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FEATURES OF *COL1A1* EXPRESSION IN BREAST CANCER TISSUE OF YOUNG PATIENTS

Background. In the last decades, the incidence of breast cancer (BCa) in young women has been increasing steadily. The quantitative indicators of expression of collagen, which play important role in stromal microenvironment, and their association with the age and survival rates of BCa patients have not been yet definitively clarified. **Aim.** To investigate the relationship between the *COL1A1* gene expression at the mRNA and protein levels in BCa tissue and the clinicopatological features and survival rates of BCa patients of different age groups. **Materials and Methods.** The study was conducted on the clinical material of 50 patients with stage I—III BCa. *COL1A1* gene expression at the mRNA and protein levels in BCa tissue were studied using the real-time PCR and immunohistochemical methods, as well as the bioinformatic analysis (UALCAN and Kaplan — Meier Plotter databases). **Results.** The bioinformatic analysis showed that BCa tissue is characterized by 6.0 times ($p < 0.05$) higher level of *COL1A1* mRNA compared to normal breast tissue. The correlation of *COL1A1* expression at the mRNA and protein levels with the molecular subtype of neoplasms was demonstrated. According to Kaplan — Meier Plotter database, a low level of expression of *COL1A1* protein level in BCa tissue is associated with lower rates of relapse-free survival of patients. The *ex vivo* study of the clinical material revealed a decrease in *COL1A1* protein expression in tumor tissue of young patients with BCa of T3 category ($p < 0.0374$), low differentiation grade ($p < 0.0163$) and basal molecular subtype ($p < 0.0001$). A correlation between the expression of *COL1A1* at the mRNA and protein levels and the expression status of estrogen receptors ($p < 0.0001$) and progesterone receptors ($p < 0.0040$) was established. The relapse-free 3-year survival rate of young BCa patients is significantly lower in the presence of a low *COL1A1* optical density index in the tumor tissue. **Conclusions.** The identified relationship between *COL1A1* expression and such indicators of BCa malignancy as tumor size, differentiation grade, molecular subtype, receptor status, and the recurrence-free survival of patients indicates the prospects of its use to predict the aggressiveness of the BCa course in young patients.

Keywords: breast cancer, collagen, *COL1A1*, young patients.

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Despite a significant progress in cancer diagnosis and treatment, the incidence of breast cancer (BCa) is constantly increasing, and the mortality rate does not show a tendency to decrease both in Ukraine and in the world. The statistical data of the last decades indicate an increase in the frequency of BCa detection in young women; BCa ranks first in the cancer incidence in women in the age group of 18—29 years and occupies one of the leading places in mortality in the age group of 15—39 years [1]. It is known that BCa in young patients is characterized by a high risk of metastasis, low sensitivity to chemotherapy, and rapid development of local recurrences compared to patients older than 45 years [2, 3].

It has been conclusively proven that the key role in tumor progression and the formation of the drug resistance phenotype of many malignant neoplasms, including BCa, is played by the stromal microenvironment of a tumor representing a complex and dynamic structure whose chemical and biophysical properties affect cell adhesion, proliferation, and migration [4, 5]. It has been established that a low percentage of stroma and high tumor-stromal ratio are characteristic features of BCa in young patients compared to older women [6].

The main fibrillar component of the stroma is collagen, which makes up to 30% of the total protein mass in the human body. The changes in the ratio and spatial structure of collagen are currently considered a key diagnostic and prognostic factor associated with the occurrence and progression of many malignant neoplasms, including BCa, which is confirmed by the data of our previous study [7].

To date, about 30 different types of collagens have been identified, which are divided into 4 groups according to their functional activity: fibrillar, fibril-associated (fibril-associated collagens with interrupted helices or FACIT), network-forming collagens, as well as proteins that are part of the basement membrane [8]. The main component of the extracellular matrix (ECM), which accounts for about 95% of the total mass of collagens in the BCa stroma, is type I collagen (COL1) [9]. COL1 consists of two COL1A1 polypeptide chains and one COL1A2 chain and is a heterotrimer in the form of a triple helix. Trimers of the procollagen molecule after enzymatic modification inside and outside the cell are exposed in ECM and form long thin fibrils with stable cross-links with each other.

It was established that an increase in the number of COL1 fibrils contributes to the increase in the stiffness of the BCa tissue and is accompanied by an increase in hypoxia, which leads to the activation of migration of disseminated cells and also correlates with cancer aggressiveness [10, 11]. Along with this, the increased *COL1A1* expression is associated with the epithelial-mesenchymal transition and high proliferative activity of malignantly transformed cells [9].

Despite the proven role of the stromal microenvironment in the development and progression of BCa, the quantitative indicators and topology of collagen expression associated with young age of patients and survival rates have not been definitively clarified and require further research. The aim of the study was to investigate the relationship between the *COL1A1* gene expression at the mRNA and protein levels in BCa tissue and the clinicopathological features and survival rates of BCa patients of different age groups.

MATERIALS AND METHODS

Patients. The study was conducted on the postoperative material of 50 patients with stage I—III BCa who were treated at the Communal Nonprofit Enterprise "Prykarpatsky Clinical Oncology Center of the Ivano-Frankivsk Regional Council" in 2017—2020. The patients gave an informed consent for the use of their clinical data for scientific purposes. All patients received neither radiation treatment nor chemotherapy before fine-needle aspiration biopsy. They were

examined using conventional clinical and laboratory methods according to the standards of diagnosis and treatment of cancer patients approved by the order of the Ministry of Health of Ukraine No. 554 from 17.09.2007. Tissue samples were encoded and depersonalized. The general clinical characteristics of the patients are presented in Table 1.

