## ORIGINAL CONTRIBUTIONS



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# EXPRESSION OF SPP1 AND SPARC GENES IN TUMOR TISSUE OF PATIENTS WITH BREAST CANCER

Background. Breast cancer (BCa) is one of the most common oncological diseases in women in Ukraine and worldwide, which determines the need to search for new diagnostic and prognostic markers. In this aspect, the study of multicellular proteins, in particular osteopontin (OPN) and osteonectin (ON), in BCa tissue is relevant. The aim of the work was to investigate the expression of SPP1 and SPARC at the mRNA and protein levels in BCa tissue and to assess their relationship with the main clinicopathological BCa characteristics and the survival rates of patients. Materials and Methods. The work was based on the analysis of the results of the examination and treatment of 60 patients with stage II—III BCa and 15 patients with breast fibroadenomas. SPP1 and SPARC mRNA levels were determined by real-time PCR. The study of the expression of protein products of the SPP1 and SPARC genes was carried out by the immunohistochemical method. Results. We have established that the BCa tissue was characterized by 3.5 (p < 0.05) and 7.4 (p < 0.05) lower levels of SPP1 and SPARC mRNA, respectively, compared to the tissue of benign neoplasms, while OPN and ON expression levels were 1.6 (p < 0.05) and 5.6 (p < 0.05) times higher, respectively, compared to fibroadenoma tissue. The analysis of the relationship between the expression of SPP1 and SPARC at the protein and mRNA levels in BCa tissue and the main clinicopathological BCa characteristics revealed its dependence on the presence of metastases in regional lymph nodes, differentiation grade, and the molecular BCa subtype. Also, high expression levels of SPP1 and OPN were associated with worse patient survival rates. **Conclusion**. The obtained results indicate the perspective of using SPP1 and SPARC expression indices in BCa tissue to assess the aggressiveness of the cancer course and optimize the tactics of treating patients.

Keywords: breast cancer, osteopontin, osteonectin, prognosis, survival.

Despite a significant progress in the diagnosis and treatment of breast cancer (BCa), it still causes the annual death of more than half a million women worldwide [1]. Most of the fatal BCa cases are associated with cancer spread and the

development of distant metastases [2]. It has been proven that the prognosis of the disease largely depends on a timely and accurate diagnosis, which is based on objective indicators of the patient's somatic condition, and especially on the biologi-

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cal characteristics of the tumor. To understand the mechanisms and key factors of cancer progression, it is considered promising to study the components of the tumor microenvironment, which provide biochemical and biomechanical signaling and are biological factors associated with the BCa progression and metastasis [3, 4].

According to the latest experimental and clinical research data, the matricellular proteins osteopontin (OPN) and osteonectin (ON) are non-collagenous proteins of the extracellular matrix that play an important role in the development and progression of BCa [5]. OPN and ON are non-fibrillar glycoproteins that belong to the bone matrix-associated factors modulating the behavior of cells and also providing regulation of the extracellular matrix [6, 7]. The expression of these proteins was detected in various tissues of the body, including bone, cartilage, vessels, and tumors of different histogenesis, which confirms a wide range of their functions, including the regulation of cell migration, adhesion, and proliferation [8, 9].

The recent research shows that disruption of OPN expression leads to an increase in the number of macrophages and T cells in the focus of inflammation. In addition, OPN has been shown to inhibit tissue calcification and overmineralization and to be involved in wound healing [10]. ON is primarily secreted by fibroblasts and macrophages in the inflammatory lesions. It regulates the regenerative processes, cell proliferation and exerts anti-adhesive properties, as well as the ability to bind with cytokines and growth factors [11].

It has been shown that the development of malignant neoplasms of various localization (cancer of the lung, stomach, pancreas, intestines, brain, and mammary gland) is accompanied by an increased expression of OPN and ON in the tumor tissue [3]. The increased expression of these matricellular proteins correlates with some clinicopathological characteristics and survival rates of patients with prostate cancer, osteosarcoma, and melanoma [12]. However, due to the disparity of data, there is still no definite unified view on the role of OPN and ON in the pathogenesis of BCa [6, 13—17].

