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Studying GATA3, FOXA1, and ELF5 transcription factors in the evaluation of prognosis in luminal breast cancer patients

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ABSTRACT

Background. The identification of predictive molecular markers of luminal breast cancer will help to assess the risk of developing distant metastases and determine a personalized approach to predicting the outcome of the disease during hormone therapy.

The aim of the study was to investigate the relationship between the transcription factors GATA3, FOXA1, and ELF5 in the tumor and the occurrence of distant metastases in patients with luminal subtype of breast cancer during adjuvant hormone therapy.

Materials and methods. The study included 101 patients with breast cancer (aged from 30 years to 81 years, average age (54.8 ± 10.3) years), with stages $T_{1–4}N_{1–3}M_0$ of the disease. The follow-up period was at least 5 years. The inclusion criteria for the study were luminal molecular genetic subtype of the tumor and lack of preoperative treatment. The exclusion criterion was stage IV disease. The study of transcription factors was carried out by the immunohistochemical method using polyclonal antibodies to GATA3, FOXA1, and ELF5, manufactured by Flarebio (Austria).

Results. Low expression of FOXA1 and ELF5 in the tumor was associated with the development of distant metastases (p = 0.000015 and p = 0.000002, respectively). In addition, it was found that high incidence of hematogenous metastases was associated with heterogeneous expression of FOXA1 ($\chi 2$ = 6.42; p = 0.01) and ELF5 ($\chi 2$ = 14.46; p = 0.0001) in the tumor. No similar differences were found in the study of GATA3 expression.

Conclusion. The level of expression of transcription factors FOXA1 and ELF5 and their distribution in the primary tumor can be considered as potential molecular markers in assessing the risk of hematogenous metastasis in patients with luminal breast cancer.

Key words: luminal breast cancer, transcription factors GATA3, FOXA1, ELF5, prognosis, distant metastases.

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

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Conformity with the principles of ethics. All patients signed informed consent to participate in the study. The work was carried out in accordance with the principles of voluntariness and confidentiality in accordance with the "Fundamentals of the legislation of the Russian Federation on the protection of public health" (Decree of the President of the Russian Federation of December 24, 1993 No. 2288) on the basis of the permission of the local committee on biomedical ethics of the Research Institute of Oncology of the Tomsk Scientific Research Center (Protocol No. 4994 of 27.10.2016).

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Исследование факторов транскрипции GATA3, FOXA1, ELF5 в оценке прогноза у больных люминальным раком молочной железы

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РЕЗЮМЕ

Актуальность. Выявление предсказательных молекулярных маркеров люминального рака молочной железы (РМЖ) позволит оценить риск развития отдаленных метастазов и определить персонализированный подход к прогнозированию течения заболевания при проведении гормонотерапии.

Цель. Изучить взаимосвязь транскрипционных факторов GATA3, FOXA1, ELF5 в опухоли с возникновением отдаленных метастазов у больных люминальным подтипом РМЖ при проведении адъювантной гормонотерапии.

Материалы и методы. В исследование включена 101 больная РМЖ (возраст от 30 лет до 81 года, средний возраст (54,8 ± 10,3) года), стадии $T_{1–4}N_{1–3}M_0$. Срок наблюдения составил не менее 5 лет. Критериями включения в исследование явились: люминальный молекулярно-генетический подтип опухоли, отсутствие предоперационного лечения. Критерий исключения – IV стадия заболевания. Исследование транскрипционных факторов проводилось иммуногистохимическим методом с использованием поликлональных антител фирмы Flarebio (Австрия) к GATA3, FOXA1 и ELF5.

Результаты. Выявлено значимое снижение процента экспрессии FOXA1 и ELF5 в опухоли при развитии отдаленных метастазов (p = 0,000015 и p = 0,000002 соответственно). Кроме того, показано, что большая частота развития гематогенных метастазов сопряжена с гетерогенной экспрессией в опухоли FOXA1 (χ^2 = 6,42; p = 0,01) и ELF5 (χ^2 = 14,46; p = 0,0001). Подобных отличий в отношении экспрессии GATA3 не обнаружено.

Заключение. Уровень и характер экспрессии транскрипционных факторов FOXA1 и ELF5 в первичной опухоли могут рассматриваться в качестве потенциальных молекулярных маркеров в оценке риска гематогенного метастазирования у больных люминальным подтипом карциномы молочной железы.

Ключевые слова: люминальный рак молочной железы, факторы транскрипции GATA3, FOXA1, ELF5, прогноз, отдаленные метастазы.

