

УДК 618.19-006.6-091.8:576.5:576.385

<https://doi.org/10.20538/1682-0363-2026-1-6-14>

The Differentiation Status of Breast Cancer Cells, its Association with Metastasis and the Production of Cytokines by the Tumor in Various Molecular Subtypes

Arkhipov S.A.^{1,2}, Mikhaylova E.S.^{1,2}, Arkhipova V.V.¹, Druzhinina Y.G.³,
Yakovleva K.I.³, Autenshlyus A.I.^{1,2}

¹ Novosibirsk State Medical University
52 Krasny Ave., 630091 Novosibirsk, Russian Federation

² Research Institute of Molecular Biology and Biophysics, Federal Research Center for Fundamental and Translational Medicine (FRC FTM)
2 Timakova St., 630117 Novosibirsk, Russian Federation

³ Vector-Best JSC
Koltsovo, 630559 Novosibirsk, Russian Federation

ABSTRACT

Aim. To investigate the relationship between the differentiation status of breast cancer (BC) tumor cells (TC) of various molecular subtypes, the ability to metastasize, and the modulation of various cytokines in BC samples *in vitro*.

Materials and methods. Biopsies from invasive breast carcinoma of a non-specific type in 50 women, classified by the presence (Met+) and absence (Met-) of metastases in regional lymph nodes, were examined and categorized into 4 groups based on their molecular subtypes: luminal A (Lum A), luminal B (Lum B), HER2/neu positive (HER2+), and triple negative (TN). Using enzyme-linked immunosorbent assay, spontaneous and mitogen complex-stimulated (MC) production of 14 cytokines was determined in the supernatant of cultured BC samples (IL-2, IL-6, IL-8, IL-10, IL-17, IL-18, IL-1 β , IL-1Ra, TNF α , IFN γ , G-CSF, GM-CSF, VEGF, and MCP-1), on the basis of which the index of the MC effect (IMCE) on cytokine production was calculated. The effect of stimulating the differentiation of TC was evaluated on the basis of cytomorphological criteria for reducing the relative content of low-grade cells (LGC) in BC samples.

Results. A statistically significant decrease in the relative content of LGC under the influence of MC was detected in the Met+ and Met- groups. MC had a differentiating effect in luminal and HER2-positive subtypes, while in TN breast cancer samples, there was only a downward trend in the proportion of LGC. To assess the role of cytokines in the mechanism of the differentiating effect of MC on LGC, a neural network analysis of IMCE in the relationship of various cytokines was carried out. According to the results obtained, in Lum A, the leading role in the manifestation of the differentiation effect was associated with IL-1Ra and IL-6; in Lum B – with MCP-1 and GM-CSF; in HER2+ – with IFN- γ and MCP-1, and in TN – with IL-1 β and IL-6.

Conclusion. The data obtained indicate that the immune mechanisms mediating the differentiating MC effect on BC may vary in different molecular subtypes of BC.

Keywords: breast cancer, cytokines, molecular subtypes, cellular differentiation

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

Source of financing. Financing of government assignment topics: state registration number 115060410035, state registration number 125031203556-7.

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 of the Research Institute of Molecular Biology and Biophysics of the Federal Research Center for Fundamental and Translational Medicine (Minutes No. 28 dated September 27, 2023).

✉ Arkhipov Sergey A., arhipowsergei@yandex.ru

For citation: Arkhipov S.A., Mikhaylova E.S., Arkhipova V.V., Druzhinina Y.G., Yakovleva K.I., Autenshlyus A.I. The Differentiation Status of breast Cancer Cells, its Association with Metastasis and the Production of Cytokines by the Tumor in Various Molecular Subtypes. *Bulletin of Siberian Medicine*. 2026;26(1):6–14. <https://doi.org/10.20538/1682-0363-2026-1-6-14>.

Дифференцировочный статус клеток рака молочной железы, его сопряженность с метастазированием и с продукцией опухолью цитокинов при различных молекулярных подтипах

Архипов С.А.^{1,2}, Михайлова Е.С.^{1,2}, Архипова В.В.¹, Дружинина Ю.Г.³, Яковлева К.И.³, Аутеншлюс А.И.^{1,2}

¹ Новосибирский государственный медицинский университет (НГМУ)
Россия, 630091, г. Новосибирск, Красный проспект, 52

² Научно-исследовательский институт молекулярной биологии и биофизики (НИИМББ), Федеральный исследовательский центр фундаментальной и трансляционной медицины (ФИЦ ФТМ)
Россия, 630117, г. Новосибирск, ул. Тимакова, 2

³ АО «Вектор-Бест»
Россия, 630559, Новосибирская область, пгт. Кольцово

РЕЗЮМЕ

Цель. Исследовать взаимосвязь между дифференцировочным статусом опухолевых клеток (далее КО) рака молочной железы (РМЖ) различных молекулярных подтипов, способностью к метастазированию и модуляции в образцах РМЖ продукции различных цитокинов *in vitro*.