Therefore, the aim of the work was to investigate the expression of the *SPP1* and *SPARC* genes at the level of mRNA and protein in BCa tissue and to establish their relationship with the main clini-

copathological characteristics of the course of the disease and the survival rates of patients.

### **Materials and Methods**

The study included the results of clinical examination and treatment of 60 patients with stage II—III BCa and 15 patients with breast fibroadenomas (FA), who were undergoing inpatient treatment at the Kyiv City Clinical Oncology Center (Kyiv, Ukraine) during 2015—2019. All patients gave informed consent for the use of the operative material and clinical data for scientific purposes. The stage of the tumor process was determined according to the International Classification of Tumors (the 8th TNM edition, 2016).

By the anamnesis data, all patients underwent general clinical, biochemical, and laboratory examinations, ultrasound examination of the abdominal organs, mammography, radiography of the chest cavity, puncture biopsy of breast tumors according to the standards of diagnosis and treatment of oncological patients approved by the orders of the Ministry of Health of Ukraine No. 140 from 07/27/1998, No. 554 dated 09/17/2007, No. 645 dated 07/30/2010, and No. 396 dated 06/30/2015.

Neoadjuvant therapy was not performed in patients with stage II—III BCa. All patients underwent surgical treatment (quadrant or lumpectomy with regional lymphodissection, radical mastectomy according to Maden). The clinicopathological characteristics of the BCa patients are shown in Table 1.

Real time polymerase chain reaction (PCR). To study the expression of the *OPN* and *ON* genes at the mRNA level (*SPP1* and *SPARC*, respectively), the reverse transcription PCR (RT-PCR) method in real time was used. The total RNA was isolated from the homogenate of 100 mg of tumor tissue using the commercial RNeasy FFPE Kit (QIAGEN, Germany). The amount of isolated RNA was determined on a NanoDrop 2000c Spectrophotometer (ThermoScientific, USA).

The purity of the isolated RNA was monitored using the ratio of optical absorbance values at 260 and 280 nm. RNA was dissolved in Tris-EDTA buffer and stored at -20 °C until PCR.

RT-PCR was performed using the hardware detection system Quant Studio DX5 Real-Time PCR System with a commercial kit for RT-PCR

LunaScript® RT SuperMix Kit (New England Biolabs, Inc., USA) according to the manufacturer's recommendations. β-actin mRNA (*ACTB1*) was used as an endogenous control. Besides, the Maxima SYBR Green/ROX qPCR Master Mix (2X) commercial kit (Thermo Fisher Scientific, USA) was used with primers synthesized by Metabion (Germany) (Table 2). Primer sequences were taken from www.origene.com.

The relative expression of the studied mRNAs was determined by the comparative dCT method and presented as fold change. The study was conducted in three replicates per sample [18].

Immunohistochemical method. Immunohistochemical (IHC) study of OPN, ON estrogen receptors (ER), progesterone (PR), human epidermal growth factor (Her2/neu), and Ki-67 in BCa tissue was performed on paraffin sections of biopsy material. Monoclonal antibodies specific for OPN (clone: 7C5H12; AbCam, UK), ON (clone: ON1-1; Thermo Scientific, USA), ER (clone: 1D5; Dako-Cytomation, Denmark), PR (clone: PgR636; Dako-Cytomation, Denmark), Her2/neu (clone: e2-400; Thermo Scientific, USA), and Ki-67 (clone: MIB-1; DakoCytomation, Denmark) were used as primary antibodies. The Lab Vision™ UltraVision™ Quanto Detection System (Thermo Fisher Scientific, USA) was used to detect the IHC reaction. The expression level of the markers was determined by the semi-quantitative H-Score method and calculated according to the formula:

$$S = 0 \times N_0(\%) + 1 \times N_1(\%) + 2 \times N_2(\%) + 3 \times N_3(\%),$$

where S is the «H-Score» index,  $N_0$  is the number of cells with no expression,  $N_1$ ,  $N_2$ , and  $N_3$  — with low, medium, and high expression, respectively [19].

*Statistical analysis*. Statistical processing of the obtained results was performed using the Graph-Pad Prism v.8.0 program (GraphPad Software Inc.,

USA) taking into account the nature of the distribution of the obtained data. In order to quantitatively compare two independent groups, Student's t-test (for parametric distribution) or the Mann — Whitney U-test (for non-parametric distribution) were used. Data are presented as  $M \pm m$  where M is the arithmetic mean, m is the standard error of the mean, or a percentage for relative values. Analysis of the survival rates of patients with BCa was performed using the Kaplan — Meier test. For this, all patients were divided into groups with low ( $\leq Me$ ) and high (>Me) levels of expression of the studied markers. The critical level of statistical significance was taken equal to 0.05.

Table 1. Clinicopathological characteristics of the BCa patients

	,		
Index	Number of patients		
mucx	N	%	
Total number of patients	60	100	
Age (years)			
Average	52.5 ± 6.3		
Range	28—89		
T category by TNM			
T2	21	35.0	
Т3	39	65.0	
N category by TNM			
N0	26	43.3	
N1	34	56.7	
Differentiation grade			
G1 (high)	8	13.3	
G2 (moderate)	32	53.3	
G3 (low)	20	33.3	
Molecular subtype			
Luminal A	12	20.0	
Luminal B	30	50.0	
Basal	18	30.0	

Table 2. Primer sequences for determination of mRNA expression

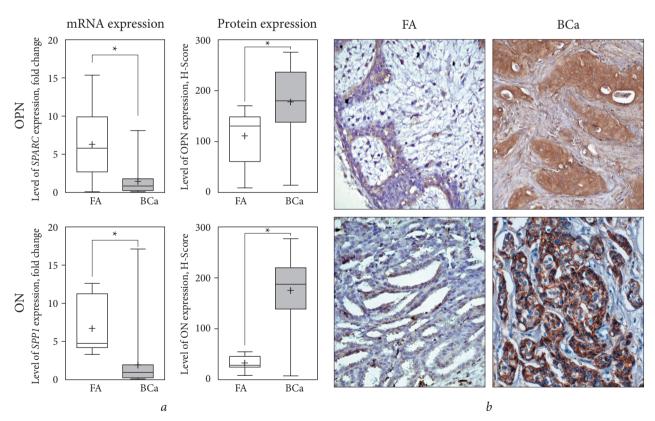
Gene	Primer		
ACTB1	Forward Reverse	5'- TGTTACCAACTGGGACGACA-3' 5'- GGGGTGTTGAAGGTCTCAAA-3'	
SPP1	Forward Reverse	5' - CGAGGTGATAGTGTGGTTTATGG- 3' 5' - GCACCATTCAACTCCTCGCTTTC- 3'	
SPARC	Forward Reverse	5' - TGCCTGATGAGACAGAGGTGGT- 3' 5' - CTTCGGTTTCCTCTGCACCATC- 3'	

### **Results and Discussion**

Features of the expression of SPP1 and SPARC in the tissues of benign and malignant neoplasms of the mammary gland at the levels of mRNA and protein. We revealed that the BCa tissue was characterized by significantly lower expression of SPP1 and SPARC compared to the similar indicators of benign neoplasms. As shown in Fig. 1 the levels of SPP1 mRNA in BCa tissue were 3.5 times lower (p < 0.05), and SPARC mRNA 7.4 times lower (p < 0.05) compared to the corresponding indices in FA tissues (Fig. 1).

In contrast, the analysis of the quantitative indicators of the IHC study of the expression of OPN and ON in the tissue of benign and malignant neoplasms of the mammary gland allowed us to establish significantly higher levels of both investigated proteins in the BCa tissue (Fig. 1). In particular, BCa tissue was characterized by 1.6-fold higher (p < 0.05) and 5.6-fold higher (p < 0.05) expression levels of OPN and ON compared to FA tissue.

Relationship of SPP1 and SPARC expression indicators in BCa tissue with clinicopathological features. We found that the BCa tissue of T3 category cases by the TNM classification was characterized by 1.7-fold higher (p < 0.05) and 1.6fold higher (p < 0.05) levels of SPP1 and SPARC mRNA compared to the similar indicators of the T2 category tumors. The levels of SPP1 and SPARC mRNA in BCa tissue of patients with metastases in the regional lymph nodes was 2.6-fold higher (p < 0.05) and 1.5-fold higher (p < 0.05), respectively, than in patients without metastases. The level of SPP1 mRNA in the basal BCa tissue was 5.0-fold higher (p < 0.05) and 2.8-fold higher (p < 0.05) compared to the neoplasms of luminal A and B molecular subtypes, respectively. The levels of SPARC mRNA in the basal BCa tissue were 1.8-fold higher (p < 0.05) and 1.7-fold higher (p < 0.05) compared to the similar indicators in the samples of BCa of luminal A and B molecular subtypes, respectively. No significant difference



*Fig. 1.* Expression of *SPP1* and *SPARC* at the mRNA and protein levels in the tissue of benign and malignant neoplasms of the breast: a — quantitative indicators of expression at the mRNA and protein levels. The median is indicated by the central line, the mean is marked by a cross, the first and third quartiles are indicated by the lower and upper limits of the "box", respectively. The whiskers emerging from the rectangles indicate the minimum and maximum values of the indicators; b — typical photomicrographs of OPN and ON expression in FA and BCa tissues. IHC, 3-diaminobenzidine tetrachloride chromogen. Staining with hematoxylin,  $\times 400$ . \*p < 0.05 — the difference is significant

Table 3. Relationship of SPP1 and SPARC expressions in BCa tissue with the main clinicopathological BCa characteristics

Index	SPP1		SPARC	
	mRNA, fold change	Protein, H-Score	mRNA, fold change	Protein, H-Score
T category by TNM classification				
$T_2$	$2.3 \pm 0.5$	$171.3 \pm 12.4$	$1.6 \pm 0.2$	$188.3 \pm 12.3$
$T_3^2$	$3.8 \pm 0.7^{1}$	$184.2 \pm 11.3$	$2.6 \pm 0.4^{1}$	$180.3 \pm 10.8$
N category by TNM classification				
N <sub>0</sub>	$1.5 \pm 0.2$	$158.6 \pm 12.3$	$1.6 \pm 0.2$	$165.5 \pm 10.4$
$N_1$	$3.9 \pm 0.9^{2}$	$194.6 \pm 9.5^2$	$2.4 \pm 0.5^{2}$	$188.6 \pm 9.4^{2}$
Differentiation grade				
High	$2.8 \pm 0.3$	155.6 ± 11.3	$2.1 \pm 0.3$	$165.0 \pm 10.2$
Moderate	$2.6 \pm 0.4$	$170.1 \pm 10.3^{3}$	$2.2 \pm 0.4$	$187.2 \pm 9.6^3$
Low	$3.0 \pm 0.8$	$192.0 \pm 8.4^3$	$2.1 \pm 0.3$	$207.3 \pm 12.2^{3}$
Molecular subtype				
Luminal A	$0.7 \pm 0.2$	$155.2 \pm 9.5$	$1.3 \pm 0.2$	$172.5 \pm 12.0$
Luminal B	$2.0 \pm 0.5^{4}$	$177.6 \pm 8.2^{4}$	$1.4 \pm 0.4^{4}$	$173.3 \pm 10.6$
Basal	$3.5 \pm 0.7^{4,5}$	$203.5 \pm 11.2^{4, 5}$	$2.4 \pm 0.3^{4, 5}$	$196.5 \pm 9.0^{4}$

*Notes*: <sup>1</sup> the difference is significant compared to category T2 (p < 0.05); <sup>2</sup> the difference is significant compared to category N0 (p < 0.05); <sup>3</sup> the difference is significant compared to high-grade neoplasms (p < 0.05); <sup>4</sup> the difference is significant compared to the luminal A molecular subtype (p < 0.05); <sup>5</sup> the difference is significant compared to the luminal B molecular subtype (p < 0.05).

was found in the mRNA indicators depending on tumor differentiation grade.

According to our results, the expression levels of the OPN and ON in BCa tissues of patients with metastases in the regional lymph nodes were by 22.7% and 14.0% higher (p < 0.05), respectively, than in patients without metastases. It was established that the level of OPN in the tissue of poorly differentiated BCa was by 12.9% and 23.4% higher (p < 0.05) compared to the similar indicators of patients with moderately and highly differentiated BCa, respectively (Table 3). When comparing the levels of OPN and ON in tumor tissue, depending on the molecular BCa subtype, we found that tumors of the basal subtype were characterized by the high expression of both investigated matricellular proteins. In particular, the OPN and ON expression levels in the basal BCa subtype were by 31.1% and 13.9% higher (p < 0.05) compared to the similar indicators in patients with the luminal A subtype, and by 14.6% and 13.4% higher (p < 0.05) compared to the indicators of patients with the luminal B subtype.

Analysis of the overall survival rates of BCa patients depending on the expression of SPP1 and SPARC at the level of mRNA and protein in the tumor tissue. Accounting the revealed relationship between the expression of matricellular genes and the clinicopathological characteristics associated with the degree of BCa malignancy, we analyzed the 5-year overall survival of the patients (Fig. 2). It was established that a high level of the SPP1 expression, both at the mRNA and protein levels in BCa tissue, was associated with a 22.0% (p = 0.0276) and 30.0% (p = 0.0182) decrease in the 5-year survival rates of patients (Fig. 2). We found no significant difference in the 5-year overall survival rates of BCa patients depending on the expression of SPARC at the mRNA and protein levels in the tumor tissue (Figs 2, b and 2, d).

Thus, our results indicate the prospects of using *SPP1* and *SPARC* as markers of the BCa aggressiveness necessitating the further study of the role of these genes in the mechanisms of BCa development and progression. The heterogeneity of the *SPP1* and *SPARC* expression ratio at the mRNA

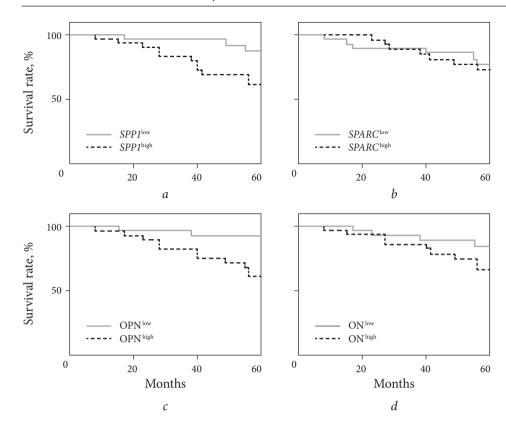


Fig. 2. Overall survival of patients depending on the SPP1 and SPARC expression indices at the level of mRNA (a, b) and protein (c, d) in BCa tissue (by the Kaplan — Meier method)

and protein levels in the tissue of benign and malignant breast neoplasms may be due to a violation of their epigenetic regulation, in particular, a change in the levels of methylation or non-coding RNAs, or caused by a decrease in the rate of the degradation of protein molecules due to the deterioration of the lysosomal and/or proteosomal system, violation of the regulation of ubiquitination processes, and ubiquitin-like modifications, such as SUMOylation and NEDDylation [20].

It is worth noting that our results for the relationship between the OPN expression and the clinicopathological BCa features are consistent with the literature. In particular, Pang et al. [21] and Allan et al. [22] proved that increased expression of OPN was associated with the metastatic lesions of the lymph nodes, a larger size and a low differentiation grade of BCa. Similarly, Wang et al. [23] demonstrated the association of a high level of OPN with the basal BCa subtype. Niedolistek et al. [24] found a relationship between the OPN expression and the presence of metastases in the regional lymph nodes. The identified relationship between the expression of matricellular proteins and the molecular subtype of BCa has been confirmed by the data of our previous studies in an in vitro system, according to which the expression levels of ON and OPN were significantly higher in cells of the MDA-MB-231 line of the basal molecular subtype, compared to the cells of the MCF-7 line, positive by the expression of steroid hormone receptors [25].

It should be noted that in the available literature, there is no agreement on the association of the *SPP1* expression with the clinicopathological characteristics and survival rates of patients with malignant neoplasms, and most of the studies are related to tumors of the head and neck [26], intestinal cancer [27], etc. Ortiz-Martínez et al. [28] showed that an increase in the level of *SPP1* mRNA was determined in the tissue of highly aggressive BCa with metastases in the regional lymph nodes, and was also associated with a low recurrence-free survival of patients.

The association of the higher ON expression with the more aggressive BCa course was reported by Watkins et al. in 2005 [29]. According to the data of an *in silico* bioinformatic study conducted by Shi et al. [30], an increase in the level of *SPARC* in BCa tissue was associated with a low differentiation grade and worse patient survival rates.

Alcaraz et al. [31] reported that ON contributed to the progression of BCa of the basal molecular subtype. The authors analyzed the prognostic value of ON expression in the parenchymal and stromal components of non-metastatic BCa of the basal subtype. The expression of this protein was observed in tumor cells, tumor-associated fibroblasts, tumor-associated macrophages, endothelial cells, and tumor-infiltrating lymphocytes. The results of the study indicated that the recurrence-free survival of patients with BCa was lower in the presence of ON expression in tumor-associated fibroblasts.

Also, interesting results of Azim et al. [32] showed that the high expression of *SPARC* mRNA was associated with the small tumor size, low differentiation grade, and luminal BCa subtype, while the high ON expression in the tissue of the basal and HER2-positive BCa correlated with an unfavorable course of the disease.

In conclusion, our results regarding the relationship between the SPP1 and SPARC expres-

sion levels and the degree of BCa malignancy and patient survival indicate the prospects of their use to assess cancer aggressiveness. Further research into the specificity of the regulation of the expression of matricellular proteins will allow us to find out the mechanisms underlying the rearrangements of tumor microenvironment during the BCa progression, which will contribute to the development of individualized treatment and the improvement of the quality of life of patients.

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#### REFERENCES

- 1. Sung H, Ferlay J, Siegel L, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*. 2021;71(3):209-249. https://doi.org/10.3322/caac.21660
- 2. Brook N, Brook E, Dharmarajan A, et al. Breast cancer bone metastases: pathogenesis and therapeutic targets. *Int J Biochem Cell Biol*. 2018;96:63-78. https://doi.org/10.1016/j.biocel.2018.01.003
- 3. Pein M, Oskarsson T. Microenvironment in metastasis: roadblocks and supportive niches. *Am J Physiol Cell Physiol*. 2015;309(10):627-638. https://doi.org/10.1152/ajpcell.00145.2015
- 4. Oskarsson T. Extracellular matrix components in breast cancer progression and metastasis. *Breast.* 2013;22(2):66-72. https://doi.org/10.1016/j.breast.2013.07.012
- 5. Lepucki A, Orlińska K, Mielczarek-Palacz A, et al. The role of extracellular matrix proteins in breast cancer. *J Clin Med.* 2022;11(5):1250. https://doi.org/10.3390/jcm11051250
- 6. Xu Y, Zhang Y, Lu F, et al. Prognostic value of osteopontin expression in breast cancer: A meta-analysis. *Mol Clin Oncol*. 2015;3(2):357-362. https://doi.org/10.3892/mco.2014.480
- 7. Delany A. M. Matricellular proteins osteopontin and osteonectin/SPARC in pancreatic carcinoma. *Cancer Biol Ther.* 2010;10(1):65-67. https://doi.org/10.4161/cbt.10.1.12452
- 8. Zhao H, Chen Q, Alam A, et al. The role of osteopontin in the progression of solid organ tumour. *Cell Death Dis.* 2018;9(3):356. https://doi.org/10.1038/s41419-018-0391-6
- 9. Rodrigues R, Teixeira A, Schmitt L, et al. The role of osteopontin in tumor progression and metastasis in breast cancer. *Cancer Epidemiol Biomarkers Prev.* 2007;16(6):1087-1097. https://doi.org/10.1158/1055-9965.EPI-06-1008
- 10. Icer MA, Gezmen-Karadag M. The multiple functions and mechanisms of osteopontin. *Clin Biochem.* 2018;59:17-24. https://doi.org/10.1016/j.clinbiochem.2018.07.003
- 11. Young MF, Kerr JM, Ibaraki K, et al. Structure, expression, and regulation of the major noncollagenous matrix proteins of bone. *Clin Orthop Relat Res.* 1992;281:275-294. PMID: 1499220
- 12. Tuck AB, Chambers AF. The role of osteopontin in breast cancer: clinical and experimental studies. *J Mammary Gland Biol Neoplasia*. 2001;6(4):419-429. https://doi.org/10.1023/a:1014734930781
- 13. Bramwell VH, Tuck AB, Chapman JA, et al. Assessment of osteopontin in early breast cancer: correlative study in a randomised clinical trial. *Breast Cancer Res.* 2014;16(1):R8. https://doi.org/10.1186/bcr3600
- 14. Shi S, Ma HY, Han XY, et al. Prognostic significance of SPARC expression in breast cancer: A meta-analysis and bio-informatics analysis. *BioMed Res Int.* 2022;8600419. https://doi.org/10.1155/2022/8600419
- 15. Lindner JL, Loibl S, Denkert C, et al. Expression of secreted protein acidic and rich in cysteine (SPARC) in breast cancer and response to neoadjuvant chemotherapy. *Ann Oncol.* 2015;26(1):95-100. https://doi.org/10.1093/annonc/mdu487

- 16. Bergamaschi A, Tagliabue E, Sørlie T, et al. Extracellular matrix signature identifies breast cancer subgroups with different clinical outcome. *J Pathol.* 2008;214(3):357-367. https://doi.org/10.1002/path.2278
- 17. Ma J, Gao S, Xie X, et al. SPARC inhibits breast cancer bone metastasis and may be a clinical therapeutic target. *Oncol Lett.* 2017;14(5):5876-5882. https://doi.org/10.3892/ol.2017.6925
- 18. Zhang JD, Ruschhaupt M, Biczok R. ddCt method for qRT-PCR data analysis. Citeseer. 2013;48(4):346-356.
- 19. McClelland RA, Wilson D, Leake R, et al. A multicentre study into the reliability of steroid receptor immunocytochemical assay quantification. British Quality Control Group. *Eur J Cancer*. 1991;27(6):711-715. https://doi.org/10.1016/0277-5379(91)90171-9
- 20. Di Gregorio J, Di Giuseppe L, Terreri S, et al. Protein stability regulation in osteosarcoma: the ubiquitin-like modifications and glycosylation as mediators of tumor growth and as targets for therapy. *Cells.* 2024; 13(6):537. https://doi.org/10.3390/cells13060537
- 21. Pang H, Lu H, Song H, et al. Prognostic values of osteopontin-c, E-cadherin and β-catenin in breast cancer. *Cancer Epidemiol.* 2013;37(6):985-992. https://doi.org/10.1016/j.canep.2013.08.005
- 22. Allan AL, George R, Vantyghem SA, et al. Role of the integrin-binding protein osteopontin in lymphatic metastasis of breast cancer. *Am J Pathol.* 2006;169(1):233-246. https://doi.org/10.2353/ajpath.2006.051152
- 23. Wang X, ChaoL, Ma G, Chen L, et al. Increased expression of osteopontin in patients with triple-negative breast cancer. *Eur J Clin Invest*. 2008;38(6):438-446. https://doi.org/10.1111/j.1365-2362.2008.01956.x
- 24. Niedolistek M, Fudalej MM, Sobiborowicz A, et al. Immunohistochemical evaluation of osteopontin expression in triple-negative breast cancer. *AMS*. 2020;20(2). https://doi.org/10.5114/aoms.2020.93695
- Zadvornyi T, Lukianova N, Borikun T, et al. Expression of osteopontin and osteonectin in breast and prostate cancer cells with different sensitivity to doxorubicin. *Exp Oncol.* 2022;44(2):107-112. https://doi.org/10.32471/exp-oncology.2312-8852.vol-44-no-2.17886
- 26. Bie T, Zhang X. Higher expression of SPP1 predicts poorer survival outcomes in head and neck cancer. *J Immunol Res.* 2021;8569575. https://doi.org/10.1155/2021/8569575
- 27. Xu C, Sun L, Jiang C, et al. SPP1, analyzed by bioinformatics methods, promotes the metastasis in colorectal cancer by activating EMT pathway. *Biomed Pharmacother*. 2017;91:1167-1177. https://doi.org/10.1016/j.biopha.2017.05.056
- 28. Ortiz-Martínez F, Perez-Balaguer A, Ciprián D, et al. Association of increased osteopontin and splice variant-c mRNA expression with HER2 and triple-negative/basal-like breast carcinomas subtypes and recurrence. *Hum Pathol*. 2014;45(3):504-512. https://doi.org/10.1016/j.humpath.2013.10.015
- 29. