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EXPRESSION PATTERNS OF MATRICELLULAR PROTEINS IN METASTATIC BREAST CANCER AT THE BACKGROUND OF METABOLIC SYNDROME

Background. Metastatic breast cancer (mBCa) is one of the main causes of mortality among postmenopausal women with malignant neoplasms. Numerous studies indicate the feasibility of using bone matrix remodeling proteins to predict the BC course. **Aim**. To investigate the relationship between osteopontin (OPN) and osteonectin (ON) expression levels in tumor tissue of patients of menopausal age with primary mBCa (pmBCa) and metabolic syndrome (MetS) taking into account the clinicopathological features of the disease. **Materials and Methods**. The study included 54 menopausal patients with pmBCa, 23 from whom had manifestations of MetS, while 31 patients were without MetS. The expression of matricellular proteins (OPN and ON) was determined immunohistochemically. **Results**. In tumor tissue of patients with MetS and pmBCa of category N3, an increase in the level of OPN expression (H-Score of 265.6 ± 7.7, $p < 0.05$) was recorded along with a decrease in the expression level of ON (H-Score of 123.2 ± 7.7, $p < 0.05$). The invasive lobular pmBCa in patients with MetS was characterized by a significant decrease in the level of OPN expression (H-Score of 219.4 ± 8.4) and an increase in ON expression (H-Score of 144.8 ± 7.5). In the patients with MetS, a significant decrease in ON expression ($p < 0.05$) was recorded in the tumor tissue of luminal A, luminal B, and Her2/neu subtypes (H-Scores of 140.2 ± 7.8, 119.3 ± 10.2, and 110.0 ± 7.7, respectively). Among the cases of pmBCa with diagnosed metastatic liver disease, ON expression in tumor tissue was lower in patients with MetS (H-Score of 146.2±9.1). **Conclusions**. The obtained data demonstrated the relationship between the expression indicators of matricellular proteins in the pmBCa tissue and the degree of malignancy and indicated the prospects for further studies of their prognostic value in the presence of MetS.

Keywords: metastatic breast cancer, matricellular proteins, osteopontin, osteonectin, metabolic syndrome.

According to the National Cancer Registry of Ukraine [1], the mortality from breast cancer (BCa) continues to occupy the first places in the structure of oncological pathologies of the female population. Such statistics is explained by the fact that 28% of patients with firstly diagnosed BCa had the disease at stages III—IV. According to the updated ESMO data, the rate of *de novo* diagnosed meta-

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static BCa (mBCa) varies from 2% to 25%, depending on nationality and race [2, 3]. Patients with mBCa face severe problems throughout the entire disease course, in particular, numerous symptoms related to the physical state of the body, psychoemotional stress, and social and everyday difficulties [4]. Such consequences of BCa progression are usually reflected in the health-related quality of life (QoL), which is increasingly defined as an important endpoint of treatment [5]. That is why the problem of mBCa has become one of the challenges for many scientists in this field today.

Sometimes the diagnosis of BCa is made only after the formation of metastases. About 3 from 100 women already have distant metastases at the time of the BCa diagnosis [6]. In recent years, the prognosis of patients with primary mBCa (pmBCa) has improved due to the use of experimental treatments, which led to an increase in 5-year survival from 28% to 55% [3, 7]. However, statistics on mBCa indicate that the 5-year survival is about 38%, and in some studies a decrease in the rate to 13% is recorded [3, 6]. It is obvious that this dynamics of mBCa is associated with an increase in the aggressive biological properties of the tumor, which arises in a clone of malignantly transformed cells in response to neoadjuvant therapy and other provoking factors. Thus, a metabolic syndrome (MetS) as a type of polyetiological endocrine pathology is recognized as one of the main factors of aggressiveness of the BCa course, which has become a serious health problem worldwide [8]. Approximately 1.9 billion adults worldwide have a body mass index (BMI) higher than 25 kg/m^2 and suffer from obesity and type 2 diabetes [9]. In addition, researchers have noticed that the increase in BMI is associated with the aggressive biological properties of the tumor, which leads to an increase in the frequency of metastases in the lymph nodes and larger tumor size [10]. According to a number of epidemiological studies, postmenopausal patients are more often diagnosed with concomitant pathology associated with diseases of the cardiovascular and hepatobiliary systems, musculoskeletal system, and carbohydrate metabolism disorders than patients with BC of reproductive age [11—13]. The incidence of MetS in elderly patients reaches 56% [11]. MetS leads to the suppression of immunological reactivity, affects the processes of angiogenesis and is associated with the development of cardiovascular and renal pathologies [14, 15].

That is why the search for new markers to improve the existing methods for the disease prognosis remains important. The matricellular glycoproteins osteopontin (OPN) and osteonectin (ON), which are expressed in normal and malignantly transformed cells of various histogenesis, belong to such markers [16, 17]. OPN is an important integrin-binding ligand in the extracellular matrix. Intracellular and extracellular variants of OPN are involved in various physiological processes (cell division and wound healing) [18]. The secreted protein ON, acidic and rich in cysteine, in addition to participating in the modulation of some intracellular signaling pathways, influencing cell migration, proliferation, and differentiation, corresponds to the profibrotic protein in its functional properties [19]. It has been shown that the pleiotropic effect of these cytokines may be associated with metabolic disorders in the human body. In particular, OPN can affect metabolic disorders via changing the intestinal microbiome and activating inflammatory processes [20]. According to the literature data, this protein is involved in the regulation of infiltration and activation of macrophages, which in turn produce interleukin-8, which further contributes to the acquisition of insulin resistance in adipose tissue [21]. An increased expression of ON contributes to the formation of adipocyte resistance to insulin and, due to fibrosis of adipose tissue, leads to the ectopic accumulation of triglycerides in the liver or skeletal muscle [22].

To date, it has been conclusively proven that impaired expression of OPN and ON is associated with regional and distant metastasis of some neoplasms, including BCa [16, 17, 23]. At the same time, the features of the expression of these matricellular proteins in BCa tissue in the presence of metabolic disorders have not been definitively elucidated. Given the above, the aim of the work was to study the relationship between the expression indicators of OPN and ON in the tumor tissue of patients of menopausal age with pmBCa and MetS, accounting the clinicopathological features of the disease.

Materials and Methods

Patients. The study was conducted on the material of 54 patients with pmBCa who were treated at the National Cancer Institute during 2016—2023 and

did not receive neoadjuvant therapy. All patients were informed and gave written consent to use clinical data for scientific purposes. The BCa stage was determined according to the International Classification of Tumors (TNM 8th edition, 2016).

MetS diagnosis was performed based on the analysis of clinical data and anamnesis according to the recommendations of the International Diabetes Federation (IDF, 2005): the presence of abdominal obesity (waist circumference > 80 cm), as well as the establishment of two or more factors such as fasting glycemia > 5.6 mmol/L or previously diagnosed type 2 diabetes, dyslipidemia (TG level ≥ 1.7 mmol/L, LDL level < 1.25 mmol/L), and arterial hypertension (BP ≥ 130/80 mm Hg). In addition to general clinical data, we determined body mass index (BMI, kg/m^2) to clarify anthropometric indicators.

Immunohistochemical method. To establish molecular BCa subtype, immunohistochemical (IHC) study of biopsy pmBC material was performed by determining the level of expression of estrogen receptors (ER), progesterone (PR), epidermal growth factor (Her2/neu), and Ki-67. The monoclonal antibodies against ER (clone: SP1, Vitro MD, Spain), PR (clone: 16, Vitro MD, Spain), Her2/neu (clone: EP3, Bio SB, USA), and Ki-67 (clone: SP6, Vitro MD, Spain) were used as primary antibodies. The analysis was performed by counting immunopositive cells (brown staining of the cytoplasm and/or nuclei) at a magnification of x100—400. An assessment of the hormone receptor status of BCa tissue was performed according to the Allred scale [24].

The *Her2/neu* gene amplification was assessed according to the current recommendations of the American Society of Clinical Oncology and College of American Pathologists guidelines for patients with BCa [25, 26]. Ki-67 expression was assessed semi-quantitatively, and the final result was expressed as a percentage [27].

For IHC study of the expression of matricellular proteins, the monoclonal antibodies specific for OPN (clone: ab21 4050, AbCam, UK) and ON (polyclonal: osteonectin, Vitro MD, Spain) were used as primary antibodies. The Master Polymer Plus Detection System (Peroxidase) (Incl. DAB Chromogen) (Vitro MD, Spain) was used to visualize the reaction results. The level of expression of matricellular proteins was determined by the semi-quantitative H-Score method and calculated by the formula:

$$S = 0\times N0\ (\%) + 1\times N1\ (\%) + 2\times N2\ (\%) + 3\times N3\ (\%),$$

where S is the H-Score, N0 is the percentage of cells without expression, N1 is the percentage of cells with weak expression, N2 — moderate expression, N3 — strong expression. The results of the assessment of the expression level of the studied markers were determined as follows: low H-Score — from 0 to 100 points, moderate — 101—200, and high — 201—300 [28, 29].

Program IBM® SPSS® Statistics v. 29.0.2.0 (USA) was used for statistical analysis. The independent-samples Kruskal — Wallis test was used to assess the significance of differences between groups. A p value of < 0.05 was considered statistically significant.

Results and Discussion

Analysis of clinical and anamnestic data showed that in patients with pmBCa, MetS was present in 43% of cases, and there was no difference by age between the groups of patients with or without MetS (Table 1). In the group of patients with MetS, the frequency of BCa of category T3, invasive lobular structure, and metastatic lung lesions prevailed, while in the group of patients without MetS, a higher frequency of tumors of categories N0 and N3 was determined. In patients of both groups, pmBCa of different molecular subtypes was diagnosed, with a predominance of the luminal A subtype in patients with MetS and the luminal B subtype in patients without metabolic disorder. A comparative analysis of the localization of distant metastases allowed us to establish that in patients with MetS, lung metastases were more common, and in patients without signs of MetS, bone lesions were mainly diagnosed (Table 1).

An analysis of the results of the IHC study allowed us to establish that the tumor tissue in both studied groups was characterized by the moderate expression of matricellular proteins (Table 2). When analyzing the features of the expression of matricellular proteins depending on the main clinicopathological features of pmBCa in the group of patients without MetS, an inverse relationship between ON expression level in tumor tissue and T category was established (Table 2).

A significant increase in OPN expression against the background of a decrease in ON expression was recorded in the tumor tissue of MetS-positive cases of category N3 (Table 2). At the same time, an increase in the degree of regional lymph node metastasis in the group of patients without MetS was associated only with a decrease in ON expression in tumor tissue.

Table 1. **Clinicopathological characteristics of pmBCa patients with and without metabolic syndrome**

Parameter	MetS +		MetS –	
	N	%	N	%
Total number of patients	23	43	31	57
Age (years)				
Average	61.7 ± 1.6		61.4 ± 1.1	
Range	52—79		50—77	
T category				
T2	6	26	12	39
T3	5	22*	3	10
T4	12	52	16	51
N category				
N0	1	4.3*	5	16.1
N1	9	39.1	7	22.6
N2	11	48	13	42
N3	2	8.6	6	19.3*
Histologic type				
Invasive Ca NST	17	74	28	90
Invasive lobular Ca	6	26*	3	10
Grade				
G2	14	60.8	20	64.5
G3	9	39.2	11	35.5
Molecular subtype				
Luminal A	10	43.5	12	39
Luminal B	8	35	13	42
Triple negative	3	13	3	9.5
Her2/neu-positive	2	8.5	3	9.5
Receptor status				
ER+PR+	18	72.2	25	80.6
ER-PR-	5	27.8	6	19.4
M category				
M1	23	100	31	100
Mts to bones	12	52*	22	71
Mts to lungs	14	60.8*	15	48
Mts to liver	7	30	12	38.7
Mts to ovaries	1	4	1	3
Combined Mts to more than 1 organ	10	43	16	51

Note: *$p < 0.05$ compared to patients without MetS.

Certain differences in the ratio of the studied matrix cellular proteins were also established depending on the histological type of pmBCa. A characteristic feature of invasive lobular pmBCa in patients with MetS was a significant decrease in the level of OPN against the background of an increase in ON in the tumor tissue. In patients without MetS, a similar ratio of matrix cellular protein indicators was recorded, on the contrary, in the cases of invasive Ca NST (Table 2).

When analyzing the relationship between the expression levels of matricellular proteins and the molecular BCa subtypes, a significant decrease in ON expression was noted in tumor tissue of luminal A, luminal B, and Her2/neu-positive BCa subtypes in patients with MetS. We found that the level of ON expression inversely depended on the receptor status of BC in patients of both studied groups (Table 2).

As seen from the data presented in Table 2, no significant relationship between the expression of matricellular proteins and the localization of distant metastases in both groups of patients was established. Along with this, in the group of pmBCa patients with diagnosed liver metastases, ON expression in tumor tissue was lower in patients with MetS.

So, we have established that the level of OPN expression in tumor tissue of patients with MetS was associated with such indicators of BCa malignancy as the degree of the involvement of the regional lymph nodes and the histological type of tumors, while ON expression indicators correlated with the histological type and molecular subtype of neoplasms as well as metastatic liver lesions.

The expression features of matricellular proteins and their relationship with the clinicopathological characteristics of pmBCa have not been fully elucidated. According to the literature, the ON molecule is able to enhance collagen cross-linking through cysteine amino acid residues in its follistatin-like domain and act as a cofactor in TGF-β signaling pathway, which leads to an increased fibrosis [15, 30]. Also, it has been established that ON initiates focal de-adhesion in cells, which secrete it, and changes the strong bond to the intermediate one due to the destruction of focal adhesive plaques.

Moreover, the violation of ON expression is associated with the destruction of stress fibers of the cell cytoskeleton, thus altering its shape [31]. So, the induction of profibrotic and antiadhesive effects of ON on the connective tissue components evidences to its role in the formation of tumor-associated extracellular matrix.

MetS with its hyperglycemic states affects the functional properties and structure of the collagen fibers of the stromal tissue. It is believed that the pathological effect is due to non-enzymatic cross-links (advanced glycation end products — AGEs), which are formed in response to the prolonged action of monosaccharides on the collagen molecule. AGEs can lead to increased stiffness and fragility of collagen fibrils due to inhibition of intrafibrillar sliding and destruction of tropocollagen molecules [32]. Thus, increased formation of AGEs is the main cause of the dysfunction of mature collagen fibers in elderly patients, which is accelerated by elevated glucose levels and the development of type 2 diabetes [33]. A central role in the development of age-related metabolic disorders (obesity, diabetes, cardiovascular diseases) is played by a chronic inflammatory process in tissues, accompanied by infiltration of leukocytes and macrophages and their

Table 2. **Relationship between OPN and ON protein expression in tumor tissue and the main clinicopathological characteristics of pmBCa patients with and without MetS**

Parameter	OPN expression (H-Score)		ON expression (H-Score)	
	MetS +	MetS –	MetS +	MetS –
Medium value	230.9 ± 9.1	231.2 ± 8.3	147.8 ± 6.3	161.3 ± 5.9
T category				
T2	231.2 ± 7.3	243 ± 8.3	149.2 ± 8.4	183.3 ± 7.1
T3	205.4 ± 10.8	207.6 ± 8.8	140.1 ± 9.9	178.4 ± 10.6
T4	243.4 ± 8.9	227.6 ± 5.5	147.2 ± 6.1	142.9 ± 7.3
N category				
N0	215.3 ± 10.4	225.3 ± 7.8	111.9 ± 10.2	168.8 ± 8.1
N1	227.8 ± 9.3	217.7 ± 7.9	155.3 ± 8.2	166.0 ± 8.7
N2	247.8 ± 8.7	245.8 ± 8.7	142.1 ± 9.5	163.1 ± 8.4
N3	265.6 ± 7.7 *	214.7 ± 7.6	123.2 ± 7.7*	146.3 ± 9.1
M category				
Mts to bones	241.3 ± 5.6	236.6 ± 7.9	152.7 ± 7.2	163.0 ± 6.6
Mts to lungs	229.8 ± 9.0	233.5 ± 8.8	150.7 ± 8.3	144.3 ± 9.3
Mts to liver	227.1 ± 7.7	224.9 ± 8.6	146.2 ± 9.1 *	161.7 ± 6.3
Combined Mets to more than 1 organ	241.3 ± 7.6	241.7 ± 7.2	152.7 ± 7.2	151.3 ± 7.3
Histologic type				
Invasive Ca NST	236.4 ± 8.7	229.9 ± 8.6	144.8 ± 7.5*	162.1 ± 5.9
Invasive lobular Ca	219.4 ± 8.4 *	250.5 ± 7.7	150.4 ± 5.4	149.1 ± 6.7
Grade				
G2	229.6 ± 7.1	232.6 ± 7.0	151.2 ± 8.0	163.6 ± 6.6
G3	235.6 ± 7.9	228.9 ± 6.6	138.4 ± 8.9	157.3 ± 9.5
Molecular subtype				
Luminal A	228.1 ± 8.1	233.5 ± 9.2	140.2 ± 7.8 *	158.3 ± 7.2
Luminal B	230.1 ± 8.8	225.0 ± 8.0	119.3 ± 10.2*	151.3 ± 7.2
Triple negative	233.7 ± 8.5	234.0 ± 8.0	195.8 ± 6.1	206.0 ± 8.2
Her2/neu-positive	256.0 ± 9.7	247.1 ± 6.9	110.0 ± 7.7*	171.0 ± 7.8
Receptor status				
ER+PR+	229.0 ± 7.8	228.9 ± 9.3	130.9 ± 8.0	154.5 ± 8.5
ER-PR-	242.6 ± 8.7	240.6 ± 7.5	201.4 ± 7.8	188.5 ± 7.5

Note: *$p < 0.05$ compared to patients without MetS.

production of pro-inflammatory cytokines, as well as matricellular proteins [30, 34].

The important role of OPN in the progression and metastasis of BC has been confirmed by numerous studies [35]. According to the literature, the OPN protein secreted by tumor cells or cells of the microenvironment is able to affect the secreting cell itself through autocrine mechanisms or can exert effects on another cell through paracrine mechanisms, provided that these cells have the corresponding receptors for integrins or CD44 [34]. OPN mediates cancer progression by counteracting anoikis, promoting cell migration in unfavorable conditions [36]. In transformed epithelial BCa cells, several OPN splicing variants are present, which induce their aggregation and activated metabolism. In particular, OPN-a signaling increases glucose levels in cells, and OPN-c uses this glucose to generate increased amounts of ATP [36, 37].

