаимосвязи с плазменной концентрацией и внутриопухолевой экспрессией галектинов 1 и 3 у больных раком толстой кишки.

Материалы и методы. Обследованы 23 больных колоректальным раком (МКБ С18–20) – 5 мужчин и 18 женщин, средний возраст (63,8 ± 9,4) лет. Группу контроля составили здоровые добровольцы, группу сравнения – пациенты с аденомами толстой кишки, сопоставимые по полу и возрасту. Материалом исследования являлись цельная кровь и биоптаты опухоли. Концентрацию галектинов 1 и 3 в крови определяли методом иммуноферментного анализа, содержание опухолевых галектин-1^+ и галектин-3^+ клеток – методом иммуногистохимии. Подсчет субпопуляций моноцитов крови выполняли методом проточной цитофлуориметрии, определение иммунофенотипов M1 ($CD68^+CD80^+$) и M2d ($CD68^+CD206^+$) макрофагов в опухолевой ткани – методом иммунофлуоресценции. Результаты исследования обрабатывали статистическими методами с применением программного пакета Jamovi 2.3.21 для Windows.

Результаты. У больных раком толстой кишки (РТК) установлена положительная связь между высокой плазменной концентрацией галектинов 1 и 3 и нарушением баланса моноцитов крови в виде снижения относительного содержания классических $CD14^{++}CD16^-$ моноцитов и, напротив, увеличения численности неклассических $CD14^+CD16^{++}$ и переходных $CD14^+CD16^-$ клеток. Относительное количество M1 ($CD68^+CD80^+$) и M2d ($CD68^+CD206^+$) макрофагов в образцах тканей РТК оказалось сопоставимым и не зависело от уровня галектинов 1 и 3 в крови и опухоли. У пациентов с аденомами толстой кишки преобладала M2d-субпопуляция опухоль-ассоциированных макрофагов ($p = 0{,}031$).

Заключение. У больных РТК галектины 1 и 3 оказывают модулирующее влияние на соотношение неклассических CD14+$CD16^{++}$, переходных $CD14^+CD16^-$ и классических $CD14^{++}CD16^-$ моноцитов в крови и не влияют на формирование M1/M2d-экспрессионного профиля опухоль-ассоциированных макрофагов.

Ключевые слова: галектины, моноциты, макрофаги, рак толстой кишки, иммунофенотип

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено этическим комитетом СибГМУ (протокол № 8881 от 29.11.2021).

Для цитирования: Курносенко А.В., Рейнгардт Г.В., Полетика В.С., Колобовникова Ю.В., Чумакова С.П., Уразова О.И., Грищенко М.Ю., Чурина Е.Г., Гамирова К.А. Фенотипический профиль моноцитов крови и опухоль-ассоциированных макрофагов во взаимосвязи с экспрессией галектинов 1 и 3 при раке толстой кишки. *Бюллетень сибирской медицины.* 2024;23(4):55–63. https://doi.org/10.20538/1682-0363-2024-4-55-63.

INTRODUCTION

Colorectal cancer (CRC) is one of the top malignancies in terms of morbidity and mortality both in Russia and worldwide [1, 2]. Despite significant efforts aimed at early detection of CRC and improvement of treatment methods, in half of cases, the disease is diagnosed at stage III–IV. One-year mortality amounts to 19.2%, and five-year survival is 57%. There is also an increase in the CRC incidence among young people [3].

The pathogenesis of CRC is based on the interaction of tumor cells with the tumor microenvironment (macrophages, endothelial and mesenchymal progenitor cells, fibroblasts, etc.). These interactions are mediated by many regulatory molecules, including galectins – proteins that bind to β-galactosides. Molecules of this family are characterized by a common carbohydrate-recognition domain and exert a variety of functions both extra- and intracellularly [4–7]. It has been shown that the pool of tumor-associated macrophages, myeloid-derived suppressor cells, and individual dendritic cells is replenished by blood monocytes [8].

The aim of the study was to identify the features of the subpopulation composition of blood monocytes and tumor-associated macrophages in relation to the plasma concentration and intratumoral expression of galectins 1 and 3 in patients with CRC.

MATERIALS AND METHODS

The study included 23 patients with verified CRC (ICD C18-20) – 5 men and 18 women (mean age 63.8 ± 9.4 years), who were registered at Tomsk Regional Cancer Center (chief physician – Maksim Yu. Grishchenko, Cand. Sci. (Med.), Associate Professor). The patients were divided into groups according to the stage of the disease: the group with stage I CRC included 4 people, with stage II – 9 patients, with stage III – 5 patients, and with stage IV – 5 patients. The comparison group encompassed patients with colon adenomas – 8 men and 7 women (mean age 60.7 ± 10.4 years). The control group included 11 healthy volunteers (6 men and 5 women aged 61.5 ± 9.7 years) from those who visited the institution for a regular health checkup.