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INTRODUCTION

In recent decades, the overall incidence of breast cancer (BC) in women has remained consistently high, with a slight increase in overall five-year survival rate. In Russia, breast cancer is the leading oncological pathology in the female population and accounts for 20.9%, occupying the largest share in the age group 30–59 years [1, 2].

Recently, transcription factors (TFs) have attracted the greatest interest as significant predictors of the course of primary breast cancer.

GATA binding protein 3 (GATA3) is a transcription factor of the zinc finger family, which normally regulates the proliferation and differentiation of breast luminal cells [3]. Mutation of this transcription factor plays an important role in breast cancer carcinogenesis, ranking third after TP53 and PIK3CA mutations [4]. A group of researchers (METABRIC Group) analyzed 2,433 and 2,000 breast carcinoma tissue samples for significant mutations and found that mutant GATA3 tumors in a subgroup of patients with ER + breast cancer had a low grade of malignancy and, therefore, a favorable prognosis and better overall 10-year survival rat [5]. Positive expression of GATA3 in breast carcinoma in the immunohistochemical study is also associated with a better prognosis and survival, and loss of expression is characterized by opposite effects [6].

The data on the relationship of this transcription factor with the development of metastases available in the literature are also contradictory and ambiguous. In one of the studies, a discrepancy between the expression level of the described transcription factor in the primary tumor and in the lymph nodes affected by metastases was found. The authors of the work showed that in some cases positive expression of GATA3 was observed in the affected lymph nodes with negative expression of the marker in the primary breast tumor and vice versa [7]. In a number of other studies, the authors evaluated the expression characteristics of GATA3 in hematogenous metastases of breast carcinoma of various localization and found that during the development of metastatic foci in the lungs, the expression of the TF under study was significantly lower in comparison with the parameters of its expression in metastases of other organs and tissues. The data obtained may indicate the possible involvement of GATA3 in the mechanisms that prevent metastasis [8, 9].

Forkhead box A1 (FOXA1), or hepatic nuclear factor 3α (HNF3α), like GATA3, is a transcription factor that not only plays a key role in the embryonic development of various organs and tissues, but also participates in breast tumorigenesis [10]. The data available in the literature suggest a possible significant potential of GATA3 in assessing the prognosis and response to hormone therapy [11, 12]. According to a number of authors, breast cancer patients in whom primary tumors were characterized by positive FOXA1 expression in ER +/PR+ status had longer overall and disease-free survival [13, 14]. The prognosis of the disease in patients was primarily determined by the fact that FOXA1 was involved in the epithelial-mesenchymal transition (EMT), interacting with the key transcription factors Twist1 and Slug, which led to a decrease in overall survival [15].

E74-like factor 5 (ELF5), a transcription factor of the E26 family (ETS), is involved in the development of breast tissue, while its primary role consists in formation of alveoli and transformation of progenitor cells into mature acinar cells [16]. This TF plays an essential role in EMT; in particular, the inhibitory effect of ELF5 on EMT in breast cancer was shown [17]. It was demonstrated in MCF-7 mouse models that the expression of this marker in the tumor can be associated with resistance to hormone therapy [18].

Thus, the data available in the literature remain controversial, which suggests the need to study the transcription factors ELF5, FOXA1, and GATA3 in patients with breast cancer in order to clarify the mechanisms of tumor progression and consider the studied markers in relation to possible assessment of prognosis for creating a personalized approach to treatment of the disease.

The aim of the study was to investigate the relationship between the expression of the transcription factors GATA3, FOXA1, and ELF5 in the tumor and the occurrence of distant metastases in patients with luminal subtype of breast cancer during adjuvant hormone therapy.

MATERIALS AND METHODS

The study included 101 patients with breast cancer (aged from 30 to 81 years, mean age 54.8 ± 10.3 years), stage $T_{1-4}N_{1-3}M_0$. All patients were treated at the Cancer Research Institute, Tomsk National Research Medical Center. The diagnosis of breast

cancer was established on the basis of core biopsy of the tumor in accordance with the WHO classification (2012). The molecular biological subtype of the neoplasm was assessed by immunohistochemistry (IHC). Antibodies to ER (clone 1D5, Dako), PR (clone PgR636, Dako), Ki-67 (clone MIB-1, Dako), and Her2 / neu (polyclonal, c-erB-2, Dako) were used. The patients did not receive preoperative treatment. In terms of surgical treatment, all women underwent surgery in form of radical mastectomy or sectoral resection (breast-conserving treatment). In 33% of patients at the time of diagnosis, menstrual function was preserved, and 68% of patients were menopausal. After the surgical stage, all patients received hormone therapy with tamoxifen or aromatase inhibitors. The observation period was at least 5 years. The primary documents were analyzed, such as medical histories and outpatient records, the presence of local recurrence of the disease and the presence and localization of distant tumor metastases were assessed. The inclusion criteria for the study were luminal molecular tumor subtype and absence of preoperative treatment. The exclusion criterion was stage IV of the disease.