Immunohistochemical method. The expression of steroid hormone receptors, human epidermal growth factor receptor 2 (HER2/neu), and Ki-67 were determined using estrogen receptor (ER) (clone EP1, Bio SB, USA), progesterone receptor (PR) (clone BSB-2, Bio SB, USA), HER2/neu (clone EP3, Bio SB, USA), and Ki-67 (clone SP6, Vitro Master Diagnostica, Spain) antibodies. The expression levels of ER, PR, and Ki-67 were determined as a percentage of positive cells. The HER2/neu expression was assessed by the results of the immunohistochemical (IHC) reaction: "+++" — high, "++" — moderate, "+" — low, or '0' — no expression. Based on the expression level of the markers, the molecular subtype was determined as follows: luminal A (ER and/or PR positive, HER2/neu negative, Ki-67 < 14%), luminal B (ER and/or PR positive, HER2/neu negative or positive, Ki-67 > 14%); HER2/neu positive (ER and PR negative, HER2/neu positive, Ki-67 — any) and basal (ER, PR and HER2/neu negative, Ki-67 — any) [12]. The expression of COL1A1 was studied on paraffin sections (4—6 μm) of fine-needle biopsy. COL1A1-Ab (clone 1B2; MyBioSource, USA) was used as a primary antibody. The Master Polymer Plus Detection System (Vitro Master Diagnostica, Spain) was used to visualize the reaction according to the manufacturer's recommendations. The sections were stained with Mayer's hematoxylin (Thermo Scientific Richard — Allan, USA). Unmasking of antigens was performed in EDTA buffer (pH = 8.0) at 96 °C. The analysis of the results of IHC studies was carried out using pre-made photomicrographs on an AxioScope A1 light microscope (Carl Zeiss, Germany) at a magnification of ×260. The analysis of COL1A1 expression was carried out using the ImageJ program, IHC Profiler plugin, and formula for calculating the optical density index [13].

Table 1. **Clinicopathological characteristics of BCa patients**

Characteristics	N	%
Number of patients	50	55.5
Patients age (years)		
Average age (range)	36.05 ± 7.3 (25—45)	
Stage		
I	14	28
II	21	42
III	15	30
Category T according to TNM		
T1	14	28
T2	18	36
T3	11	22
T4	7	14
Category N according to TNM		
N0	14	28
N1—3	36	72
Differentiation grade		
G2	28	56
G3	22	44
Molecular subtype		
Luminal A	18	36
Luminal B	16	32
Her2/neu-positive	9	18
Basal	7	14
ER expression		
+	33	66
–	17	34
PR expression		
+	22	44
–	28	56
Her2/neu expression		
+	14	28
–	36	72
Recurrence status		
Negative	33	66
Positive	17	34

Real-Time quantitative reverse transcription polymerase chain reaction (qRT-PCR). Total RNA from BCa tissue was isolated using the commercial kit "TRI" (QIAGEN, Germany) according to the manufacturer's recommendations. The amount of isolated RNA was determined using a spectrophotometer "NanoDrop 2000c Spectrophotometer" (Thermo Scientific, USA). To study the mRNA expression of *COL1A1* gene, single-stranded DNA was synthesized from 100 ng of total RNA using the LunaScript RT SuperMix Kit (New England Biolabs, Inc., USA) for reverse transcription. For qRT-PCR, the verified primers [14], synthesized by Metabion, Germany, were used. The relative expression of *COL1A1* mRNA was determined by the comparative CT method using β-actin as internal control. qRT-PCR was performed on a QuantStudio 5 Dx Real-Time PCR System (Thermo Scientific, USA).

Bioinformatics method. Comparison of the studied gene expression levels (mRNA and protein) in BCa tissue in relation to similar data in non-malignant breast cells, as well as the relationship of their expression with the main clinicopathological characteristics of the tumor process, was carried out using The University of Alabama at Birmingham CANcer data analysis Portal UALCAN (http://ualcan.path.uab.edu/index.html). The study of relapse-free survival indicators of BCa patients depending on the COL1A1 expression level was conducted using the Kaplan — Meier Plotter online resource (https://kmplot.com/analysis/index.php?p=service).

Statistical methods. Statistical analysis of the results was performed using the GraphPad Prism v.8.0 program (GraphPad Software Inc., USA) taking into account the nature of the distribution of the obtained data. Testing of the statistical hypothesis about the normality of the distribution was carried out using a Student's *t*-test (for a parametric distribution) or a Mann — Whitney U-test (for a non-parametric distribution). $p < 0.05$ was considered to indicate statistically significant differences. A recurrence-free survival analysis was carried out by the Mantel — Cox test. The critical level of significance was taken as equal to 0.05.

Results and Discussion

Bioinformatic analysis of collagen expression in BCa tissue. At the first stage, a comparative study of *COL1A1* expression at the mRNA and protein levels in normal and malignantly transformed BCa was conducted.

As shown in Fig. 1, the level of *COL1A1* mRNA in BCa tissue was 1432.7 transcripts per million (TPM) ranging from 6.8 to 5576.4 TPM, which was 6 times higher ($p < 0.05$) than in the normal breast tissue (239.0 TPM ranging from 17.3 to 1128.2 TPM). The expression values of COL1A1 protein level in normal and malignantly transformed breast tissue did not differ statistically (–0.04 a.u. ranging from 0.435 to –0.766 a.u. and 0 a.u. ranging from 2.617 to –1.778 a.u., respectively).