The exclusion criteria were: chronic inflammatory non-infectious or infectious (in the acute phase) and autoimmune or allergic (without achieving control) diseases, purulent processes, other malignant neoplasms, antitumor therapy (at the time of the study and in medical history), as well as refusal to participate in the study. Each subject signed an informed consent. The study was approved by the Ethics Committee at Siberian State Medical University (Protocol No. 8881 of 29.11.2021). Information about the patient's age, diagnosis, tumor localization, and cancer stage was obtained from medical records.

The study material was whole venous blood and tumor biopsy samples (malignant and benign) obtained from the colon. The concentration of galectin 1 and galectin 3 in blood plasma was measured by enzyme-linked immunosorbent assay using the Boster Bio ELISA kits (USA). The expression of galectins in tumor biopsy samples was determined by immunohistochemistry using a standard technique. The number of monocyte subpopulations was counted by flow cytometry on the BD Accuri flow cytometer (USA) using antibodies to CD14 labeled with R-Phycoerythrin and antibodies to CD16 labeled with FITC.

Sample preparation involved lysis of erythrocytes, washing of leukocytes, addition of fluorochrome-labeled antibodies to the cell suspension according to the manufacturer's instructions, incubation of the solution, and addition of the staining buffer. The number of each monocyte subpopulation was evaluated according to the expression of CD14 and CD16 on the cell membranes. Monocytes expressing $CD14^{+}CD16^{++}$ were classified as non-classical, $CD14^{+}CD16^{-}$ – as classical, and $CD14^{++}CD16^{+}$ – as intermediate cells.

To assess the colocalization of CD68, CD206, and CD80 molecules on the surface of macrophages, triple immunofluorescence staining of paraffin sections (according to the standard technique) was performed, followed by confocal microscopy. For immunofluorescence staining, the following primary antibodies were used: mouse monoclonal anti-CD68 (Novus Biologicals, NBP2 445-39, clone KP1, 1:200 working dilution, USA), rabbit polyclonal anti-CD80 (Abcam, ab64116, 1:70 working dilution, USA), goat polyclonal anti-CD206 (R&D Systems, AF2634, 1:40 working dilution, USA). Additionally, the following combinations of secondary antibodies were utilized: anti-mouse Alexa Fluor 488-labeled, anti-rabbit Alexa Fluor 555-labeled, anti-goat Alexa Fluor 647-labeled (working dilution of all secondary antibodies was 1:400). When staining cell nuclei, DAPI fluorescent stain (ab104135, Abcam, USA) was used for their subsequent visualization on the Carl Zeiss LSM 780 NLO confocal microscope (Germany). The results were analyzed using the Black Zen and Qupath 0.4.4 software (Fig.1).

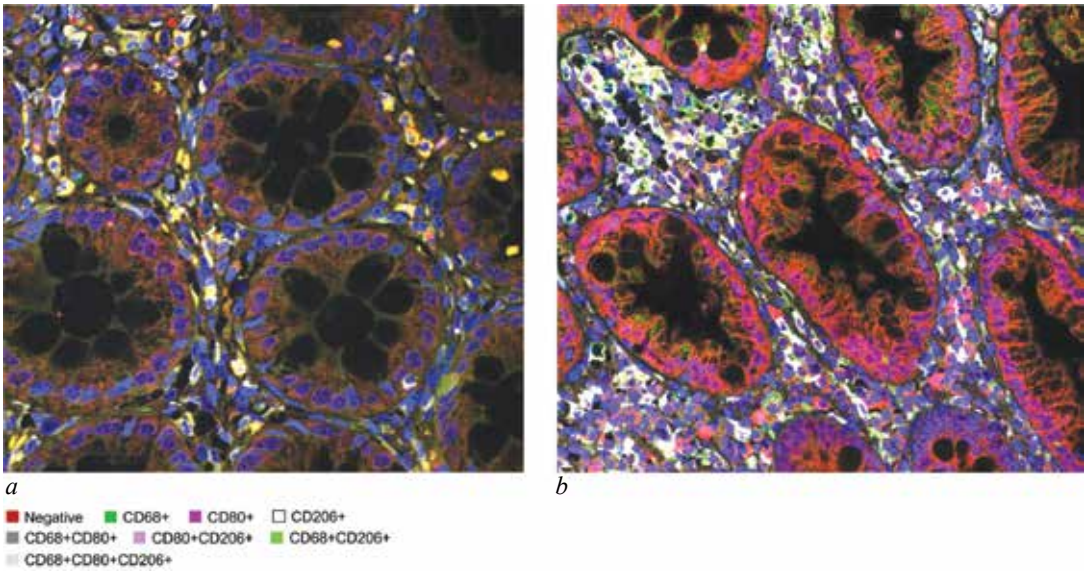

Fig. 1. Immunofluorescence staining of paraffin sections: *a* – colorectal cancer, *b* – colon adenoma

Based on the expression of the CD68/CD206/CD80 markers, $CD68^+CD80^+$ macrophages were considered as cells with M1 immunophenotype, and $CD68^+CD206^+$ – as cells with M2d immunophenotype. Since CD68 is a common marker for macrophages [9], the number of $CD68^+$ cells was initially assessed as a percentage of all stromal cells, and then the relative number of $CD68^+CD206^+$ and $CD68^+CD80^+$ cells was determined.