Metastases usually develop within the first few years after the diagnosis of BCa and are most commonly localized in the bone tissue, liver, and lung [38]. Although invasive lobular carcinoma (ILC) arises from the same ductal epithelium as non-specific type IBC (NST), this type of tumor is of particular interest due to its regulatory pathways, specific histological presentation, and biological properties [39, 40]. The most characteristic features of lobular carcinomas include the loss of E-cadherin, leading to a discohesive growth pattern. In addition, most of these tumors are ER+/PR+ and Her2/neu-negative [41]. ILC is the second most common type of BC with a high incidence of distant metastases. In addition, it has an unusual pattern of metastasis, due to the predominance of hematogenous spread, which poses a diagnostic challenge for clinicians and pathologists [42, 43]. IBC NST and ILC metastases are somewhat different in tropism to target organs, as well as in metabolic behavior, prompting the use of specific diagnostic strategies [38, 44].

Our data on the relationship between the expression of matricellular proteins in pmBCa tissue and cancer malignancy indicates the prospect of further studies of their prognostic value in the presence of metabolic syndrome. Such an approach will allow us to expand an understanding of the role of the tumor microenvironment in the pmBCa progression, which will contribute to the development of individualized treatment and improvement of the quality of life of patients.

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ОСОБЛИВОСТІ ЕКСПРЕСІЇ МАТРИЦЕЛЮЛЯРНІХ БІЛКІВ ПРИ МЕТАСТАТИЧНОМУ РАКУ МОЛОЧНОЇ ЗАЛОЗИ НА ТЛІ МЕТАБОЛІЧНОГО СИНДРОМУ

Метастатичний рак грудної залози (МРГЗ) — одна з головних причин смертності серед жінок постменопаузального віку, хворих на злоякісні новоутворення. Численні дослідження свідчать про доцільність використання білків ремоделювання кісткового матриксу для прогнозування перебігу раку грудної залози. **Мета**. Дослідити зв'язок показників експресії остеопонтину (OPN) та остеонектину (ON) у пухлинній тканині хворих на первинний МРГЗ (пМРГЗ) з метаболічним синдромом (МетС) менопаузального віку з урахуванням клініко-патологічних особливостей пухлинного процесу. **Матеріали та методи**. В дослідження включено 54 пацієнтки менопаузального віку з пМРГЗ, які попередньо не отримували неоад'ювантного лікування. Дослідну групу складали 23 пацієнтки з наявними ознаками МетС; решта хворих, 31 особа, не мали ознак МетС та входили в групу контролю. Імуногістохімічне дослідження проводилось для визначення експресії матрицелюлярних білків (OPN та ON). Для оцінки значущості відмінностей між групами застосовувався критерій Крускала — Уолліса. **Результати**. Зафіксовано підвищення рівня експресії OPN (265,6 ± 7,7 балів H-Score, $p < 0,05$) на тлі зниження рівня ON (123,2 ± 7,7 балів H-Score, $p < 0,05$) у тканині пМРГЗ за наявності МетС у пацієнток категорії N3. Встановлено, що інвазивний дольковий пМРГЗ у хворих з МетС характеризується суттєвим зниженням рівня OPN (219,4 ± 8,4 балів H-Score) на тлі підвищення ON (144,8 ± 7,5 балів H-Score). Відзначено достовірне зниження ($p < 0,05$) ON у пухлинній тканині люмінального А, люмінального Б та Her2/neu пМРГЗ у хворих з МетС (відповідно 140,2 ± 7,8, 119,3 ± 10,2, 110 ± 7,7 балів H-Score). У групі хворих на пМРГЗ з діагностованим метастатичним ураженням печінки експресія ON в пухлинній тканині була нижчою, ніж у хворих з наявними ознаками МетС (146,2 ± 9,1 балів H-Score). **Висновки**. Отримані дані демонструють зв'язок показників експресії матрицелюлярних протеїнів у тканині пМРГЗ зі ступенем злоякісності пухлинного процесу та вказують на перспективність подальших досліджень їх прогностичного значення за наявності МетС.

Ключові слова: метастатичний рак грудної залози, матрицелюлярні білки, остеопонтин, остеонектин, метаболічний синдром.