Материалы и методы. Исследовали биоптаты РМЖ (инвазивной карциномы молочной железы неспецифического типа) 50 женщин при наличии (Мет+) и отсутствии (Мет-) метастазов в регионарных лимфатических узлах, разделенных на четыре группы по молекулярным подтипам РМЖ: люминальный А (Люм А), люминальный В (Люм В), HER2/neu позитивный (HER2+) и тройной негативный (ТН). С помощью иммуноферментного анализа в супернатанте культивируемых образцов РМЖ определяли спонтанную и стимулированную комплексом митогенов (КМ) продукцию 14 цитокинов (интерлейкин (IL) 2, IL-6, IL-8, IL-10, IL-17, IL-18, IL-1 β , IL-1Ra, фактор некроза опухоли альфа, интерферон-гамма (IFN γ), гранулоцитарный колониестимулирующий фактор, гранулоцитарно-макрофагальный колониестимулирующий фактор (GM-CSF), фактор роста эндотелия сосудов и моноцитарный хемотаксисный белок-1 (MCP-1)), на основе которой рассчитывали индекс влияния КМ на продукцию цитокинов (ИБКМ). Эффект стимуляции дифференцировки КО *in vitro* оценивали на основе цитоморфологических критериев по снижению относительного содержания низкодифференцированных клеток (НДКО) в образцах РМЖ.

Результаты. Статистически значимое снижение НДКО под воздействием КМ выявлено в группах Мет+ и Мет-. Комплекс митогенов оказывал дифференцировочный эффект при люминальных и HER2-позитивном подтипах, в образцах ТН наблюдалась тенденция к снижению НДКО. Для оценки роли цитокинов в механизме дифференцирующего действия КМ проведен нейросетевой анализ ИБКМ в отношении различных цитокинов. Согласно полученным результатам, при Люм А ведущая роль в проявлении дифференцировочного эффекта была связана с IL-1Ra и IL-6, при Люм В – MCP-1 и GM-CSF, при HER2+ – с IFN γ и MCP-1, а при ТН – с IL-1 β и IL-6.

Заключение. Полученные данные свидетельствуют о том, что иммунные механизмы, опосредующие дифференцировочное воздействие КМ на КО, РМЖ могут отличаться при различных молекулярных подтипах РМЖ.

Ключевые слова: рак молочной железы, цитокины, молекулярные подтипы, клеточная дифференцировка

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

Источники финансирования. Финансирование тем государственных заданий: № госрегистрации 115060410035, 125031203556-7.

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено этическим комитетом НИИМББ ФИЦ ФТМ (протокол № 28 от 27.09.2023).

Для цитирования: Архипов С.А., Михайлова Е.С., Архипова В.В., Дружинина Ю.Г., Яковлева К.И., утеншлюс А.И. Дифференцировочный статус клеток рака молочной железы, его сопряженность с метастазированием и с продукцией опухолью цитокинов при различных молекулярных подтипах. *Бюллетень сибирской медицины*. 2026;26(1):6–14. <https://doi.org/10.20538/1682-0363-2026-1-6-14>.

INTRODUCTION

Despite significant advances in developing new treatments for breast cancer (BC), including targeted therapy and immunotherapy, the problem of resistance of breast cancer tumor cells (TC) to anticancer therapy continues to be a pressing concern [1]. Traditional approaches to BC treatment, such as chemotherapy and radiation therapy, target rapidly dividing cells, but they are not always effective against TC that are dormant at the time of treatment but retain the potential for tumor progression. In this regard, alternative strategies aimed at changing the biological properties of TC rather than just eliminating them are gaining increased interest. One such strategy is differentiation therapy [2, 3].

Differentiation therapy is an approach aimed at stimulating the differentiation of TCs, leading to a reduction in their proliferative activity and malignant potential. This approach is based on the idea that TCs retain the capacity for differentiation, and that targeting specific signaling pathways can restore a normal cell phenotype [3]. Differentiation therapy is already used in the treatment of certain types of cancer, such as acute promyelocytic leukemia, where the use of retinoic acid leads to the differentiation of blast cells and disease remission [4]. However, the application of differentiation therapy to solid tumors, such as BC, presents a more complex challenge. BC is a heterogeneous disease that is divided into different molecular subtypes, characterized by different prognoses and sensitivity to therapy [5, 6]. These subtypes differ in the expression of estrogen receptors (ER), progesterone receptors (PR), and human epidermal growth factor receptor 2 (HER2), as well as in the levels of expression of the proliferation marker Ki-67. Each BC subtype has a unique gene expression profile, and, therefore, its response to differentiation therapy may vary [7]. One of the factors regulating the differentiation of BC cells is the tumor cell microenvironment producing cytokines [8]. Cytokines can affect directly the differentiation of TC, both stimulating and inhibiting this process. For example,

interleukin-6 (IL-6) can promote TC proliferation and BC metastasis, while interferon gamma (IFN- γ) can have an anti-tumor effect, inducing differentiation or apoptosis of BC TC [9, 10].