Statistical analysis of the results was performed using the Jamovi 2.3.21 software package for Windows. The Kolmogorov – Smirnov and the Shapiro – Wilk tests were used to check the normality of distribution of the studied variables. The Mann – Whitney *U*-test was used to assess the statistical significance of differences in quantitative variables between the samples. The Spearman's rank correlation coefficient (r_s) was calculated to identify relationships between paired quantitative variables. The results were considered statistically significant at $p < 0.05$.

RESULTS

According to our previous study [10], the content of galectin 1-expressing cells in the tumor tissue of patients with CRC was higher than in the comparison group: 33.5 (27.0; 55.0) % in CRC and 11.0 (5.0; 21.0) % in colon adenomas ($p < 0.001$). Similarly, the concentration of galectins 1 and 3 in the peripheral blood of individuals with CRC was higher than in patients with colon adenoma and healthy donors by 2.5 and 6.6 times, respectively ($p < 0.001$). A positive correlation was found between the concentration of galectin 1 and galectin 3 in peripheral blood plasma ($rs = 0.843$, $p < 0.01$).

Statistically significant differences in the number of classical, intermediate, and non-classical blood monocytes were found between the studied groups (Table 1). At the same time, the total number of leukocytes ($p = 0.201$) and monocytes ($p = 0.673$) in the blood was comparable.

Table 1

Subpopulation composition of blood monocytes in patients with colorectal cancer, *Me* (Q_1; Q_3)

Subpopulations of monocytes, %	Patients with CRC	Healthy donors
Classical $CD14^+CD16^-$	75.53 (71.41; 78.12)	88.66 (86.06; 93.29)
	$p < 0.001$	
Intermediate $CD14^{++}CD16^+$	8.76 (6.19; 10.53)	1.87 (0.93; 2.6)
	$p < 0.001$	
Non-classical $CD14^+CD16^{++}$	15.08 (11.80; 19.91)	4.09 (1.26; 5.51)
	$p < 0.001$	

The increase in the number of non-classical $CD14^+CD16^{++}$ monocytes had a direct strong correlation with the level of galectin 1 ($rs = 0.557$) and galectin 3 ($rs = 0.780$) in blood plasma. A similar relationship was observed for the number of intermediate $CD14^{++}CD16^+$ monocytes: a direct strong correlation was identified with the concentration of galectin 1 ($rs = 0.618$) and galectin 3 ($rs = 0.617$) in blood plasma.

Therefore, in patients with CRC, an increase in the number of $CD16^+$ monocytes in the blood was revealed compared to the group of healthy volunteers.

A direct strong correlation was determined between the number of non-classical monocytes and low tumor differentiation grade (G3), i.e. a high malignant potential of the tumor. On the other hand, an inverse strong relationship was identified between the number of classical monocytes ($CD14^{++}CD16^{-}$) in the blood and tumor differentiation grade ($rs = –0.469$, $p < 0.01$), the level of galectin 1 ($rs = –0.663$, $p < 001$) and galectin 3 ($rs = –0.804$, $p < 0.001$) in the blood plasma.

Following the analysis of the subpopulation composition of macrophages in tumor biopsy samples in patients with CRC and colon adenomas, no differences in the content of tumor-associated $CD68^{+}CD80^{+}$ macrophages (with the M1 phenotype) were found between the groups. The number of $CD68^{+}CD206^{+-}$ cells (macrophages with the M2d phenotype) in patients with CRC was 1.9 times smaller than in patients with colon adenomas (Table 2). At the same time, in patients with colon adenomas, relative predominance of the M2d macrophages in the tumor tissue was discovered ($p = 0.030$), while in CRC, the ratio of tumor-associated M1- and M2d macrophages was comparable.

Table 2

Subpopulation composition of tumor-associated macrophages in patients with colorectal cancer, *Me* (Q_1; Q_3)

Immunophenotype of macrophages, %	Colorectal cancer	Colon adenoma
M1 ($CD68^{+}CD80^{+}$)	4.34 (1.80; 6.74)	4.26 (1.19; 6.53)
	$p = 0.454$	
M2d ($CD68^{+}CD206^{+}$)	3.21 (2.01; 4.79)	6.10 (3.97; 7.71)
	$p = 0.031$	

The example of stained tumor tissue samples is shown in Fig. 2.

