ORIGINAL CONTRIBUTION

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ROLE OF miR-26b-5p AND miR-186-5p IN BREAST CANCER PATIENTS OF YOUNG AGE: CLINICAL ASSOCIATIONS AND RELATION TO ANTHRACYCLINE RESPONSE

Background. Age-specific biological differences in breast cancer (BC) shape the disease course, therapeutic sensitivity, and prognosis. The microRNAs hsa-miR-26b-5p and hsa-miR-186-5p are considered promising biomarkers of tumor aggressiveness and treatment response, yet their age-dependent expression features remain insufficiently characterized. **Aim.** To evaluate age-related expression patterns of hsa-miR-26b-5p and hsa-miR-186-5p in the BC tissue samples and their relation to the response to neoadjuvant 4AC chemotherapy. **Materials and Methods.** Expression levels of hsa-miR-26b-5p and hsa-miR-186-5p were analyzed by qRT-PCR in formalin-fixed paraffin-embedded tissue samples of BC patients (n = 56) divided into two age groups: ≤45 and >45 years. MiRNAs expression patterns were analyzed in relation to molecular BC subtype, disease stage, T and N categories by TNM, and treatment response grades assessed by the Miller — Payne system. **Results.** Patients ≤45 years exhibited significantly higher miR-26b-5p levels (1.78-fold; $p = 0.0005$), especially in the luminal B subtype tumors (7.26—8.45-fold). The reduced miR-26b-5p and miR-186-5p levels in younger patients were associated with a locoregionally advanced disease (stage III) and lymph-node metastasis. In patients ≥45 years, miR-186-5p levels were significantly higher in the HER2/neu-positive subtype (2.9-fold; $p = 0.0278$) and in the younger patients responding to 4AC NACT compared to the older responders. **Conclusions.** In BC, hsa-miR-26b-5p and hsa-miR-186-5p exhibited pronounced age-specific regulatory patterns and could be considered potential markers of BC course and efficacy of anthracycline-based therapy.

Keywords: breast cancer, neoadjuvant polychemotherapy, young patients, microRNA.

Breast cancer (BC) remains one of the leading causes of morbidity and mortality among women worldwide, despite substantial progress in diagnosis and treatment [1]. The early detection, identification of the prognostic and predictive factors, and personalized therapeutic approach are considered key components determining the effectiveness of clinical management of BC patients. In this context, the analysis of molecular biomarkers, allowing for not only diagnosing BC but also predicting the disease course, treatment response, and the potential risk of progression, is of particular importance.

MicroRNAs represent small non-coding RNAs that exert regulatory functions at both the tran-

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scriptional and translational levels, influencing the expression of numerous genes involved in proliferation, apoptosis, angiogenesis, and metastasis [2]. Due to their stability in biological fluids, including plasma, miRNAs are considered promising non-invasive biomarkers for cancer detection and monitoring of tumor progression [3]. Moreover, the expression of specific miRNAs is associated with molecular BC subtypes and the sensitivity of tumors to standard chemotherapy regimens [4—8].

The statistical data of the recent decades indicate a steady rise in BC incidence among young women. BC occupies first place in the structure of cancer morbidity in females aged 18—29 years and is one of the leading causes of death in the 15—39-year age group. It is well established that BC in young patients differs from that in older women in terms of clinical aggressiveness, histopathological characteristics, and molecular profiles. In Ukrainian women aged 18—24, BC ranks third in cancer incidence (10.8%), while in the 30—74 age group, it ranks first (27.6—20.6%). In the structure of cancer-related mortality among women, BC predominates in the age groups 30—54, 55—74, and 75+, accounting for 24.5, 20.6, and 18.5%, respectively [9]. Given these trends, the search for the factors that may help predict tumor aggressiveness and assess the effectiveness of medical treatment, taking into account the biological characteristics of tumor cells, is a priority research direction.