We have previously obtained data indicating that a mitogen complex (MC) consisting of lectins (phytohemagglutinin M, phytohemagglutinin P, concanavalin A) and lipopolysaccharide (LPS) has the ability to reduce the content of low-grade tumor cells (LGC) and, accordingly, increase the content of more differentiated forms of TCs in samples of invasive breast carcinoma *in vitro* [11]. In the present study, we investigated the effect of MC on the differentiation status of TC in BC samples of various molecular subtypes, as well as on the production of various cytokines by BC biopsy specimens. It is known that counting BC TC with varying grades of differentiation is critical for both diagnosis and prognosis of malignant neoplasms [12]. LGC contribute to a higher level of malignancy and aggressiveness of BC compared to more differentiated cells [13]. Clinical studies show that the presence of LGC in the tumor correlates with a poor prognosis. Their level is associated with a lower survival rate of patients and an increased risk of recurrence [14]. Therefore, when analyzing the results obtained, we primarily focused on assessing changes in the relative content of LGC, the counting accuracy of which due to their morphological characteristics is higher compared to differentiated TC.

The aim of the study was to investigate the relationship between the differentiation status of TC of different molecular subtypes of BC, the BC ability to metastasize, and the modulation of the production of various cytokines in BC samples *in vitro*.

MATERIALS AND METHODS

The study material comprised biopsy samples of BC (grade 2-3 invasive breast carcinoma of non-specific type) collected from 50 women aged 35–76 years with (Met+) or without (Met–) metastases to regional lymph nodes, as well as supernatants obtained during *in vitro* culturing of biopsy samples in a medium without MC

and in a medium containing MC, who were treated at Novosibirsk Regional Oncology Dispensary. The Ethics Committee of the Research Institute of Molecular Biology and Biophysics of the Federal Research Center for Fundamental and Translational Medicine approved this study (Minutes No. 28 dated September 27, 2023). The inclusion criterion for the study was newly diagnosed BC. Exclusion criteria were the presence of distant metastases, exacerbation of chronic diseases, and neoadjuvant therapy. Patients were divided into 4 groups depending on a particular molecular subtype of the tumor: Luminal A (Lum A), Luminal B (Lum B), HER2/neu positive (HER2+), and triple negative (TN).

To stimulate the differentiation of BC TC and modulate cytokine production *in vitro*, we used a composition of four MCs, specifically: phytohemagglutinin M at a concentration of 2.0 µg/ml, phytohemagglutinin P at a concentration of 2.0 µg/ml, concanavalin A at a concentration of 4.0 µg/ml, and lipopolysaccharide (LPS) at a concentration of 2.0 µg/ml [11]. Tumor biopsy samples obtained by core needle biopsy with an 8 mm³ volume were placed into two glass vials. One vial contained only DMEM-F12 nutrient medium, and the other contained MC in the same volume of the medium. All samples were then cultured for 72 hours at 37 °C. Next, the biopsy samples were removed from the culture medium and fixed in a neutral formalin solution for further morphological examination, and cytokine concentrations in the obtained supernatants were determined using solid-phase enzyme-linked immunosorbent assay: IL-2, IL-6, IL-8, IL-10, IL-17, IL-18, IL-1b, IL-1Ra, TNFα, IFNγ, G-CSF, GM-CSF, VEGF, and MCP-1. The index of the MC effect on cytokine production by tumor samples was calculated using the formula: $IMCE = A / B$, where A is the concentration of the cytokine in the supernatant of the BC biopsy sample after stimulation of the tumor with CM (CM+), B is the concentration of the cytokine in the supernatant of the BC biopsy sample without MC stimulation (MC-). For morphological examination, BC biopsy samples fixed in formalin were dehydrated and embedded in paraffin, followed by preparation of sections with thickness of 4–5 µm. Deparaffinization and rehydration of BC samples were carried out according to the standard method using a xylene – ethanol sequence. The obtained sections were stained with hematoxylin and eosin according to the standard method. The study was conducted using light microscopy at ×400 magnification. The grade of differentiation of TC in BC samples was evaluated according to the cytomorphological criteria described

in our study [11], taking into account the Nottingham histologic grade [15]. The relative content (%) of low-grade TC (LGC), as well as intermediate-grade (IGC) and high-gradeC (HGC), was counted. The presence of the stimulating effect of BC TC differentiation when exposed to MC was determined by a decrease in the relative content of LGC in BC samples and expressed as an index of the MC effect on the differentiation of (IEMC LGC) equal to the ratio of the amount of LGC in BC samples without MC exposure to the amount of LGC after MC exposure [11].

The level of statistical significance of the differences between the groups was determined using the non-parametric paired Wilcoxon test. Indicators were expressed as the median (*Me*), lower and upper quartiles (Q_1 ; Q_3). Calculations of medians and lower and upper quartiles were performed using the Statistica v. 7.0 software package. Neural network analysis was performed using the IBM SPSS Statistics v. 22.0 software package. The study used a neural network model (NNM) generated based on the Multilayer Perceptron module. The input layer included 15 neurons, one hidden – three neurons, and the output – one neuron. The activation function of the hidden layer is “hyperbolic tangent”, the activation function of the output layer is “sigmoid”. The use of a “hyperbolic tangent” in the hidden layer allowed the NNM to learn on non-linear dependencies between input and output data. The activation function of the output layer “sigmoid” allowed to interpret the output value as the probability of belonging to the class “differentiation stimulation” (value 0 or 1). The choice of the Multilayer Perceptron architecture (one hidden layer with three neurons) was due to the relatively small amount of data, in order to reduce the probability of overfitting the NNM. The input parameters for the perceptron were the values of the IMCE. The target variable in the NNM was the presence or absence of the effect of TC differentiation stimulation in the corresponding BC samples (values 0 and 1). Since the relationship between cytokines and the effect of TC differentiation could vary for different subtypes of BC, a separate NNM was formed for each subtype. The data of each group of patients were divided into training and test samples in an approximate ratio of 80/20 (%) or 70/30 (%). The division was performed randomly, but with the preservation of the proportions of classes (0 and 1) in both samples (stratified division). The neural network was trained on the training sample, and the accuracy of the NNM was evaluated on the test sample. For Lum A, the data were randomly divided into training and test samples in a ratio of 14/4. For

Lum B, HER2+, and TN subtypes, the data were randomly divided into training and test samples in the ratios 9/3, 5/2, and 9/3, respectively. The batch training method and the gradient descent algorithm were used. NNM training was performed under similar training parameters for different BC subtypes: standardization of input predictors using normalization: $(x - \min) / (\max - \min)$; initial learning rate was 0.4; moment – 0.9; center of the interval – 0; interval bias – ± 0.5 . The accuracy of the neural network model (ANNM) for determining class 1 – “presence of differentiation effect” (Positive Predictive Value) was calculated using the formula: $\text{ANNM} = (\text{TP} / (\text{TP} + \text{FP}))$, where TP (True Positive) is the number of samples with a “differentiation effect” that are correctly classified by the model as having a “differentiation effect”, FP (False Positive) – the number of samples without a “differentiation effect” that are erroneously classified by the model as having a “differentiation effect”. The ANNM, obtained on the training and test sets was the

following: for NNM LumA – 92.31% and 100%, for NNM LumB – 87.5% and 100%, for HER2+ – 80% and 100%, for NNM TN – 75% and 66.7%, respectively. After training the NNM, the “normalized importance” (NI) of each input predictor was calculated, which is an estimate of the relative strength of the influence of the input variable on predicting the target variable – the presence or absence of the effect of stimulation of TC differentiation in BC samples after MC exposure.

RESULTS

Table 1 presents data demonstrating the effect of MC on the relative content of LGC, IGC, and HGC in BC samples from patients with (Met+) and without (Met–) metastases to regional lymph nodes, as well as in patients not separated by the presence or absence of lymphogenous metastasis.

In all subgroups of patients (with or without metastases), a statistically significant decrease in the

Table 1

Effect of MC on the Relative Content of LGC, IGC, and HGC in BC Samples, <i>Me</i> (Q_1 ; Q_3), %			
BC Patients	Samples	Relative content of LGC, %	Relative content of HGC+IGC, %
Without separation for metastasis	MC – ($n = 50$)	22.32 (16.67; 27.06)	77.68 (72.94; 83.33)
	MC+ ($n = 50$)	16.36*** (12.07; 21.55)	83.64*** (78.45; 87.93)
Without metastasis (Met–)	MC – ($n = 29$)	18.57 (15.00; 24.59)	81.43 (75.41; 85.00)
	MC + ($n = 29$)	14.89*** (12.07; 18.22)	85.11*** (81.78; 87.93)
With metastasis (Met+)	MC – ($n = 21$)	23.45 (21.11; 30.38)	76.55 (69.62; 78.89)
	CM+ ($n = 21$)	17.64*** (15.12; 23.61)	82.36*** (76.39; 84.88)

Differences are statistically significant when comparing MC+ samples with MC– samples. ** $p < 0.0001$ (paired Wilcoxon test)

relative content of LGC was observed in BC samples in response to MC exposure (MC+), which indicates that MC can affect the differentiation of BC TC, shifting them towards more mature forms. Analysis of the data obtained, depending on the metastatic status (Met– and Met+), showed that the effect of MC on TC differentiation was maintained regardless of the presence or absence of metastases in the lymph nodes. The differences between the MC+ and MC– sample groups were statistically significant for both patients with metastases (Met+) and patients without metastases (Met–). Comparing the medians (*Me*) for each sample group, one can estimate the degree of a decrease in LGC content under the influence of MC. In the overall patient cohort, without segregating by the presence or absence of metastases, the median decreased from 22.32% (MC–) to 16.36% (MC+). In the group of patients without metastases (Met–), the median relative content of LGC

decreased from 18.57% (MC–) to 14.89% (MC+), and in the group of patients with metastases (Met+) – from 23.45% (MC–) to 17.64% (MC+).