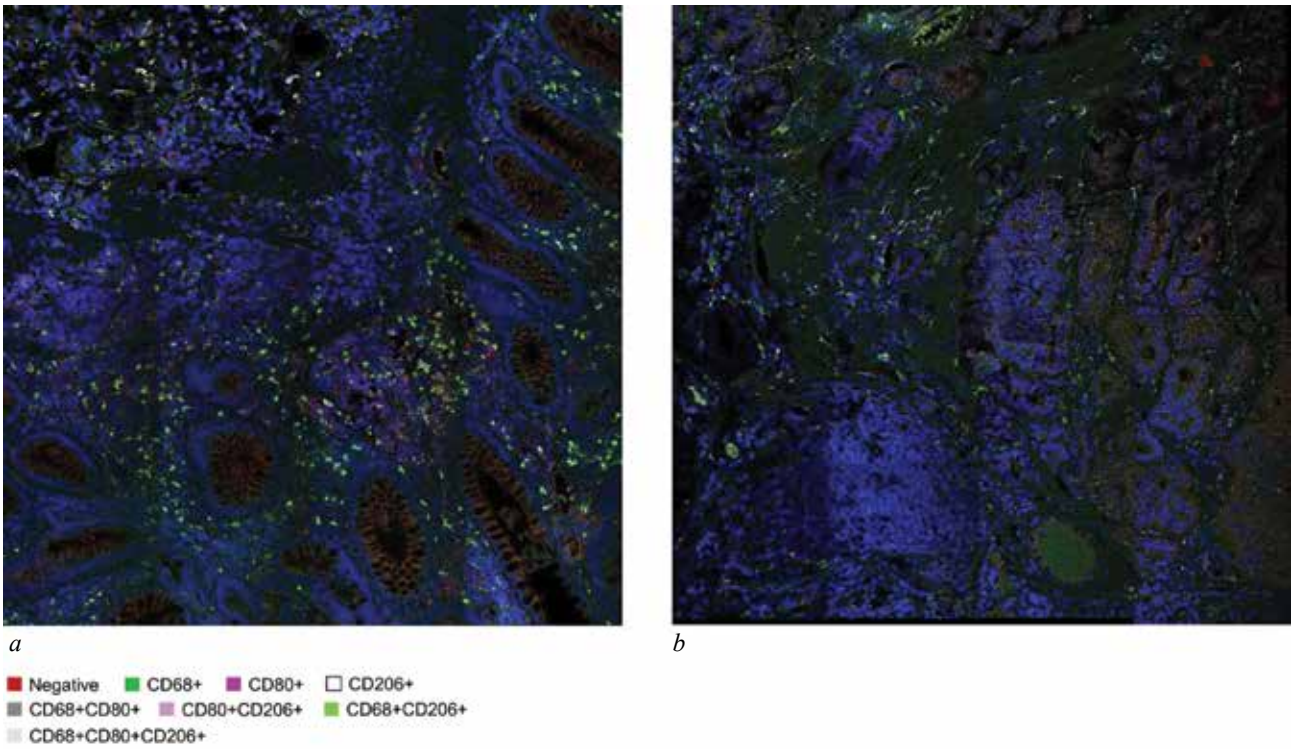

Fig. 2. Staining of tumor tissue specimens

The correlation analysis of the M1 / M2 expression profile of tumor-associated macrophages and the content of galectins 1 and 3 in peripheral blood and tumor tissue found no statistically significant relationships.

DISCUSSION

Galectins are a family of proteins expressed by a variety of cells, including cells in a tumor [11]. Among the lectins involved in the pathogenesis of cancer, galectin 1 and galectin 3 are of particular importance [12, 13]. These molecules not only affect the properties of tumor cells, such as the ability to proliferate and metastasize (detachment from the primary tumor, aggregation, adhesion, intra- and extravasation, migration, and invasion) and susceptibility to programmed cell death [14], but also participate in the interaction of the tumor with immunocompetent

cells having immunosuppressive properties, which contributes to tumor escape from immune surveillance [4]. The mechanism underlying this effect is based on binding of galectins to CD receptors on leukocytes, which regulates maturation of polarization, activation, and apoptosis of these cells [15, 16]. Galectins-1 and 3 also exhibit predominantly anti-inflammatory and tolerogenic (suppressive) effects toward macrophages, directing their polarization along the alternative M2 pathway [17, 18]. Macrophages differentiated in this way, in turn, actively synthesize galectin 1 and galectin 3 themselves, while classical M1 macrophages, on the contrary, are characterized by low expression of these lectins [19].

According to N.N. Sarbaeva (2016), the identification of two distinct macrophage subpopulations (M1 and M2) does not fully characterize the diversity of macrophage immunophenotypes due to their plasticity [20]. Macrophages with the anti-inflammatory M2 phenotype are classified into M2a, M2b, M2c, and M2d subpopulations. Such differences between the cells are due to the set of intracellular and membrane molecules expressed by them (transcription factors, differentiation clusters, receptors for cytokines and chemokines, etc.), secreted cytokines (mainly interleukin (IL) 10 and transforming growth factor β (TGFβ)), and functions (phagocytosis, activation of cell growth and regeneration processes, maintenance of tissue homeostasis, polarization of the immune response toward the Th2 (humoral) or Th17 (autoimmune) pathway, or Treg-mediated immunosuppression, etc.). According to the literature, macrophages expressing CD68 and CD206 markers (i.e. with the M2d phenotype) promote tumor growth, angiogenesis (including tumor vascularization through production of proangiogenic mediators), and extracellular matrix remodeling [21, 22].