Table 1. **Clinicopathological characteristics of patients with BC**

Characteristics	≤45 years group		>45 years group	
	N	%	N	%
Number of patients	56	46.6	64	53.3
Patient's age (years)				
Average age	39.05 ± 5.9		64.3 ± 10.5	
Age range	28—45		46—89	
BC stage				
II	20	35.7	20	31.2
III	36	64.3	44	68.8
Category N according to TNM				
N0	36	64.3	40	62.5
N1	20	35.7	24	37.5
Molecular subtype				
Luminal A	16	28.7	16	25.0
Luminal B	20	35.7	22	34.4
Her2/neu-positive	10	17.8	14	21.8
Basal	10	17.8	12	18.8

Neoadjuvant chemotherapy (NACT) is standardly used for patients with locally advanced BC and in initially inoperable cases. According to BC treatment protocols, the most commonly used NACT regimens are AC and CAF, which include anthracyclines (primarily doxorubicin) and cyclophosphamide. However, despite the long clinical history of NACT, the optimal drugs and chemotherapy regimens remain clearly defined, particularly for young women, where minimizing adverse effects and preserving reproductive potential are essential treatment considerations [9].

Among many miRNAs, particular attention has been drawn to hsa-miR-26b-5p and hsa-miR-186-5p due to their role in important processes in cancer cells. According to the literature [10—13], hsa-miR-26b-5p may function as a tumor-suppressive miRNA, including through regulation of genes that control apoptosis and the cell cycle. The reduced expression of this miRNA is associated with increased cell proliferation, more aggressive tumor phenotype, and poor prognosis [11, 12]. In turn, hsa-miR-186-5p may act as a regulator of stress-induced signaling pathways involved in the development of anthracycline resistance [11, 13]. An assessment of the levels of these two miRNAs could be useful for evaluating prognosis and prediction of treatment response in BC patients receiving chemotherapy by the 4AC regimen.

Therefore, the aim of this study was to evaluate age-related expression patterns of hsa-miR-26b-5p and hsa-miR-186-5p in the BC tissue samples and their relation to the response to neoadjuvant 4AC chemotherapy. Identifying the associations between miRNA levels and clinicopathological BC features, as well as assessing their ability to predict treatment response, may represent an important step toward personalized medicine and optimization of therapeutic strategies for BC patients.

Material and Methods

In the study, the data and clinical materials (formalin-fixed paraffin-embedded tissue samples) of patients with BC who were treated at the National Can-

cer Institute of the Ministry of Health of Ukraine in 2019—2022 were analyzed. The clinical and pathological characteristics of the studied BC cases are presented in Table 1. All patients received NACT according to the 4AC regimen (Table 2). The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. All patients provided written informed consent for the use of clinical data for research purposes.

Therapeutic response in BC cases was evaluated using the Miller — Payne grading system, which provides a standardized morphological measure of treatment efficacy (Table 2).

MicroRNA expression was analyzed using real-time PCR. Total RNA was extracted from formalin-fixed paraffin-embedded (FFPE) tissue sections using the RNeasy FFPE Kit (QIAGEN, Germany) and the NucleoSpin kit (MACHEREY-NAGEL, Germany) according to the manufacturer's instructions. RNA concentration was measured using a NanoDrop 1000 Spectrophotometer (Thermo Scientific, USA), and the purity was assessed by the 260/280 nm absorbance ratio. Isolated RNA was dissolved in Tris-EDTA buffer and stored at –20 °C until further use.

Complementary DNA was synthesized from 100 ng of total RNA using the LunaScript® RT SuperMix Kit (New England Biolabs, USA). RNU48 small nucleolar RNA was employed as an endogenous control to normalize miRNA expression levels. Primers for the RNAs were synthesized by YP Biotech (Ukraine): RNU48 F: 5`-AGT GAT GAC CCC AGG TAA CTC-3` and R: 5`-CTG CGG TGA TGG CAT CAG-3`; hsa-miR-26b-5p F: 5`-GTT TGG GTT CAA GTA ATT CAG G-3` and universal R; hsa-miR-186-5p F: 5`-GTT TGG GTT CAA GTA ATT CAG G-3` and universal R.

A quantitative real-time PCR was performed on a QuantStudio 5 Dx Real-Time PCR System (Thermo Fisher Scientific, USA) using a LunaScript® MasterMix Kit (New England Biolabs, USA). Each sample was analyzed in triplicate, and the threshold cycle (Ct) values were averaged. Relative miRNA expression was calculated using the $2^{-\Delta Ct}$ method.

Comparisons between two groups were performed using the Mann — Whitney U test. Statistical analyses were conducted using GraphPad Prism 10 (GraphPad Software, USA), and p-values <0.05 were considered statistically significant.

Resultgs and Discussion

To evaluate the clinical relevance of the studied miRNAs in different age groups, the patients were divided into two groups: those younger than 45 years and those older than 45 years. We assessed the expression of miR-26b-5p and miR-186-5p in BC tumor tissue in these subgroups (Fig. 1). We demonstrated that a characteristic feature of BC in patients under 45 years is the significantly higher (1.78-fold, $p = 0.0005$) expression level of miR-26b-5p compared to the women older than 45. No age-associated differences in miR-186-5p expression in tumor tissue were observed.

Then we analyzed expression of these miRNAs in different molecular BC subtypes (Fig. 2). As shown, expression of miR-26b-5p in luminal B BC in younger women was significantly higher compared to luminal A, HER2/neu-positive, and basal-like subtypes by 7.26-fold, 8.40-fold, and 8.45-fold ($p < 0.05$), respectively. Tumor tissue from HER2/neu-positive BC patients older than 45 years showed a 2.9-fold ($p = 0.0278$) elevation of miR-186-5p levels compared to samples of the luminal B subtype in this age group (Fig. 2).

We further established that BC tissue from younger patients with stage III disease exhibited a

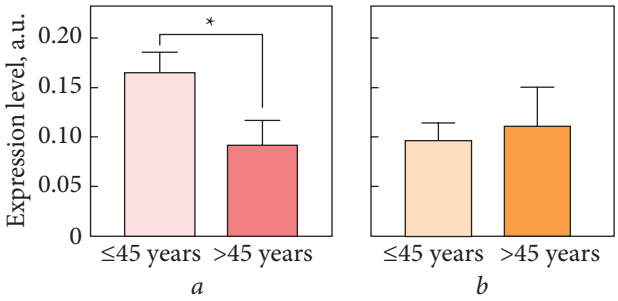

Fig. 1. Expression levels of miR-26b-5p (*a*) and miR-186-5p (*b*) in BC tissue, dependent on patient age

Table 2. **Distribution of BC patients by the clinical response to NACT (4AC regimen) according to the Miller — Payne criteria**

Miller — Payne criteria	≤ 45 years group		> 45 years group	
	N (56)	% (100)	N (64)	% (100)
I	4	7.1	6	9.4
II	8	14.3	7	10.9
III	17	30.4	21	32.8
IV	15	26.8	16	25.0
V	12	21.4	14	21.9

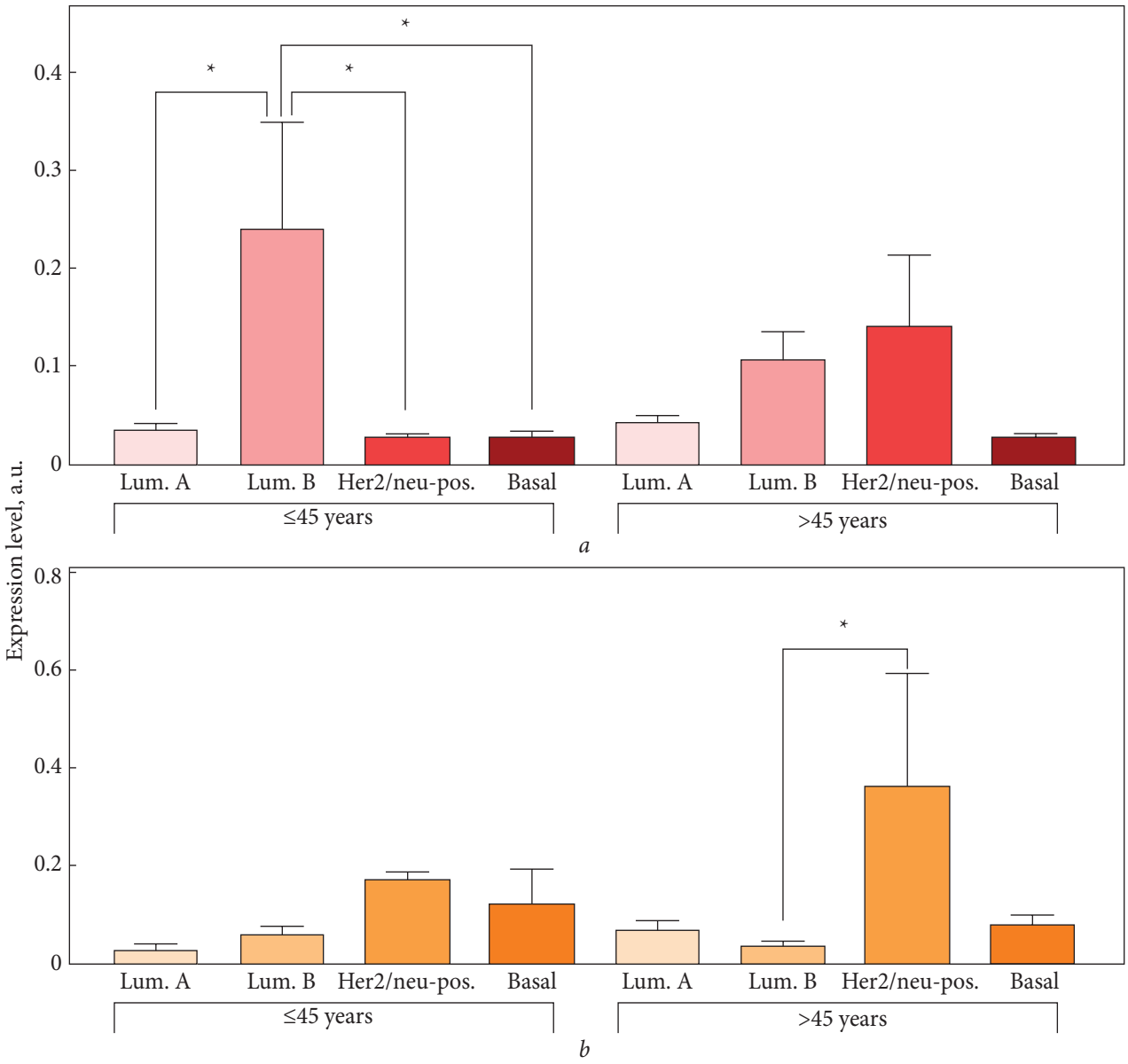

Fig. 2. Expression levels of miR-26b-5p (*a*) and miR-186-5p (*b*) in tumor tissue samples of different BC molecular subtypes and age groups

10.14-fold ($p = 0.0003$) and 3.31-fold ($p = 0.0310$) reduction in miR-26b-5p and miR-186-5p levels, respectively, compared to patients with stage II BC of the same age group (Fig. 3). No significant differences in miRNA levels between age groups depending on tumor grade were observed.

In tumor tissue of the younger BC patients with T3 tumors, a decrease in miR-26b-5p and miR-186-5p expression by 4.34-fold ($p = 0.0163$) and 6.59-fold ($p = 0.0369$), respectively, was detected compared to T2 cases. A significant 3.53-fold ($p = 0.0299$) decrease in miR-26b-5p expression was also recorded in samples from the younger patients with metastatic lesions in regional lymph nodes (Fig. 4).

Then, we assessed the expression of the miRNAs in relation to the effectiveness of 4AC NACT, dependent on the patient age. We showed that BC tissue from the younger patients who responded to treatment was characterized by significantly higher expression of miR-186-5p compared to the older responders (Fig. 5).

Taken together, the results indicated that miR-26b-5p expression exhibited clear age- and biology-dependent specificity, showing an increased expression in tumor tissue of the younger BC patients, especially in luminal B tumors. Reduced miR-26b-5p and miR-186-5p levels in younger women were associated with greater locoregional tumor spread

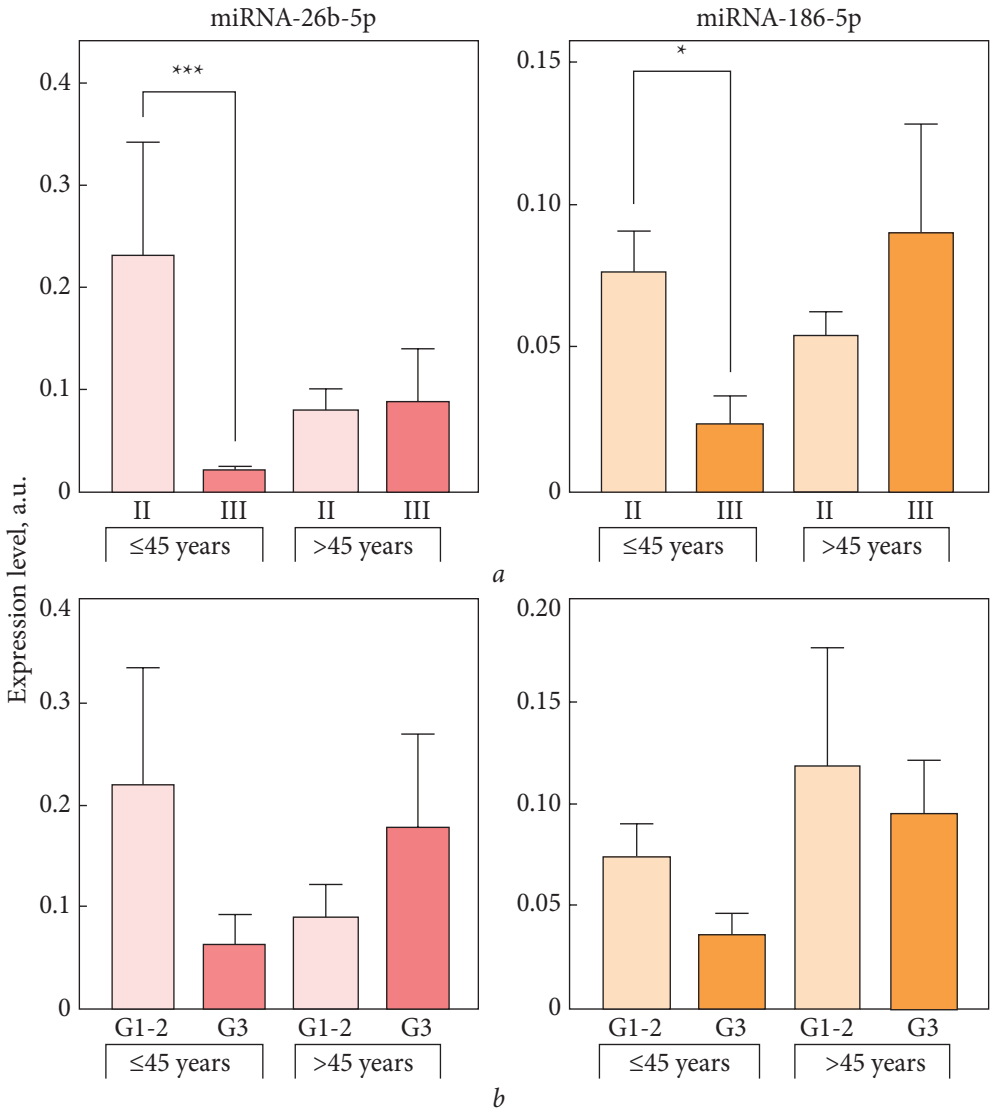

Fig. 3. Expression levels of miR-26b-5p and miR-186-5p in tumor tissue of BC patients of different age groups, dependent on disease stage (*a*) and tumor grade (*b*)

(stage III, T3 category) and metastatic lymph node involvement. In the older patients, an increased miR-186-5p expression was characteristic of the HER2/neu-positive subtype. Additionally, we identified potential predictive significance for miR-186-5p: among the younger patients responsive to neoadjuvant 4AC therapy, its expression was significantly higher than in the older responders. Altogether, these findings highlight the age-specific regulation of miR-26b-5p and miR-186-5p and underscore their potential as biomarkers of some BC patterns.

In this study, we demonstrated for the first time the age-related features of hsa-miR-26b-5p and hsa-miR-186-5p expression in BC patients, as well as their potential as predictive markers in the context of response to anthracycline-based therapy. The data obtained are consistent with current understanding of the features of BC in young women, i.e., a more aggressive clinical behavior, a higher prevalence of unfavorable molecular subtypes, and a tendency toward early systemic dissemination [9]. The expression patterns of both miRNAs evidenced their potential impact on age-dependent therapy responses.

The elevated miR-26b-5p levels in the luminal B subtype of young patients may be beneficial, as this subtype is generally characterized by high proliferative activity and a propensity for developing chemoresistance [12]. The decrease in miR-26b-5p expression in tumor tissue of young patients with locally advanced disease (stage III, T3) and lymph node metastasis aligns with the literature data on

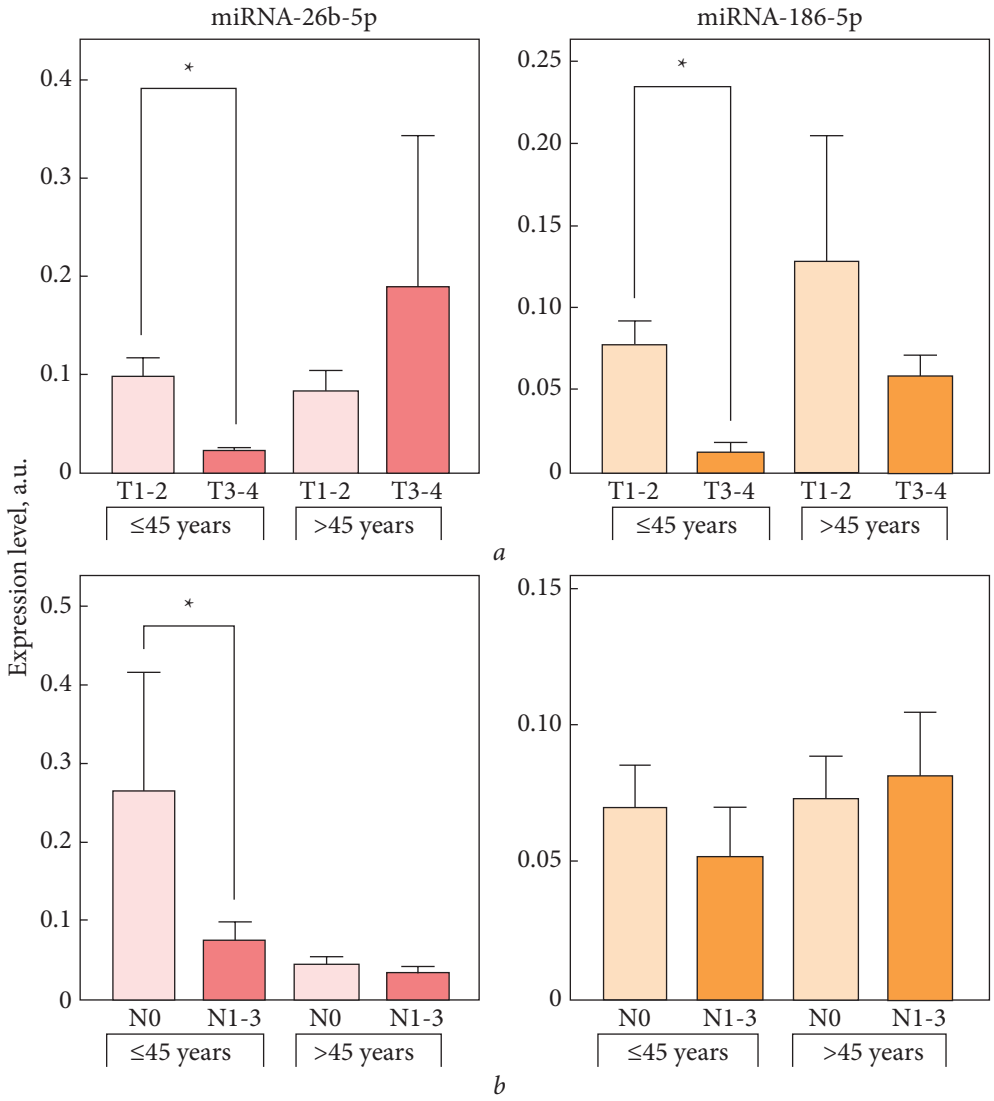

Fig. 4. Expression levels of miR-26b-5p and miR-186-5p in BC patients of different age groups, dependent on T (*a*) and N (*b*) categories by TNM

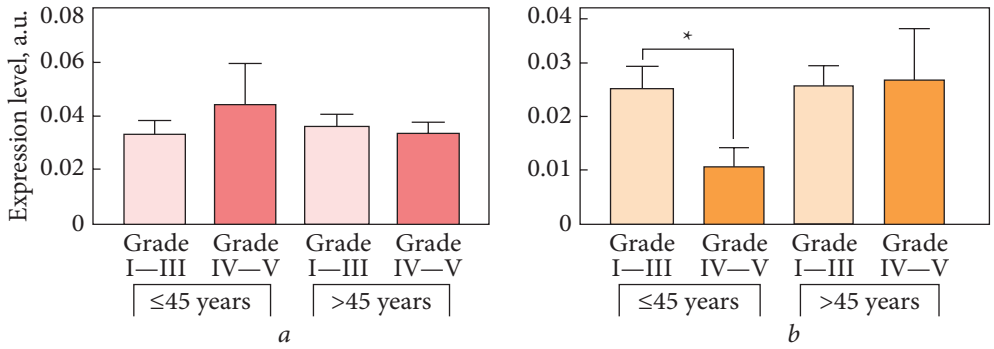

Fig. 5. Expression levels of miR-26b-5p (*a*) and miR-186-5p (*b*) in BC patients of different age groups, dependent on treatment response assessed by the Miller — Payne system

its involvement in the control of proliferation and epithelial–mesenchymal transition [12, 15, 16].