Table 2 presents the results of studying the MC effect on the relative content of cells with varying grades of differentiation in BC samples of different molecular subtypes: Lum A, Lum B, HER2+, and TN. It was established that in Lum A samples, a statistically significant decrease in the relative content of LGC was observed in MC+ samples compared to MC– samples ($p = 0.00159$). This suggests that MC may promote TC differentiation in this BC subtype. Similarly, in the Lum B subtype, a statistically significant decrease in LGC content was also observed in MC+ samples ($p = 0.00963$). The median decreased from 22.18% to 17.26%, which also indicates the ability of MC to induce differentiation in Lum B cells. In the HER2+ subtype, a statistically significant decrease in the

Table 2

Effect of MC on the Relative Content of LGC, HGC and MGC in BC Samples of Different Molecular Subtypes			
Molecular BC Subtype	Samples	Relative content of LGC, %	Relative content of HGC+IGC, %
Lum A	MC– (<i>n</i> = 18)	18.52 (15.61; 25.32)	81.48 (74.62; 84.39)
	MC+ (<i>n</i> = 18)	15.77** (11.01; 20.08)	84.23** (79.92; 88.99)
Lum B	MC– (<i>n</i> = 12)	22.18 (18.37; 27.06)	77.82 (72.94; 81.63)
	MC+ (<i>n</i> = 12)	17.26** (13.19; 22.5)	82.74** (77.5; 86.81)
HER2+	CM– (<i>n</i> = 7)	17.38 (12.71; 24.57)	82.62 (75.43; 87.30)
	CM+ (<i>n</i> = 7)	15.67* (9.68; 24.55)	84.33* (75.45; 90.32)
TN	CM– (<i>n</i> = 12)	23.26 (21.87; 29.98)	76.74 (70.03; 77.69)
	CM+ (<i>n</i> = 12)	15.79 (14.23; 18.76)	84.21 (77.84; 85.48)

Differences are statistically significant when comparing MC+ samples with MC– samples. * $p < 0.05$; ** $p < 0.01$ (paired Wilcoxon test)

relative content of LGC was also observed in MC+ samples compared to MC– samples ($p = 0.03125$). In the TN group, although there is a trend towards a decrease in the content of LGC in MC+ samples compared to MC– samples, this decrease did not reach statistical significance ($p = 0.05974$).

In order to assess the likely role of cytokines in stimulating BC cell differentiation under the influence of MC, a study was conducted on the effect of MC on cytokine production by BC samples when they were cultured in a medium containing MC. The indices of mitogen complex effect (IMCE) were calculated equal to the ratio of the concentration of the studied cytokines in the supernatants with MC to the concentrations of cytokines in the supernatants of BC samples cultured in a medium without MC. At the same time, the LGC effect index was calculated, equal to the ratio of the number of LGC in samples cultured in a medium without MC to the number of LGC in samples cultured in a medium containing MC. On the basis of neural network analysis methods, NNMs were formed and trained separately for each BC subtype, on the basis of which the NI of each input predictor of the independent variable – the index of the MC effect on the production of a cytokine – was calculated in predicting the target dependent variable, to which only two values were assigned – the presence of differentiation stimulation (1) or its absence (0). When the LGC IMCE value exceeded 1.1, the presence of a differentiation effect was noted, and when the value was less than or equal to 1.1 – its absence. The validity of this choice of assessment was justified by the fact that, in the group comparison of the relative content of LGC in BC samples after culturing in a medium with MC and without MC, provided that their ratio exceeds

1.1, the differences (evaluated by the paired Wilcoxon test) were significant (Table 3).

Table 3

Results of Neural Network Analysis of the Role of IMCE of Various Cytokines in the Development of the Differentiation Effect of LGC BC under the Action of MC in BC of Various Molecular Subtypes				
EICM	Molecular BC Subtype			
	Lum A	Lum B	Her2+	TN
	Normalized importance (NI), %			
IL-2	30.0	48.7	44.0	47.4
IL-4	44.3	18.7	36.5	56.7
IL-6	87.3	14.1	67.9	81.9
IL-8	74.3	70.9	52.3	32.5
IL-10	34.2	45.0	45.6	41.8
IL-17	51.0	32.1	91.9	67.6
IL-18	36.3	68.7	43.2	45.8
IL-1β	28.0	13.2	84.0	100.0
IL1-Ra	100.0	28.7	54.8	41.0
TNFα	69.7	17.7	21.5	47.0
IFNγ	80.9	12.2	100.0	63.9
G-CSF	40.8	30.9	58.2	45.7
GM-CSF	29.1	76.5	73.2	44.9
VEGF	69.6	21.9	42.4	42.7
MCP-1	57.8	100.0	96.4	35.7