The analysis of *COL1A1* expression according to the clinicopathological features of BCa demonstrated that the levels of *COL1A1* mRNA in the tissue of BCa of the luminal A and B molecular subtypes were 1.1 ($p < 0.05$) and 2.3 ($p < 0.05$) times higher compared to neoplasms of HER2/neu–positive and basal molecular subtypes, while in the HER2/neu-positive molecular subtype it was 2.2 ($p < 0.05$) times higher than in the basal molecular subtype. The *COL1A1* mRNA expression in BCa tissue did not depend on the age of patients, tumor stage, and the status of the regional lymph nodes (Fig. 2).

Analysis of COL1A1 protein expression showed its significant increase in BCa tissue of patients aged 41—80 years compared to patients aged 81—100 years (Fig. 3). The highest levels of COL1A1 expression were identified in tissue of stage I BCa ($p < 0.05$). The level of COL1A1 expression in BCa tissue of both luminal molecular subtypes was significantly hig-

her ($p < 0.05$) compared to the basal molecular subtype.

Also, according to the UALCAN data, the high expression of COL1A1 protein in BCa tissue is associated with low rates of relapse-free survival of patients. In particular, the 10-year survival of patients with a high level of COL1A1 in BCa tissue was 37% lower ($p < 0.05$) compared to patients with a low level of COL1A1 expression (Fig. 4). There was no significant difference in the recurrence-free survival rate of BCa patients depending on the level of *COL1A1* mRNA expression in tumor cells.

Therefore, the bioinformatic analysis and systematization of information from the UALCAN and KMplotter databases made it possible to establish that the development of BCa is ac-

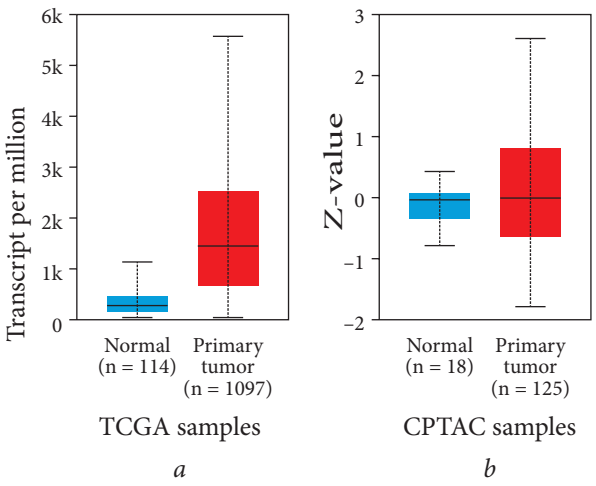

Fig. 1. Expression of *COL1A1* in normal and malignantly transformed breast tissue at the mRNA (*a*) and protein (*b*) levels according to UALCAN