In tumor tissue from the younger patients sensitive to neoadjuvant 4AC therapy, higher miR-186-5p expression was observed compared to the older responders. As reported earlier [11, 17, 18], this miRNA is involved in regulating drug resistance through the effects on transport proteins and

DNA repair mechanisms. Supposedly, miR-186-5p may serve as a marker of anthracycline sensitivity in young BC patients, whereas its reduction could be indicative of resistance development.

In conclusion, our findings indicate that hsa-miR-26b-5p and hsa-miR-186-5p can be considered potential markers of BC features and NACT efficacy. However, interpretation of their age-specific expression patterns requires further analysis accounting for the hormonal status, metabolic changes, and ECM remodeling in young patients.

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REFERENCES

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71:209-249.
2. Shang R, Lee S, Senavirathne G, Lai EC. MicroRNAs in action: biogenesis, function and regulation. *Nat Rev Genet*. 2023;24(12):816-833. https://doi.org/10.1038/s41576-023-00611-y
3. Sandau US, Wiedrick JT, McFarland TJ, et al. Analysis of the longitudinal stability of human plasma miRNAs and implications for disease biomarkers. *Sci Rep*. 2024;14:2148. https://doi.org/10.1038/s41598-024-52681-5
4. Lukianova N, Zadvornyi T, Kashuba E, et al. Expression of markers of bone tissue remodeling in breast cancer and prostate cancer cells in vitro. *Exp Oncol*. 2022;44(1):39-46. https://doi.org/10.32471/exp-oncology.2312-8852.vol-44-no-1.17354
5. Zadvornyi T, Lukianova N, Borikun T, et al. Mast cells as a tumor microenvironment factor associated with the aggressiveness of prostate cancer. *Neoplasma*. 2022;69(6):1490-1498. https://doi.org/10.4149/neo_2022_221014N1020
6. Chekhun V, Borikun T, Mushii O, et al. Expression profile of miR-145, -182, -21, -27a, -29b, and -34a in breast cancer patients of young age. *Exp Oncol*. 2023;45(4):421-431. https://doi.org/10.15407/exp-oncology.2023.04.421
7. Borikun T, Mushii O, Pavlova A, et al. Tumor microenvironment-associated miR-7-5p, miR-19a-3p, and miR-23b-3p expression in prostate cancer with different progression risk. *Exp Oncol*. 2023;45(4):432-442. https://doi.org/10.15407/exp-oncology.2023.04.432
8. Mushii O, Pavlova A, Bazas V, et al. Mast cells as a factor in regulation of breast cancer stromal component associated with breast cancer aggressiveness. *Exp Oncol*. 2024;46(4):311-323. https://doi.org/10.15407/exp-oncology.2024.04.311
9. Chekhun V, Martynyuk O, Lukianova Y, et al. Features of breast cancer in patients of young age: search for diagnosis optimization and personalized treatment. *Exp Oncol*. 2023;45(2):139-150. https://doi.org/10.15407/exp-oncology.2023.02.139
10. Fukumoto I, Kikkawa N, Matsushita R, et al. Tumor-suppressive microRNAs (miR-26a/b, miR-29a/b/c and miR-218) concertedly suppressed metastasis-promoting LOXL2 in head and neck squamous cell carcinoma. *J Hum Genet*. 2016;61(2):109-118. https://doi.org/10.1038/jhg.2015.120
11. Lu Q, Chen W, Ji Y, et al. Ursolic acid enhances cytotoxicity of doxorubicin-resistant triple-negative breast cancer cells via ZEB1-AS1/miR-186-5p/ABCC1 axis. *Cancer Biother Radiopharm*. 2021;37:673. https://doi.org/10.1089/cbr.2020.4147
12. Ma S, Wei H, Wang C, Han J, Chen X, Li Y. miR-26b-5p inhibits cell proliferation and EMT by targeting MYCBP in triple-negative breast cancer. *Cell Mol Biol Lett*. 2021;26(1):52. https://doi.org/10.1186/s11658-021-00288-3
13. Lnc00462717 regulates the permeability of the blood-brain tumor barrier through interaction with PTBP1 to inhibit the miR-186-5p/Occludin signaling pathway. *FASEB J*. 2020;34(8):9941-9958. https://doi.org/10.1096/fj.202000045R
14. Perou CM, Sørlie T, Eisen MB, et al. Molecular portraits of human breast tumours. *Nature*. 2000;406(6797):747-752. https://doi.org/10.1038/35021093
15. Ding Q, Wang Y, Zuo Z, et al. Decreased expression of microRNA-26b in locally advanced and inflammatory breast cancer. *Hum Pathol*. 2018;77:121-129. https://doi.org/10.1016/j.humpath.2018.04.002
16. Zhou A, Pan H, Sun D, et al. miR-26b-5p inhibits proliferation, migration and invasion of human papillary thyroid cancer in a β-catenin-dependent manner. *Onco Targets Ther*. 2020;13:1593-603. https://doi.org/10.2147/OTT.S236319
17. Wang Z, Zhou X, Deng X, et al. miR-186-ANXA9 signaling inhibits tumorigenesis in breast cancer. *Front Oncol*. 2023;13:1166666. https://doi.org/10.3389/fonc.2023.1166666
18. Tessitore A, Cicciarelli G, Del Vecchio F, et al. MicroRNAs in the DNA damage/repair network and cancer. *Int J Genomics*. 2014;2014:820248. https://doi.org/10.1155/2014/820248

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РОЛЬ miR-26b-5p ТА miR-186-5p У ХВОРИХ НА РАК МОЛОЧНОЇ ЗАЛОЗИ МОЛОДОГО ВІКУ: КЛІНІЧНІ АСОЦІАЦІЇ ТА ВПЛИВ НА ВІДПОВІДЬ НА АНТРАЦИКЛІНВМІСНУ ТЕРАПІЮ

Вступ. Вікові особливості біології раку молочної залози (РМЗ) зумовлюють різний перебіг захворювання, чутливість до терапії та прогностичні характеристики пухлин. hsa-miR-26b-5p та hsa-miR-186-5p розглядаються як перспективні біомаркери пухлинної агресивності та відповіді на лікування, однак їхні вікові патерни експресії залишаються недостатньо вивченими. **Мета.** Оцінити вікові особливості експресії hsa-miR-26b-5p та hsa-miR-186-5p у тканині РМЗ та визначити їх прогностичне й предиктивне значення для відповіді на неоад'ювантну хіміотерапію (НХТ) за схемою 4АС. **Матеріали та методи.** Рівні експресії miRNA визначали в пухлинній тканині хворих на РМЗ, розподілених на групи ≤45 та >45 років. Проведено аналіз залежно від молекулярного підтипу, стадії, категорій T і N за TNM та ступеня лікувального патоморфозу за Miller — Payne. Статистичну значущість оцінювали за $p < 0,05$. **Результати.** Пацієнтки віком < 45 років мали достовірно вищі рівні miR-26b-5p (в 1,78 рази; $p = 0,0005$). Для молодих жінок характерне різке підвищення експресії цієї miRNA в люмінальному B підтипі (у 7,26—8,45 рази). Зниження рівнів miR-26b-5p та miR-186-5p у молодих пацієнток асоційоване з локорегіонально поширеним процесом (стадія III, T3) та метастатичним ураженням лімфатичних вузлів. У жінок ≥45 років зафіксовано підвищення miR-186-5p у HER2/neu-позитивному підтипі (у 2,9 рази; $p = 0,0278$). Встановлено предиктивне значення miR-186-5p: у молодих пацієнток, чутливих до НХТ (4АС), її експресія була значно вищою порівняно зі старшими хворими. **Висновки.** hsa-miR-26b-5p та hsa-miR-186-5p демонструють виражену вікову специфічність і можуть слугувати комплексними біомаркерами, що відображають біологічні особливості РМЗ, ступінь пухлинної агресивності та потенційну чутливість до антрациклінвмісної терапії. Дані підкреслюють важливість урахування віку при інтерпретації профілю miRNA та формуванні індивідуалізованих підходів до лікування.

Ключові слова: рак молочної залози, неоад'ювантна поліхіміотерапія, хворі молодого віку, мікроРНК.