The results of the neural network analysis of the association of IMCE with the production of various cytokines by BC samples and the LGC IMCE, depending on the molecular genetic subtype of BC, are presented in Table 3. It is shown that in Lum A BC, the IMCE IL-1Ra had a maximum NI value (100%), which indicates the leading role of IL-1Ra in the manifestation of the effect of differentiation of TC under the action of MC in tumor samples of this subtype. At the same time, high NI values of the IMCE were established for a number of other cytokines: IFNγ (NI = 80.9%), IL-6

(NI = 87.3%), TNF α (NI = 69.7%), and VEGF (NI = 69.6%). In Lum B, the largest value of NI was shown by MCP-1 (100%). A relatively high level of NI was also identified for cytokines: GM-CSF (NI = 76.5%), IL-8 (NI = 70.9%), and IL-18 (NI = 68.7%).

For HER2+ BC, other combinations of cytokines with large NI values were identified: IFN γ (NI = 100%), MCP-1 (NI = 96.4%), IL-17 (NI = 91.9%), and IL-1 β (NI = 84.0%). In TN BC, the highest NI was shown by IL-1 β (NI = 100%). At the same time, highly significant cytokines in the development of the effect of TC differentiation under the MC effect were IL-6 (NI = 81.9%), IL-17 (NI = 67.6%), and IFN γ (NI = 63.9%). Thus, the neural network analysis of the importance of the IMCE values of various cytokines in the development of the effect of BC LGC differentiation exposed to MC showed significant differences between the molecular BC subtypes.

DISCUSSION

Currently, the prospects of differentiation therapy as an effective treatment method and its application remain limited due to a lack of understanding of the possible pathways of TC differentiation in solid malignant neoplasms, including a lack of sufficient information about the immunological mechanisms that can regulate these processes [1–3]. This study was aimed at investigating the likely immune mechanisms of the MC effect on the differentiation of BC cells, and in particular, on the formation of the cytokine network underlying this process.

The MC effect on the differentiation of BC cells turned out to depend on the molecular subtype of the tumor. The most pronounced effect was observed in Lum A and Lum B, where MC caused a statistically significant decrease in the relative content of LGC. Similar trends were observed in the HER2-positive subtype, although the effect was less pronounced. It is noteworthy that in (TN BC), only a trend towards a decrease in LGC was observed, which did not reach statistical significance. These data are consistent with the understanding of the molecular heterogeneity of BC and differences in the mechanisms of regulation of differentiation in various BC subtypes. Presumably, MC affects specific signaling pathways that play a more important role in the differentiation of luminal and HER2-positive tumors than in TN BC. This may also be due to the heterogeneity of TN BC and its known resistance to various types of therapy [16].

The results of the neural network analysis showed significant differences in the NI of various cytokines

depending on the molecular subtype of BC. Differences in the NI of cytokines between BC subtypes reflect the diversity of the tumor's immune microenvironment. Thus, for Lum A BC, IL-1Ra, IFN γ , IL-6, TNF α , and VEGF played a key role in the effect of stimulating the differentiation of BC cells. The role of IL-1Ra may be related to its ability to block the pro-inflammatory effect of IL-1 β , thereby shifting the regulatory balance towards the differentiation of Lum A TC [17, 18]. Presumably, IFN γ can directly induce the expression of genes involved in the differentiation of BC cells [19], and IL-6 and TNF α can have both pro-inflammatory and differentiation effects depending on the baseline level of their production [9, 20]. VEGF, as an angiogenesis factor, can affect the vascular microenvironment of the tumor and, indirectly, the differentiation of BC TC.

In BC Lum B, MCP-1, a chemokine involved in the recruitment and activation of macrophage cells [21], had the highest NI of IMCE. GM-CSF, IL-8, and IL-18 were also significant. As is known, GM-CSF stimulates the differentiation of myeloid cells [6], while IL-8 and IL-18 are involved in the regulation of inflammation and immune response [22, 23]. In HER2+, IFN γ had the highest IMCE NI, which may be due to its ability to activate a complex anti-tumor immune response and induce differentiation. At the same time, MCP-1, IL-17, and IL-1 β may also play an important role in this process [24]. It should be noted, however, that due to the small sample of the HER2+ group, the results obtained for this HER2+ group by the neural network analysis method should be considered as preliminary and requiring confirmation with a larger sample.

In TN BC, the highest NI values were identified for the IMCE of IL-1 β , IL-6, IL-17, and IFN γ , which may reflect the peculiarities of the immune microenvironment of this subtype. The fact that the IL-1 β IMCE was of great importance for stimulating differentiation in triple-negative BC suggests that targeting the IL-1 β signaling pathway may be a promising therapeutic approach for this particularly aggressive BC subtype.