Galectins 1 and 3 act as chemoattractants for monocytes newly recruited to the tumor site, thereby forming a vicious circle that promotes tumor progression [23, 17]. There is evidence that galectin 1 promotes M2 polarization of macrophages by regulating arginine metabolism and reducing nitric oxide production [24].

According to the results of the present study, patients with colitis showed a slight trend toward an increase in the proportion of M1 macrophages compared to patients with colon adenomas. This could be attributed to the involvement of inflammation in the mechanisms of malignant tumor growth. Inflammation can both precede the development of colitis-associated CRC and accompany the formation and progression of a tumor not associated with colitis. A colon tumor formed in the context of chronic inflammation is morphologically characterized by pronounced infiltration with immunocompetent cells, which in turn are a source of pro- and anti-tumorigenic and proinflammatory cytokines [25].

In a benign tumor (adenoma) of the colon, we found an imbalance in the ratio of M1 / M2 macrophages with the predominance of the M2d subpopulation, which, according to many authors, is more characteristic of malignant tumors [26–28]. Interpreting the obtained results, it should be noted that in most studies, the authors evaluate the immunophenotype of tumor-associated macrophages in comparison to healthy tissue samples. In our study, we assessed the ratio of M1 / M2 macrophages in CRC compared to their content in patients with colon adenoma.

Division of macrophages into polarized subpopulations (M1 and M2) is more consistent with *in vitro* conditions and does not fully reflect the heterogeneity of these cells *in vivo*. Markers that determine the M1 and M2 immunophenotypes can be simultaneously expressed on a single cell. For example, macrophages of human skin wounds, along with cytokines characteristic of M1 cells, synthesize IL-10 and express CD206, CD163, CD36, and the receptor for IL-4 on their membranes [29]. The possibility of direct and reverse transformation of M1 and M2 immunophenotypes accompanied by changes in the spectrum of secreted cytokines and efferocytosis (the process of burying dead cells – removal of apoptotic cells by phagocytosis) has been described [30, 31].

At the same time, there are no standardized methods for the quantitative assessment of macrophages, and significant differences in the protocols for their immunofluorescence typing exist [32]. To assess the subpopulation composition of macrophages, researchers use various combinations of surface molecules [33–35] due to the lack of highly specific markers. In our study, to level out the subjective component of the assessment, we used the QuPath software, which allows to automate cell counting or exclude the "tumor" component from the analysis and assess only the cells of the tumor microenvironment.

The pool of tumor-associated macrophages, immunosuppressive cells of myeloid origin, and immunogenic and tolerogenic dendritic cells is replenished via migration of monocytes originated from the bone marrow into tissues. The influence of the tumor on monocytes can manifest itself not only

within the tumor site, but also directly at the level of their precursors in the bone marrow or spleen. In turn, monocytes themselves produce cytokines and chemokines, thereby participating in the recruitment of new immune cells into the tumor site and maintaining inflammation [8].

According to some authors, the properties of tissue-associated macrophages are determined by the status of blood monocytes, while others claim that the role of monocytes in the formation of macrophage immunophenotypes is ambiguous [8, 30], and their differentiation depends on the interaction of cells with elements of the microenvironment and is mediated by growth factors, extracellular matrix components, etc. [23]. Galectin 1 is an inducer of monocyte chemotaxis and can modulate the expression of major histocompatibility antigens and FcγRI receptors, which mediate phagocytosis [28].

In the present study, we hypothesized that galectins 1 and 3 are capable of influencing the direction of polarization of monocytes as precursors of tumor-associated macrophages. According to our results, patients with CRC were characterized by a decrease in the content of classical monocytes in the blood, while the number of cells with alternative immunophenotypes – non-classical and intermediate monocytes – was increased.

According to the literature, an increase in the number of circulating non-classical $CD14^{+}CD16^{++}$ monocytes may be associated with their specific function of synthetizing growth factors, and an increase in the intermediate $CD14^{++}CD16^{+}$ monocyte count – with the production of tumor necrosis factor alpha (TNFα), which acts as a mediator of tumor-associated inflammation [29]. The positive relationship between high content of galectin 1 and galectin 3 and the increase in the number of non-classical $CD14^{+}CD16^{++}$ and intermediate $CD14^{++}CD16^{+}$ monocytes in the blood identified in our study indicates the possible effect of these lectins on the polarization of monocyte subpopulations toward CD16-positive cells.

The obtained quantitative data were not associated with the clinical and morphological parameters of the tumor, gender, and age of patients. We believe that this can be attributed to the variability of the clinical course of the underlying disease and the diversity of its symptoms, which depend on the location of the primary tumor node, the features of tumor growth, the presence of complications, the severity of inflammation-related manifestations of alteration accompanying cancer, etc.

The absence of a relationship between the content of galectins 1 and 3 in the blood and in the tumor with the M1 / M2 expression profile of tumor-associated macrophages in patients with CRC, on the one hand, indicates that galactoside-binding proteins do not have a significant distant and local effect on the maturation and functional specialization of tissue macrophages. On the other hand, this might be due to an insufficient sample size, which requires further, more detailed studies with an increase in sample data and expansion of the spectrum of tested regulatory biomolecules capable of polarizing the differentiation of myeloid cells within the tumor microenvironment.