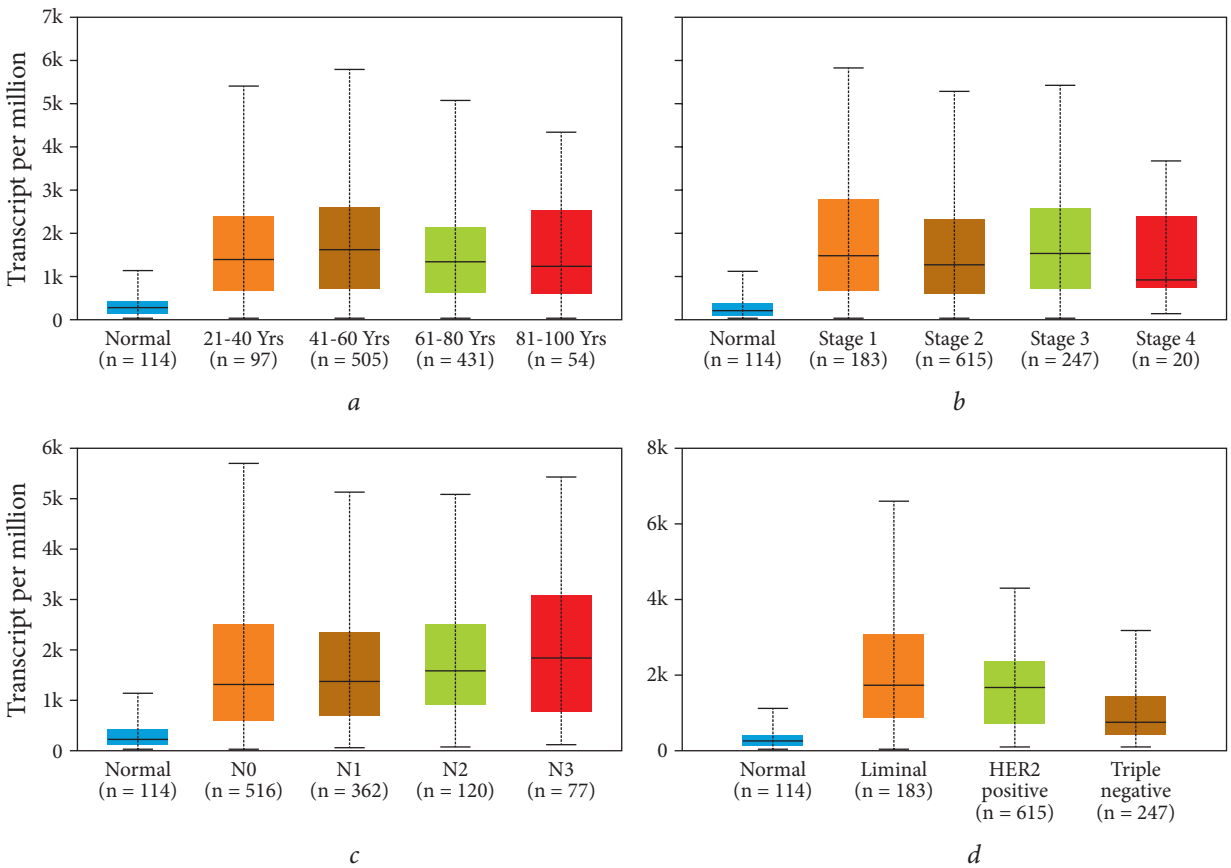

Fig. 2. *COL1A1* mRNA levels depending on clinical and pathological characteristics of BCa according to UALCAN: *a* — age of patients; *b* — tumor stage; *c* — regional lymph node status; *d* — molecular subtype

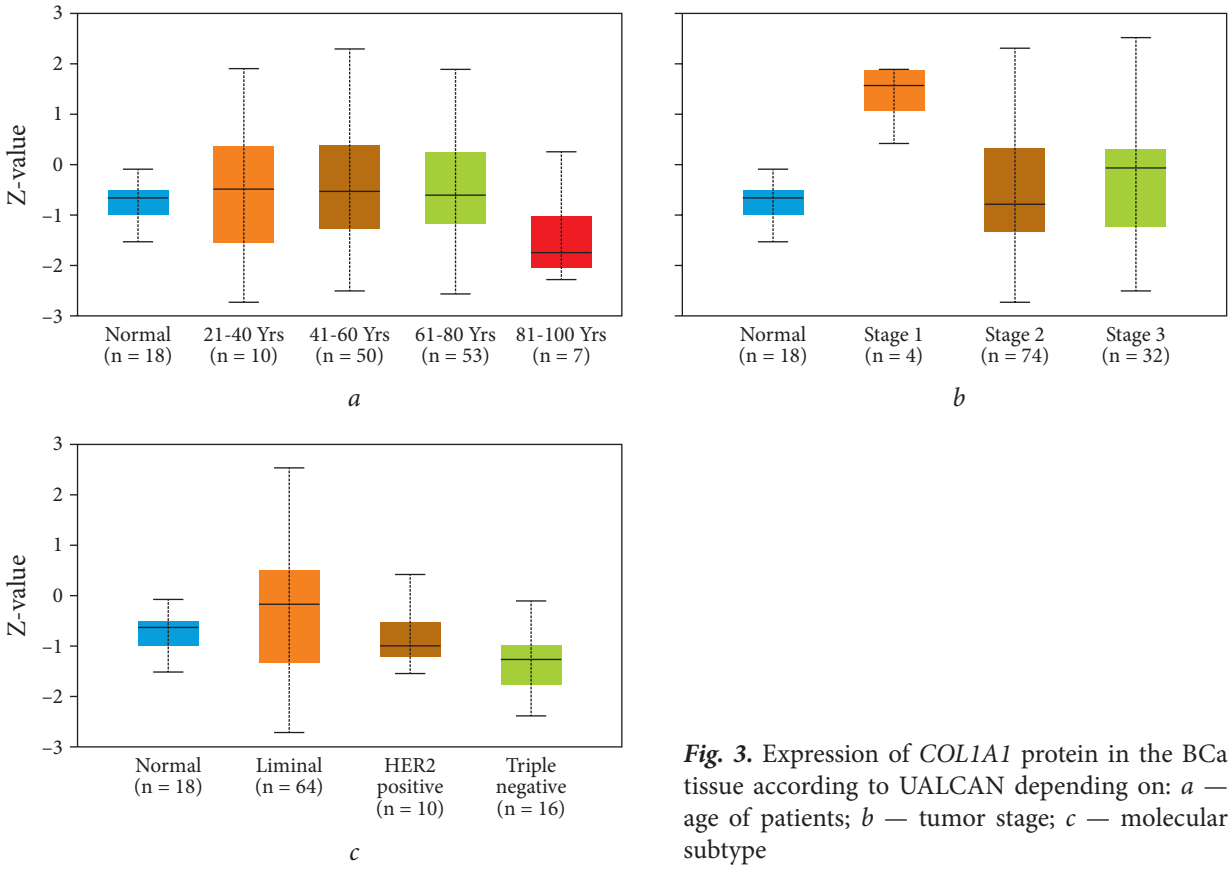

Fig. 3. Expression of *COL1A1* protein in the BCa tissue according to UALCAN depending on: *a* — age of patients; *b* — tumor stage; *c* — molecular subtype