The NI in neural network analysis is a tool for assessing the relative strength of the influence of variables on the expected effect, and not their share in the overall effect. Therefore, there is no universal, rigidly defined boundary for dividing predictors into "significant" and "insignificant" based on NI. Given that in our study, NI assesses the prognostic strength of each individual predictor (IMCE) in the

development of the TC differentiation effect, it is only possible to conditionally identify (for example, with NI > 65%) the most significant cytokines that affect the development of this effect in various BC subtypes: in Lum A – IL1-Ra, IL-6, IFN γ , IL-8, TNF α , and VEGF; in Lum B – MCP-1, GM-CSF, and IL-8; in HER2+ – IFN γ , MCP-1, IL-17, and IL-1 β ; in the TN subtype – IL-1 β and IL-6.

Thus, the differences in the relationships we identified between the IMCE and the development of the differentiation effect under the action of MC may reflect complex interactions between various cells of the immune system that form the tumor microenvironment and the intratumoral cytokine network, which controls the processes that regulate the differentiation of BC cells. Some cytokines may directly affect differentiation, while others may modulate this process indirectly, through interaction with other components of the tumor microenvironment.

CONCLUSION

The results obtained are important for understanding the mechanisms underlying the effect of MC on the differentiation of BC cells. Identifying the key cytokines involved in this process can serve as the basis for developing new therapeutic strategies aimed at inducing the differentiation of tumor cells and increasing the effectiveness of BC treatment. The study results emphasize the importance of a personalized approach to the treatment of BC within the framework of differentiation therapy, taking into account the molecular subtype of the tumor.

REFERENCES

1. Terpinskaya T.I. Mechanisms of Drug Resistance of Tumors: a Brief Review of Modern Data. *News of Biomedical Sciences*. 2022; 22(2): 160–174. (In Russ.).
2. Yan M., Liu Q. Differentiation therapy: a promising strategy for cancer treatment. *Chin. J. Cancer*. 2016;35(3). DOI: 10.1186/s40880-015-0059-x
3. Bar-Hai N., Ishay-Ronen D. Engaging plasticity: differentiation therapy in solid tumors. *Front Pharmacol*. 2022;13:944773. DOI: 10.3389/fphar.2022.944773
4. Madan V., Koeffler H.P. Differentiation therapy of myeloid leukemia: four decades of development. *Haematologica*. 2021;106(1). DOI:10.3324/haematol.2020.262121
5. Turner K.M., Yeo S.K., Holm T.M., Shaughnessy E., Guan J.L. Heterogeneity within molecular subtypes of breast cancer. *Am. J. Physiol. Cell Physiol*. 2021;321(2):343–C354. DOI: 10.1152/ajpcell.00109.2021
6. Carvalho E., Canberk S., Schmitt F., Vale N. Molecular subtypes and mechanisms of breast cancer: precision medicine approaches for targeted therapies. *Cancers*. 2025;17(7):1102. DOI:10.3390/cancers17071102
7. Li X., Zhou J., Xiao M., Zhao L., Zhao Y., Wang S. et al. Uncovering the subtype-specific molecular characteristics of breast cancer by multiomics analysis of prognosis-associated genes, driver genes, signaling pathways, and immune activity. *Front. Cell Dev. Biol*. 2021;9:689028. DOI: 10.3389/fcell.2021.689028
8. Akinsipe T., Mohamedelhasan R., Akinpelu A., Pondugula S.R., Mistriotis P., Avila L.A. et al. Cellular interactions in tumor microenvironment during breast cancer progression: new frontiers and implications for novel therapeutics. *Front. Immunol*. 2024;15:1302587. DOI: 10.3389/fimmu.2024.1302587
9. Chen J., Wei Y., Yang W., Huang Q., Chen Y., Zeng K. et al. IL-6: The link between inflammation, immunity and breast cancer. *Front. Oncol*. 2022;12:903800. DOI: 10.3389/fonc.2022.903800
10. Jorgovanovic D., Song M., Wang L., Zhang Y. Roles of IFN- γ in tumor progression and regression: a review. *Biomarker Research*. 2020;8:49. DOI: 10.1186/s40364-020-00228-x
11. RF Patent for Invention No. 2697199 08/13/2019. Bulletin No. 23. Autenshlyus A.I., Arkhipov S.A., Mikhajlova E.S., Marinkin I.O., Varaksin N.A. Composition for Increasing Content of Highly Differentiated Cells in Breast Adenocarcinoma. (In Russ.)
12. Nascimento R.G., Otoni K.M. Histological and molecular classification of breast cancer: what do we know? *Mastology*. 2020;30:e20200024. DOI: 10.29289/259453942020200024
13. Zhen H., Yang L., Li L., Yu J., Zhao L., Li Y. et al. Correlation analysis between molecular subtypes and Nottingham Prognostic Index in breast cancer. *Oncotarget*. 2017;8(43):74096–74105. DOI: 10.18632/oncotarget.18242
14. Ali S., Hamam D., Liu X., Lebrun J.J. Terminal differentiation and anti-tumorigenic effects of prolactin in breast cancer. *Front. Endocrinol*. 2022;13:993570. DOI: 10.3389/fendo.2022.993570
15. Rakha E.A., Reis-Filho J.S., Baehner F., Dabbs D.J., Decker T., Eusebi V. et al. Breast cancer prognostic classification in the molecular era: the role of histological grade. *Breast Cancer Res*. 2010;12(4):207. DOI: 10.1186/bcr2607
16. Asleh K., Riaz N., Nielsen T.O. Heterogeneity of triple negative breast cancer: Current advances in subtyping and treatment implications. *J. Exp. Clin. Cancer Res*. 2022;41:265. DOI: 10.1186/s13046-022-02473-z
17. Tan C., Hu W., He Y., Zhang Y., Zhang G., Xu Y., et al. Cytokine-mediated therapeutic resistance in breast cancer. *Cytokine*. 2018, 108: 151–159. DOI: 10.1016/j.cyt.2018.03.020
18. Baker K.J., Houston A., Brint E. IL-1 family members in cancer, two sides to every story. *Front. Immunol*. 2019;10:1197. DOI: 10.3389/fimmu.2019.01197
19. Garcia-Tunon I., Ricote M., Ruiz A., Fraile B., Paniagua R., Royuela M. Influence of IFN-gamma and its receptors in human breast cancer. *BCM Cancer*. 2007;7:158. DOI: 10.1186/1471-2407-7-158
20. Cruceriu D., Baldasici O., Balacescu O., Berindan-Neagoe I. The dual role of tumor necrosis factor-alpha (TNF- α) in breast cancer: molecular insights and therapeutic approaches. *Cell Oncol*. 2020;43:1–18. DOI: 10.1007/s13402-019-00489-1
21. Singh S., Anshita D., Ravichandiran V. MCP-1: Function, regulation, and involvement in disease. *Int. Immunopharmacol*. 2021;101:107598. DOI: 10.1016/j.intimp.2021.107598