CONCLUSION

We revealed galectin-1, 3-dependent changes in the subpopulation composition of monocytes: a decrease in the number of classical $CD14^{++}CD16^{-}$ cells and an increase in the number of non-classical $CD14^{+}CD16^{++}$ and intermediate $CD14^{+}CD16^{-}$ cells in the peripheral blood of patients with CRC. Elevated content of galectin 1-expressing tumor cells and high concentration of galectins 1 and 3 in the blood plasma did not influence the ratio of M1 ($CD68^{+}CD80^{+}$) and M2d ($CD68^{+}CD206^{+}$) tumor-associated macrophages.

A detailed study of the immunoregulatory effect of galectins on individual subpopulations of blood monocytes and tissue macrophages opens up prospects for the use of these lectins as new biomarkers for CRC and molecular targets for innovative methods of targeted and immune (via reprogramming of the tumor microenvironment) therapy for colon cancer.

REFERENCES

1. Sung H., Ferlay J., Siegel R.L., Laversanne M., Soerjomataram I., Jemal A. et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin*. 2021;71(3):209–249. DOI: 10.3322/caac.21660.
2. Siegel R.L., Wagle N.S., Cercek A., Smith R.A., Jemal A. Colorectal cancer statistics, 2023. *CA Cancer J. Clin.* 2023;73(3):233–254. DOI: 10.3322/caac.21772.
3. Kaprin A.D., Starinsky V.V., Shakhzadova A.O. The state of cancer care for the Russian population in 2021. M: P.A.Hertsen Moscow Oncology Research Institute (MORI) – branch of the National Medical Research Center of Radiology of the Ministry of Health of the Russian Federation. 2022:239 (in Russ.).
4. Cherdyntseva N.V., Mitrofanova I.V., Buldakov M.A., Stakheeva M.N., Patysheva M.R., Zavjalova M.V., et al. Macrophages and tumor progression: on the way to macrophage-specific therapy. *Bulletin of Siberian Medicine*. 2017;16(4):61–74 (in Russ.).
5. Chou F.C., Chen H.Y., Kuo C.C., Sytwu H.K. Role of galectins in tumors and in clinical immunotherapy. *Int. J. Mol. Sci.* 2018;19(2):430. DOI: 10.3390/ijms19020430.