When examining the surgical material, the size of the tumor node, breast tissue outside the formation, the state of the resection margins, as well as all removed axillary lymph nodes were assessed for the presence of metastatic lesions. The tumor grade was determined according to the Nottingham Histologic Grade (Elston modification of the Bloom–Richardson grading system). In the tumor stroma, the severity of infiltration by immune cells was assessed in points (1 point – no infiltration or weakly pronounced, 2 points – moderately pronounced, 3 points – strongly pronounced).

IHC of transcription factors was carried out according to the standard technique. We used polyclonal rabbit antibodies to GATA3 (dilution 1 : 200), FOXA1 (dilution 1 : 200), and ELF5 (dilution 1 : 150) produced by Flarebio (Austria). The interpretation of the staining results included the following features: the presence of marker expression (positive or negative expression) and the intensity of expression of the marker under study (on a scale from 1 to 3 points). The percentage of tumor cells with positive immunostaining was counted (the count was performed per 1,000 cells in 10 fields of view at ×40). In addition, the type of the distribution of the expression of the studied transcription factors in the tumor was assessed. In case of uniform staining in the tumor cells, regardless of the intensity of the marker expression, the expression was considered homogeneous. The presence of foci with positive and negative expression in the tumor section, as well as foci with varying degrees of staining intensity, the distribution pattern was considered heterogeneous.

Statistical analysis of the data was carried out using the Statistica v.10 package using analysis of variance, χ^2 test, and nonparametric Mann–Whitney *U*-test. Thee data were presented as the median and interquartile range Me ($Q_1 \div Q_3$). Results with significant differences at $p < 0.05$ were discussed.

RESULTS AND DISCUSSION

At the first stage, we analyzed the frequency of expression of the studied transcription factors in the tumor and possible combinations of these markers in patients with luminal BC. The frequency of occurrence of positive staining was as follows: in 96 (95.1%) patients, positive nuclear staining of the GATA3 marker was noted, in 5 cases (4.9%), the expression of the marker was negative. Positive expression of FOXA1 was detected in 91 (90.1%) patients, negative – in 10 (9.9%) patients; at the same time, positive expression of the ELF5 factor was noted in 89 (88.1%) cases, in 12 (11.9%) cases, this factor was not detected in the tumor.

Analysis of the combination of the expression of all three studied transcription factors showed fairly high variability of their co-expression. The phenotype GATA3 + FOXA1 + ELF5 + was dominant in the study group of patients and amounted to 78.2% (79 / 101). In 8 cases, the tumor showed positive staining only with antibodies to GATA3 and FOXA1 (GATA3 + FOXA + ELF5). In 4 cases, the tumor cells had the GATA3-FOXA1 + ELF5 + phenotype. In 3 cases, the neoplasm had the GATA3 + FOXA1-ELF5- phenotype, and in 5 patients the neoplasm had GATA3 + FOXA1-ELF5 +. It should be emphasized that 1 case in the study group was characterized by the presence of only positive expression of ELF5 (GATA3-FOXA1-) and 1 case had negative expression of all three studied markers in the primary tumor (GATA3-FOXA1-ELF5-).

Our analysis showed that the frequency of positive expression and the variant of the co-expression of all three studied transcription factors did not have any significant differences in the follow-

ing clinical and morphological signs: the age of the patients, menstrual status, the luminal subtype (A, B1, B2), the stage of cancer. It was also shown that the percentage of positively stained tumor cells for GATA3, FOXA1, and ELF5 did not statistically differ depending on the above mentioned clinical and morphological parameters.

We have previously shown that there is a relationship between the expression of markers ELF5 and FOXA1 and such an important parameter as the size of the primary tumor. In addition, it was found that the character of GATA3 and ELF5 expression was associated with the rate of lymph node metastases [19].

At the follow-up stages, local tumor recurrence was diagnosed in 9 out of 101 (8.9%) patients. In 8 (88.9%) cases, local tumor recurrence developed in the area of the postoperative scar, and in 1 (11.1%) case in the remaining breast tissue after organ-preserving treatment. In 7 (77.8%) cases, tumor recurrence was represented by a single node, while in 2 (22.2%) cases, multiple tumor lesions were noted. Assessment of the expression of transcription factors in the tumor did not reveal significant dependence of the expression on the rate of recurrence in the studied group of patients.

Distant metastases were diagnosed in 15 out of 101 (14.8%) patients. The site of metastatic foci and the time of their occurrence after surgery were assessed. Isolated bone metastases were found in 9 out of 15 cases (60%). In 6 cases (40%), multiple metastatic lesions of the bones and visceral organs were observed. It was of interest to study the clinical, morphological, and molecular characteristics of the tumor in patients with luminal BC depending on the development of hematogenous spread. The incidence of distant metastases was associated with the size of the primary tumor (p = 0.002) and regional lymph node metastases (χ^2 = 10,9, p = 0.00095). There was a clear tendency towards a higher rate of hematogenous dissemination with an increase in the grade of tumor (χ^2 = 4.9, p = 0.08). However, the development of distant metastases was not associated with the Ki67 proliferative activity index of tumor cells (p = 0.8) and the luminal subtype of the neoplasm (p = 0.6). At the same time, significant differences were revealed in the expression of estrogen and progesterone receptors. Thus, in the group of patients with distant metastases, the expression (%) of both types of receptors was significantly lower (Table 1).

Table 1

Indicators of expression of sex hormone receptors in the tumor depending on the presence of distant metastases in patients with luminal breast cancer

Hormone receptors	Expression in the tumor, %, *Me* $Q_1 \div Q_3$	
	Absence of hematogenous metastases	Presence of hematogenous metastases
ER	94.7 (70÷100)	69.3 (51.5÷93) p_{1-2} = 0.02
PR	83 (59.5÷98)	49 (32÷74.4) p_{1-2} = 0.038

Analysis of the relationship between the expression of the studied transcription factors in the tumor in groups of patients and the presence or absence of hematogenous metastases revealed significant differences in the percentage of FOXA1 and ELF5 expression. It was found that with the development of distant metastases, the percentage of FOXA1 and ELF5 expression in the cells of the primary tumor is significantly lower than in patients with no signs of disease progression. No such differences in the expression of the GATA3 marker were recorded (Table 2).

Table 2

Indicators of expression of transcription factors in the tumor depending on the presence of distant metastases in patients with luminal breast cancer

Transcription factors	Expression in the tumor, % (*Me* $Q_1 \div Q_3$)	
	Absence of hematogenous metastases	Presence of hematogenous metastases
GATA3	100 (98 ÷ 100)	100 (100 ÷ 100)
FOXA1	100 (90.3 ÷ 100)	70 (42 ÷ 76) p_{1-2} = 0.000015
ELF5	100 (100 ÷ 100)	76 (65 ÷ 100) p_{1-2} = 0.000002

The study showed a relationship between the nature of the distribution of the studied marker expression and the frequency of hematogenous metastases.

Most often, hematogenous metastases were observed with heterogeneous expression of factors FOXA1 (Fig. 1) (χ^2 = 6.42, p = 0.01) and ELF5 (χ^2 = 14.46, p = 0.0001) in the tumor (Table 3).

When studying the distribution pattern of GATA3 expression in the tumor (Fig. 2) and the rate of distant metastases, no significant differences were revealed. Infiltration of a tumor by immune cells is an important component of the microenvironment that affects the processes of proliferation, angiogenesis,

Table 3

Rate of distant metastasis depending on the distribution of FOXA1 and ELF5 expression in patients with luminal breast cancer

Distribution of the marker expression in the primary tumor	Distant metastases, abs. number (%)	
	Absence of hematogenous metastases	Presence of hematogenous metastases
	FOXA1	
Homogeneous (n = 45)	44 (97%)	1(3%)
Heterogeneous (n = 46)	36 (78.2%)	10 (21.8%)
	ELF5	
Homogeneous (n = 56)	54 (96.4%)	2 (3.6%)
Heterogeneous (n = 33)	21 (63.6%)	12 (36.4%)

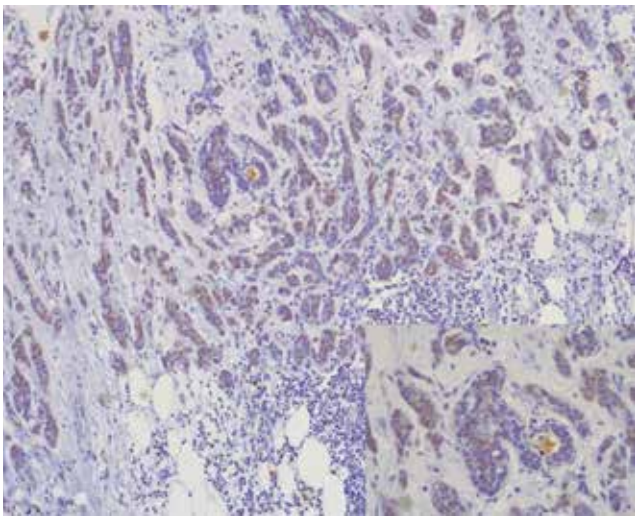

Fig. 1. Moderately pronounced heterogeneous nuclear expression of FOXA1 in tumor cells of invasive breast carcinoma, ×100. The lower right corner shows the presence of tumor cells with positive and negative expression, ×400. ICH

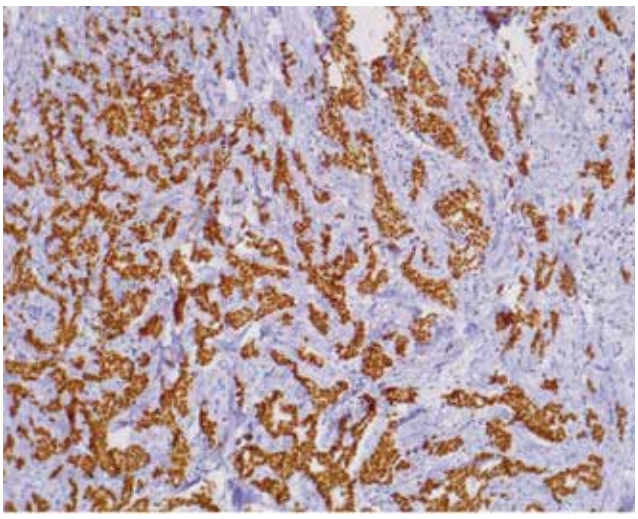

Fig. 2. Positive homogeneous nuclear expression of GATA3 in tumor cells of invasive breast carcinoma, ×100. ICH

and invasion and can determine the characteristics of tumor progression, since it is involved in the mechanisms of the metastatic cascade. The expression of the studied transcription factors in the tumor was analyzed depending on the degree of inflammatory infiltration in the neoplasm stroma. The study showed that the expression of GATA3, FOXA1, and ELF5 did not differ in tumors with different degree of infiltration with immune cells. The expression of the latter had no significant association with the incidence of distant metastases (p = 0.57).

The study of GATA3, FOXA1, and ELF5 in the primary tumor in patients with the luminal subtype of breast cancer showed a clear relationship between their expression and the development of hematogenous metastasis. The identified relationship between the low expression of ELF5 and FOXA1 and the rate of hematogenous metastasis can be explained in different ways. On the one hand, the revealed patterns can be caused by the pathogenetic influence of these factors on proliferation of tumor cells and angiogenesis, being a manifestation of EMT activation as a key mechanism in the development of hematogenous progression. It is known that the transcription factors ELF5 and FOXA1 have a suppressive effect on EMT. Therefore, a decrease in the level of their expression in the tumor may promote the activation of important factors Twist1 and Slug. However, these aspects require further study. On the other hand, hematogenous progression in the group of patients with low expression of FOXA1 and ELF5 with their heterogeneous distribution in the tumor along with low expression of ER and PR can determine the development of resistance to hormonal therapy and, therefore, become the pathogenetic basis of treatment inefficiency and disease progression.

CONCLUSION

The results of this study demonstrate the clinical significance of the transcription factors FOXA1 and ELF5 in assessing the risk of distant metastasis in patients with luminal BC subtype and can be used to predict the course of the disease when choosing the management tactics for this category of patients. Analysis of these transcription factors in the tumor can be performed at the preoperative stage during a standard immunomorphological study and considered as additional tumor parameters when planning the patient's treatment strategy.

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Authors contribution

Vtorushin S.V. – conception and design, analysis and interpretation of data. Vasilchenko D.V. – analysis and interpretation of data, review of publications on the topic of the manuscript. Zavyalova M.V. – analysis and interpretation of data. Krakhmal' N.V. – carrying out of immunohistochemical studies, analysis of data. Patalyak S.V. – selection of cases for research, work with medical records, analysis of data.

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