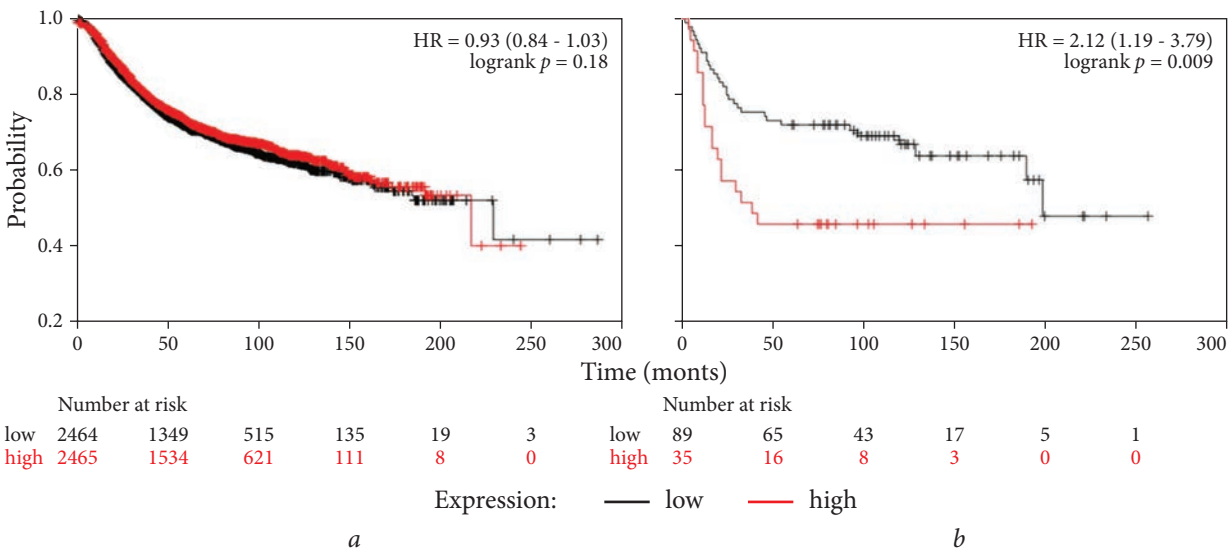

Fig. 4. Relapse-free survival of BCa patients depending on *COL1A1* expression indicators at the level of mRNA (*a*) and protein (*b*) according to KMplotter

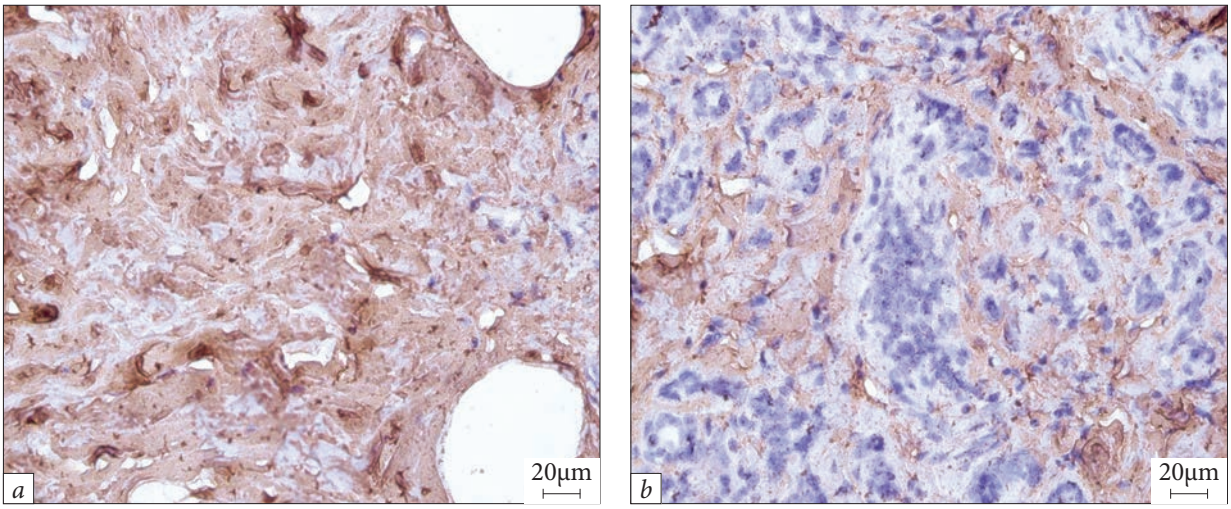

Fig. 5. IHC detection of COL1A1 in BCa tissue of young patients: *a* – high level of expression; *b* – low level of expression. × 260

companied by a significant increase in the level of *COL1A1* mRNA in tumor cells. The highest *COL1A1* expression at the mRNA and protein levels were recorded in the tissue of both luminal BCa subtypes compared to neoplasms of the HER2/neu-positive and basal molecular subtypes. It was found that the expression of COL1A1 protein level is inversely correlated with the age of patients and the stage of BCa, and is associated with low survival rates.

Study of features of type 1 collagen expression in young BCa patients. At the next stage, the specifics of *COL1A1* gene expression at the mRNA and protein levels in BCa tissue of young patients were investigated. The analysis of COL1A1 expression revealed that in BCa tissue of patients < 45 years, the *COL1A1* mRNA level was 0.293 ± 0.161 dCT, while the optical density index of COL1A1 expression assayed by IHC was 1.514 ± 0.017 a.u.

The analysis of the results of the IHC study made it possible to establish that a positive reaction with antibodies to COL1A1 was determined in the form of brown diffuse staining of the stromal component of BCa of varying intensity (from light brown to dark brown). COL1A1 expression of low intensity was also detected in the cytoplasm of individual malignantly transformed cells (Fig. 5).

When investigating the relationship between *COL1A1* expression and clinicopathological characteristics of BCa, we found that *COL1A1* mRNA levels in the tumor tissue of young BCa patients do not depend on the tumor stage, the size of neoplasms, the presence of metastases in regional lymph nodes, the molecular subtype of tumors, and the HER2/neu expression status (Table 2).

In BCa tissue, negative for PR expression and positive for ER expression we recorded an increase in *COL1A1* mRNA by 9.8 and 12.6 times, respectively ($p < 0.004$ and $p < 0.001$, respectively) (Table 2).

The highest levels of COL1A1 protein were characteristic for patients with T1 category and neoplasms with a moderate differentiation grade (Fig. 6). A significantly higher optical density was found in the BCa tissue of young patients with the luminal A molecular subtype compared to luminal B, Her2/neu-positive and basal subtypes, as well as ER+, PR+ BCa (Fig. 6).

Taking into account the obtained results, at the next stage we analyzed a recurrence-free survival of young BCa patients depending on *COL1A1* expression at the protein and mRNA

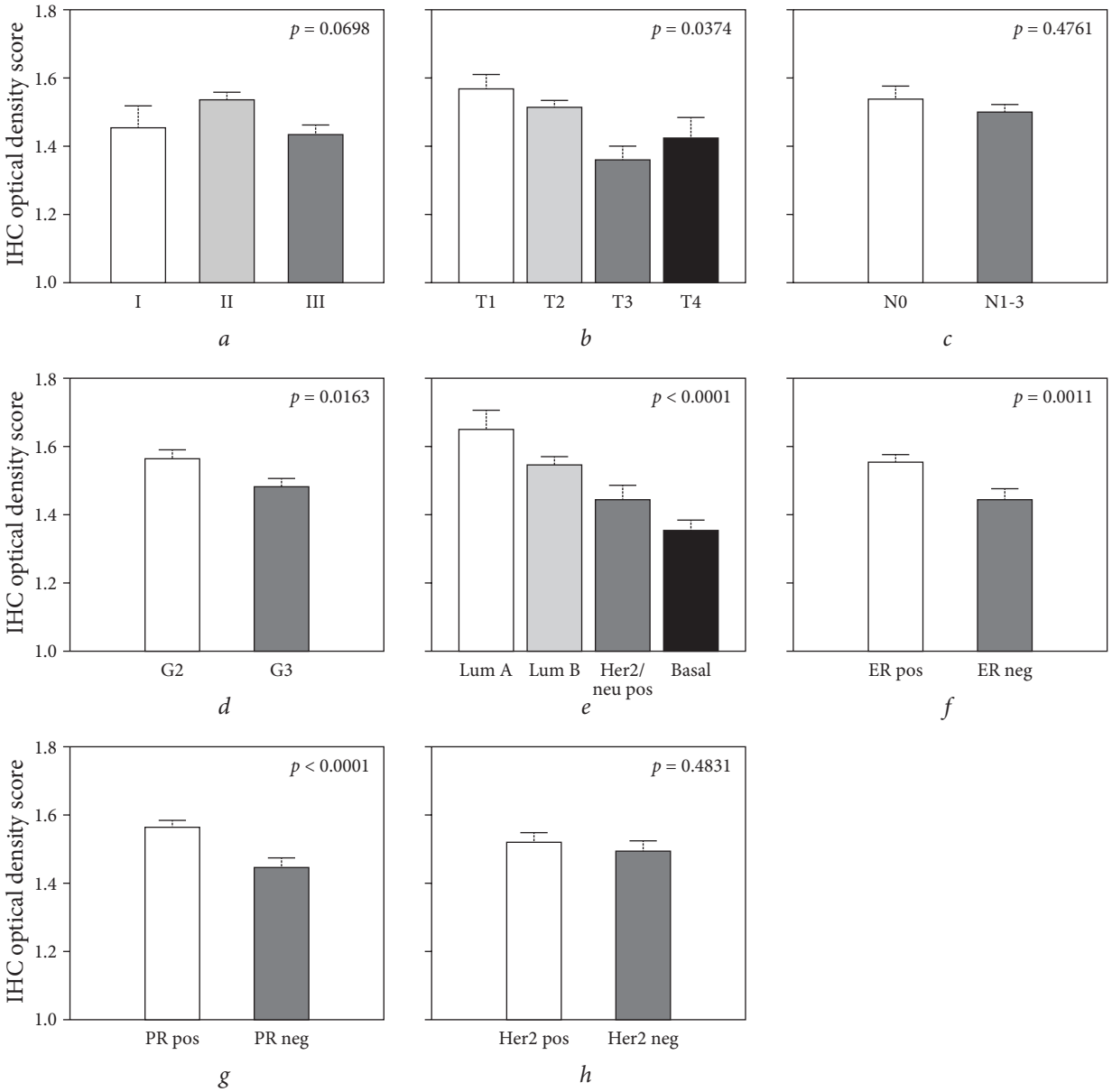

Fig. 6. Dependence of *COL1A1* protein level on the main clinical and pathological characteristics and receptor status of BCa: *a* — tumor stage; *b* — tumor size; *c* — regional lymph node status; *d* — tumor differentiation grade; *e* — molecular subtype of BCa; *f* — ER expression status; *g* — PR expression status; *h* — HER2/neu expression status

levels. As seen from the data shown in Fig. 7, a significant decrease in 3-year recurrence-free survival rates by 22.8% ($p < 0.0460$) was recorded in young BCa patients with low values of the optical density COL1A1 index in tumor tissue. Nevertheless, no relationship between patient survival rates and the level of *COL1A1* mRNA expression in BCa tissue was found.