6. Orozco C.A., Martinez-Bosch N., Guerrero P.E., Vinaixa J., Dalotto-Moreno T., Iglesias M. et al. Targeting galectin-1 inhibits pancreatic cancer progression by modulating tumor-stroma crosstalk. *Proc. Natl. Acad. Scio USA.* 2018;115(16):e3769–778. DOI: 10.1073/pnas.1722434115.
7. Lin Y.H., Qiu D.C., Chang W.H., Yeh Y.Q., Jeng U.S., Liu F.T. et al. The intrinsically disordered N-terminal domain of galectin-3 dynamically mediates multisite self-association of the protein through fuzzy interactions. *J. Biol. Chem*. 2017;292(43):17845–17856. DOI: 10.1074/jbc.M117.802793.
8. Patysheva M.R., Stakheeva M.N., Larionova I.V., Tarabanovskaya N.A., Grigorieva E.S., Slonimskaya E.M., et al. Monocytes and cancer: promising role as a diagnostic marker and application in therapy. *Bulletin of Siberian Medicine.* 2019;18(1):60–75 (in Russ.). DOI: 10.20538/1682-0363-2019-1-60-75.
9. Zwager M.C., Bense R., Waaijer S., Qiu S.Q., Timmer-Bosscha H., de Vries E.G.E., et al. Assessing the role of tumour-associated macrophage subsets in breast cancer subtypes using digital image analysis. *Breast Cancer Res. Treat.* 2023;198(1):11–22. DOI: 10.1007/s10549-022-06859-y.
10. Kolobovnikova Yu.V., Urazova O.I., Poletika V.S., Reyngardt G.V., Romanova E.V., Kurnosenko A.V., et al. Galectin-1 and galectin-3 expression in colon cancer and its correlation with tumor invasion, differentiation, and metastatic spread. *Fundamental and Clinical Medicine.* 2021;6(4):45–53 (in Russ.).
11. Ge X.N., Ha S.G., Liu F.T., Rao S.P., Sriramarao P. Eosinophil-expressed galectin-3 regulates cell trafficking and migration. *Front. Pharmacol.* 2013;4:37. DOI: 10.3389/fphar.2013.00037.
12. Cornejo-García J.A., Romano A., Guéant-Rodríguez R.M., Oussalah A., Blanca-López N., Gaeta F., et al. A non-synonymous polymorphism in galectin-3 lectin domain is associated with allergic reactions to beta-lactam antibiotics. *Pharmacogenomics J.* 2016;16(1):79–82. DOI: 10.1038/tpj.2015.24.
13. Chetry M., Thapa S., Hu X., Song Y., Zhang J., Zhu H. et al. The role of galectins in tumor progression, treatment and prognosis of gynecological cancers. *J. Cancer.* 2018;9(24):4742–4755. DOI: 10.7150/jca.23628.
14. Ito K., Stannard K., Gabutero E., Clark A.M., Neo S.Y., Onturk S. et al. Galectin-1 as a potent target for cancer therapy: role in the tumor microenvironment. *Cancer Metastasis Rev.* 2012;31(3–4):763–778. DOI: 10.1007/s10555-012-9388-2.
15. Iqbal A.J., Sampaio A.L.F., Maione F., Greco K.V., Niki T., Hirashima M. et al. Endogenous galectin-1 and acute inflammation: emerging notion of a galectin-9 pro-resolving effect. *Am. J. Pathol.* 2011;178(3):1201–1209. DOI: 10.1016/j.ajpath.2010.11.073.
16. Rabinovich G.A., Conejo-García J.R.. Shaping the immune landscape in cancer by galectin-driven regulatory pathways. *J. Mol. Biol*. 2016;428(16):3266–3281. DOI: 10.1016/j.jmb.2016.03.021.
17. Yakushina V.D., Vasilyeva O.A., Ryazantseva N.V., Novitsky V.V., Savelyeva O.E., Prokhorenko T.S., et al. Galectin-1 and its role in development of innate and adaptive immunity. *Medical Immunology*. 2012;14(1-2):21–32 (in Russ.). DOI: 10.15789/1563-0625-2012-1-2-21-32.
18. Kianoush F., Nematollahi M., Waterfield J.D., Brunette D.M. Regulation of RAW264.7 macrophage polarization on smooth and rough surface topographies by galectin-3. *J. Biomed. Mater. Res. A*. 2017;105(9):2499–2509. DOI: 10.1002/jbm.a.36107.
19. Novak R., Dabelic S., Dumic J. Galectin-1 and galectin-3 expression profiles in classically and alternatively activated human macrophages. *Biochim. Biophys. Acta*. 2012;1820(9):1383–1390. DOI: 10.1016/j.bbagen.2011.11.014.
20. Dragomir A.C.D., Sun R., Choi H., Laskin J.D., Laskin D.L. Role of galectin-3 in classical and alternative macrophage activation in the liver following acetaminophen intoxication. *J. Immunol*. 2012;189(12):5934–5941. DOI: 10.4049/jimmunol.1201851.
21. Sarbaeva N.N., Ponomareva J.V., Milyakova M.N. Macrophages: diversity of phenotypes and functions, interaction with foreign materials. *Genes & Cells*. 2016;11(1):9–17 (in Russ.). DOI: 10.23868/gc120550.
22. Kapitanova K.S., Naumenko V.A., Garanina A.S., Mel'nikov P.A., Abakumov M.A., Alieva I.B. Prospects of applying nanoparticles to reprogram tumor-associate macrophages in immunotherapy of cancer. *Biochemistry*. 2019;84(7):934–952 (in Russ.). DOI: 10.1134/S0320972519070054.
23. Zhguleva A.S., Zementova M.S., Selkov S.A., Sokolov D.I. M1/M2 macrophages: origin, phenotype, methods of production, interaction with natural killer cells and trophoblast. *Medical Immunology*. 2024;26(3):425–448 (in Russ.). DOI: 10.15789/1563-0625-MMO-2877.
24. Correa S.G., Sotomayor C.E., Aoki M.P., Maldonado C.A., Rabinovich G.A. Opposite effects of galectin-1 on alternative metabolic pathways of L-arginine in resident, inflammatory, and activated macrophages. *Glycobiology*. 2003;13(2):119–128. DOI: 10.1093/glycob/cwg010.
25. Grachev A.N., Samoylova D.V., Rashidova M.A., Petrenko A.A., Kovaleva O.V. Tumor-associated macrophages: current research and perspectives of clinical use. *Advances in Molecular Oncology*. 2018;5(4):20–28 (in Russ.).
26. Barrionuevo P., Beigier-Bompadre M., Ilarregui J.M., Toscano M.A., Bianco G.A., Isturiz M.A. et al. A novel function for galectin-1 at the crossroad of innate and adaptive immunity: galectin-1 regulates monocyte/macrophage physiology through a nonapoptotic ERK-dependent pathway. *J. Immunol.* 2007;178(1):436–445. DOI: 10.4049/jimmunol.178.1.436.
27. Baran B., Bechyne I., Siedlar M., Szpak K., Mytar B., Sroka J. et al. Blood monocytes stimulate migration of human pancreatic carcinoma cells in vitro: the role of tumour necrosis factor- alpha. *Eur. J. Cell Biol*. 2009;88(12):743–752. DOI: 10.1016/j.ejcb.2009.08.002.
28. Wu K., Lin K., Li X., Yuan X., Xu P., Ni P. et al. Redefining tumor-associated macrophage subpopulations and functions in the tumor microenvironment. *Front. Immunol.* 2020;11:1731. DOI: 10.3389/fimmu.2020.01731.
29. Sindrilaru A., Peters T., Wieschalka S., Baican C., Baican A., Peter H. et al. An unrestrained proinflammatory M1 macrophage population induced by iron impairs wound healing in humans and mice. *J. Clin. Invest.* 2011;121(3):985–997. DOI: 10.1172/JCI44490.
30. Gong D., Shi W., Yi S., Chen H., Groffen J., Heisterkamp N. TGFβ signaling plays a critical role in promoting alternative macrophage activation. *BMC Immunol*. 2012;13:31. DOI: 10.1186/1471-2172-13-31.