22. Long X., Ye Y., Zhang L., Liu P., Yu W., Wei F., et al. IL-8, a novel messenger to cross-link inflammation and tumor EMT via autocrine and paracrine pathways. *Int J. Oncol.* 2016;48(1):5–12. DOI: 10.3892/ijo.2015.3234
23. Cirella A., Olivera I., Luri-Rey C., Bolaños E., Berraondo P., Melero I. Interleukin-18 in cancer immunology and immunotherapy. *Expert Opin. Ther. Targets.* 2023;27(11):1035–1042. DOI: 10.1080/14728222.2023.2287574
24. Akinsipe T., Mohamedelhasan R., Akinpelu A., Pondugula S.R., Mistriotis P., Avila L.A., et al. Cellular interactions in tumor microenvironment during breast cancer progression: new frontiers and implications for novel therapeutics. *Front. Immunol.* 2024;15:1302587. DOI: 10.3389/fimmu.2024.1302587

Author Contribution

Autenshlyus A.I., Arkhipov S.A. – conception and design, data analysis and interpretation, substantiation of the manuscript and critical revision for important intellectual content, final approval of the manuscript for publication. Mikhaylova E.S., Arkhipova V.V., Druzhinina Y.G., Yakovleva K.I. – data analysis and interpretation, final approval of the manuscript for publication.

Author Information

Arkhipov Sergey A. – Dr. Sci. (Biol.), Leading Researcher, Central Research Laboratory, Novosibirsk State Medical University, Novosibirsk; Senior Researcher, Research Institute of Molecular Biology and Biophysics, Federal Research Center for Fundamental and Translational Medicine, Novosibirsk, arhipowsergei@yandex.ru, <https://orcid.org/0000-0002-1390-4426>

Mikhaylova Elena S. – Researcher, Central Research Laboratory, Novosibirsk State Medical University, Novosibirsk; Researcher, Research Institute of Molecular Biology and Biophysics, Federal Research Center for Fundamental and Translational Medicine, Novosibirsk, elena.michajlova.58@mail.ru, <https://orcid.org/0000-0002-8364-819X>

Arkhipova Valentina V. – Junior Researcher, Central Research Laboratory, Novosibirsk State Medical University, Novosibirsk, valia.arkhipova@yandex.ru, <https://orcid.org/0009-0000-0172-0905>

Druzhinina Yulia G. – Senior Researcher, Vector-Best JSC, Novosibirsk, druzhinina@vector-best.ru, <https://orcid.org/0009-0000-9279-8395>

Yakovleva Ksenia I. – Senior Researcher, Vector-Best JSC, Novosibirsk, yakovlevak@vector-best.ru, <https://orcid.org/0009-0003-7802-5486>

Autenshlyus Alexander I. – Dr. Sci. (Biol.), Professor, Head of Central Research Laboratory, Novosibirsk State Medical University, Novosibirsk; Chief Researcher, Research Institute of Molecular Biology and Biophysics, Federal Research Center for Fundamental and Translational Medicine, Novosibirsk, lpciip@211.ru, <https://orcid.org/0000-0001-7180-010X>

(✉) **Arkhipov Sergey A.**, arhipowsergei@yandex.ru

Received on May 15, 2025;
approved after peer review on July 01, 2025;
accepted on September 04, 2025