The features of *COL1A1* expression identified in our study in the BCa tissue of young patients indicated that a decrease in the COL1A1 protein level is associated with the size, differentiation

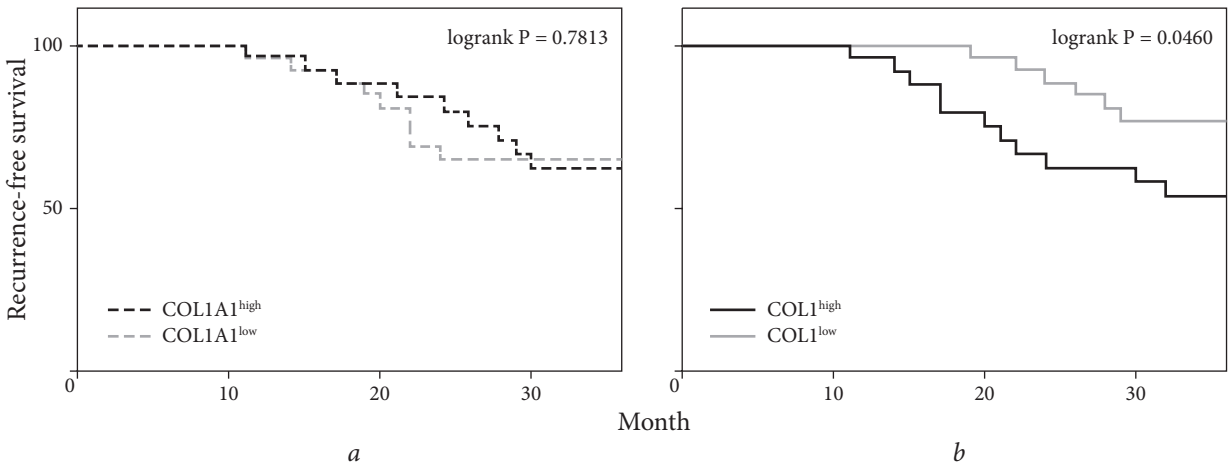

Fig. 7. Relapse-free survival of BCa patients depending on the expression of *COL1A1* in tumor tissue at the levels of mRNA (*a*) and protein (*b*) according to the Kaplan — Meier method (log-rank test)

Table 2. **Relationship of *COL1A1* mRNA expression with clinicopathological and molecular-biological features of BCa in young patients**

Clinicopathological characteristics		*COL1A1*					
		Min (dCT)	Q1 (dCT)	Median (dCT)	Q3 (dCT)	Max (dCT)	*p*
Stage	I	0.09	0.09	0.22	0.36	0.36	0.5822
	II	0.02	0.04	0.09	0.22	3.49	
	III	0.09	0.09	0.15	0.23	0.23	
T	1	0.03	0.05	0.09	0.29	0.36	0.996
	2	0.02	0.04	0.16	0.23	3.49	
	3	0.09	0.09	0.10	0.10	0.10	
N	0	0.10	0.12	0.21	0.32	3.49	0.246
	1—3	0.03	0.06	0.145	0.22	0.56	
Molecular subtype	Lum A	0.03	0.05	0.12	0.30	0.36	0.995
	Lum B	0.02	0.04	0.13	0.23	3.49	
	HER2/neu	0.09	0.09	0.10	0.19	0.19	
	Basal	0.04	0.04	0.13	0.23	0.23	
Differentiation grade	2	0.03	0.06	0.15	0.24	0.36	0.751
	3	0.02	0.04	0.09	0.22	3.49	
ER expression	positive	0.02	0.12	**1.26**	1.64	3.49	**0.001**
	negative	0.03	0.09	**0.10**	0.13	0.23	
PR expression	positive	0.02	0.08	**0.59**	0.23	3.49	**0.004**
	negative	0.03	0.03	**0.06**	0.12	0.22	
HER2/neu expression	positive	0.02	0.04	0.09	0.22	3.49	0.807
	negative	0.03	0.04	0.15	0.23	0.36	

grade and molecular subtype of neoplasms, as well as with the recurrence-free survival of the patients.

Therefore, the analysis of the results of the bioinformatic study and the data obtained *ex vivo* on the clinical material made it possible to establish that the development of BCa is accompanied by an increase in the level of *COL1A1* mRNA in the tumor tissue, while the BCa tissue of young patients is characterized by an increase in the expression of COL1A1 protein. According to the data provided in the UALCAN database, the highest levels of *COL1A1* expression at the mRNA and protein levels were recorded in the tissue of both luminal BCa subtypes compared to the Her2/neu-positive and basal molecular subtypes. In contrast, in young BCa patients, a high level of *COL1A1* mRNA is associated only with the presence of ER and PR expression.

A bioinformatic study showed that the expression of COL1A1 protein is inversely related to the BCa stage and, according to our data, to the T category and tumor differentiation grade. We recorded the highest COL1A1 level in the tumor tissue of young patients with luminal A and B molecular subtypes, which coincides with the data presented in UALCAN. We also noted a significantly increased COL1A1 level in patients with the positive steroid hormone receptor status.

It is also worth noting that high COL1A1 expression in BCa was associated with a worse prognosis according to the Kaplan — Meier Plotter data. At the same time, our results indicate that in young BCa patients, high expression of COL1A1, on the contrary, directly correlates with the indicators of the recurrence-free survival.

Importance of disturbances in the expression and spatial organization of collagen as a key component of the stromal microenvironment of breast tumors in patients of different age groups has not been definitively clarified to date [15, 16]. It is known that the tissue of BCa is more rigid compared to the tissue of benign neoplasms, which is associated with the presence of a desmoplastic extracellular matrix, the main structural component of which is COL1. COL1 is known to play a key role in many biological processes, such as cell morphogenesis, proliferation, migration, differentiation, apoptosis, and carcinogenesis [17]. Despite a large volume of data on the role of COL1 in the occurrence and progression of malignant neoplasms, today its prognostic value in young BCa patients remains debatable.