31. Bill R., Wirapati P., Messemaker M., Roh W., Zitti B., Duval F., et al. CXCL9:SPP1 macrophage polarity identifies a network of cellular programs that control human cancers. *Science.* 2023;381(6657):515–524. DOI: 10.1126/science.ade2292.
32. Jayasingam S.D., Citartan M., Thang T.H., Mat Zin A.A., Ang K.C., Ch'ng E.S. Evaluating the polarization of tumor-associated macrophages into M1 and M2 phenotypes in human cancer tissue: technicalities and challenges in routine clinical practice. *Front. Oncol*. 2020;24(9):1512. DOI: 10.3389/fonc.2019.01512.
33. Dunstan R.W., Wharton K.A., Quigley C., Lowe A. The use of immunohistochemistry for biomarker assessment--can it compete with other technologies? *Toxicol. Pathol*. 2011;39(6):988–1002. DOI: 10.1177/0192623311419163.
34. Da C., Mc K., Va M., An O., Yv B. CD68/macrosialin: not just a histochemical marker. *Lab Invest*. 2017;97(1):4–13. DOI: 10.1038/labinvest.2016.116.
35. Fan W., Yang X., Huang F., Tong X., Zhu L. The Second Clinical Medical College ZCMU. Identification of CD206 as a potential biomarker of cancer stem-like cells and therapeutic agent in liver cancer. *Oncology Letters.* 2019;18(3):3218–3226. DOI: 10.3892/ol.2019.10673.

Authors' contribution

Kurnosenko A.V., Reingardt G.V., Gamirova K.A. – carrying out of the research, analysis and interpretation of the data. Poletika V.S., Grishchenko M.Yu., Churina E.G. – conception and design, justification of the research aim, main provisions, and conclusion of the manuscript. Kolobovnikova Yu.V., Chumakova S.P., Urazova O.I. – critical revision of the manuscript for important intellectual content and final approval of the manuscript for publication. All members of the research team meet the criteria and requirements for authorship.

Authors' information

Kurnosenko Anna V. – Post-Graduate Student, Pathophysiology Division, Siberian State Medical University, Tomsk; Oncologist, Tomsk Regional Cancer Center, Tomsk, kurnosenko.av@ssmu.ru, http://orcid.org/0000-0002-3210-0298

Reingardt Gleb V. – Teaching Assistant, Pathophysiology Division, Siberian State Medical University, Tomsk; Oncologist, Tomsk Regional Cancer Center, Tomsk, glebreyngardt@gmail.com, http://orcid.org/0000-0003-3148-0900

Poletika Vadim S. – Cand. Sci. (Med.), Associate Professor, Pathophysiology Division, Siberian State Medical University, Tomsk, vpoletika@yandex.ru, http://orcid.org/0000-0002-2005-305X

Kolobovnikova Yulia V. – Dr. Sci. (Med.), Associate Professor, Head of Normal Physiology Division, Professor of the Pathophysiology Division, Siberian State Medical University, Tomsk, kolobovnikova.julia@mail.ru, http://orcid.org/0000-0001-7156-2471

Chumakova Svetlana P. – Dr. Sci. (Med.), Associate Professor, Pathophysiology Division, Siberian State Medical University, Tomsk, chumakova_s@mail.ru, http://orcid.org/0000-0003-3468-6154

Urazova Olga I. – Dr. Sci. (Med.), Professor, Corresponding Member of the RAS, Head of the Pathophysiology Division, Siberian State Medical University, Tomsk, urazova.oi@ssmu.ru, http://orcid.org/0000-0002-9457-8879

Grishchenko Maksim Yu. – Cand. Sci. (Med.), Associate Professor, Head of the Surgery Division with a Course in Mobilization Training and Disaster Medicine, Siberian State Medical University; Chief Physician, Tomsk Regional Cancer Center, Tomsk, grischenko.mj@ssmu.ru, http://orcid.org/0000-0002-0961-7336

Churina Elena G. – Dr. Sci. (Med.), Professor, Pathophysiology Division, Siberian State Medical University, Tomsk, lena1236@yandex.ru, http://orcid.org/0000-0002-8509-9921

Gamirova Kristina A. – Post-Graduate Student, Division of Health Organization and Public Health, Siberian State Medical University; Oncologist, Tomsk Regional Cancer Center, Tomsk, k.a.gamirova@tomonco.ru, http://orcid.org/0009-0007-7947-360X

(✉) **Kurnosenko Anna V.,** kurnosenko.av@ssmu.ru

Received 19.07.2024;
approved after peer review 09.08.2024;
accepted 12.09.2024