Confirmation of the importance of *COL1A1* expression disorders in the formation of the molecular profile of BCa in patients younger than 45 years is also evidenced by the inverse relationship of *COL1A1* expression indicators at the mRNA and protein levels with the ER and PR status of tumor cells. It is worth noting that similar results were previously published by Kim et al. [18] who in an in vitro study found significantly lower levels of *COL1A1* expression in cells of the hormone-refractory BCa line MDA-MB-231 compared to cells of the hormone-sensitive line MCF-7. However, according to the data of Antoni et al. [19] and Barcus et al. [20], the presence of a dense collagen matrix in ER-positive BCa can contribute to the activation of cell migration and metastasis. In particular, in the study by Liu et al. [21], it is reported that in patients with ER-positive BCa, a high level of *COL1A1* expression is associated with an unfavorable clinical course, which, according to the authors, is probably due to the COL1A1 involvement in the mechanisms of the resistance to hormonal therapy.

There are reports that inhibition of PR signaling leads to a decrease in the expression level of *COL1A1* and also activates the expression of the extracellular matrix remodeling genes [22].

It is also worth noting that the inverse relationship between expression indicators of COL1 and HER2/neu in BCa tissue demonstrated in this study can explain the results of clinical observations, according to which the low mammographic density of the tumor lesion is a char-

acteristic feature of neoplasms of the HER2/neu-positive molecular subtype [23, 24].

Thus, a decrease in the expression of *COL1A1* in BCa tissue is associated with an unfavorable BCa course in young patients, which probably leads to remodeling of the extracellular matrix and promotes the activation of proliferation and metastasis. The obtained data indicate the perspective of further studies of the prognostic value of *COL1A1* expression in tumor tissue, taking into account the molecular subtype of neoplasms. This will contribute to the development of the individualized treatment tactics and improve the survival rates of young BCa patients.

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ОСОБЛИВОСТІ ЕКСПРЕСІЇ COL1A1 У ТКАНИНІ РАКУ МОЛОЧНОЇ ЗАЛОЗИ ХВОРИХ МОЛОДОГО ВІКУ

Стан проблеми. Статистичні дані останніх десятиріч свідчать про зростання частоти виявлення раку молочної залози (РМЗ) у жінок молодого віку, який посідає перше місце у структурі захворюваності жінок у віковій групі 18—29 років та є однією із провідних причин смертності у віковому діапазоні 15—39 років. Незважаючи на доведену роль стромального мікрооточення у розвитку та прогресуванні РМЗ, кількісні показники та топологія експресії колагену, асоційовані з віком та показниками виживаності хворих, остаточно не з'ясовані та потребують подальших досліджень. **Мета роботи:** дослідити зв'язок показників експресії гену *COL1A1* на рівні мРНК та білка у тканині РМЗ із клініко-патологічними особливостями та показниками виживаності хворих різних вікових груп. **Матеріали та методи.** Дослідження проведено на клінічному матеріалі 50 хворих на РМЗ I—III стадії, що знаходились на лікуванні у Комунальному некомерційному підприємстві «Прикарпатський клінічний онкологічний центр» продовж 2017—2020 років. Показники експресії гену *COL1A1* на рівні мРНК та білка у тканині РМЗ досліджували із застосуванням методів ПЛР у реальному часі та імуногістохімії, а також біоінформативного аналізу (бази даних UALCAN та Kaplan — Meier Plotter). Статистичний аналіз результатів виконували за допомогою GraphPad Prism v.8.0. **Результати.** У ході

біоінформатичного аналізу встановлено, що тканина РМЗ характеризується в 6,0 разів ($p < 0,05$) вищим рівнем експресії мРНК *COL1A1* у порівнянні з нормальною тканиною молочної залози. Продемонстровано зв'язок показників експресії *COL1A1* на рівні мРНК та білка з молекулярним підтипом новоутворень. Згідно з даними Kaplan — Meier Plotter низький рівень експресії *COL1A1* на рівні білка в пухлинній тканині РМЗ асоціюється з нижчими показниками безрецидивної виживаності пацієнтів. *Ex vivo* на клінічному матеріалі зниження рівня експресії *COL1A1* на рівні білка зафіксовано у тканині РМЗ хворих молодого віку категорії Т3 ($p < 0,0374$), низького ступеня диференціювання ($p < 0,0163$) та базального молекулярного підтипу ($p < 0,0001$). Встановлено зв'язок експресії *COL1A1* на рівні мРНК та білка з такими молекулярно-біологічними характеристиками РМЗ у хворих молодого віку як статус експресії рецепторів естрогену ($p < 0,0001$) та прогестерону ($p < 0,0040$). Безрецидивна 3-річна виживаність хворих на РМЗ молодого віку є достовірно меншою за наявності низького індексу оптичної щільності *COL1A1* у пухлинній тканині. **Висновки.** Ідентифікований зв'язок експресії *COL1A1* з такими показниками злоякісності РМЗ, як розмір новоутворень, ступінь диференціювання, молекулярний підтип, рецепторний статус, а також із безрецидивною виживаністю хворих, свідчить про перспективність його використання з метою прогнозування агресивності перебігу пухлинного процесу у хворих молодого віку.

Ключові слова: рак молочної залози, колаген, *COL1A1*, хворі молодого віку.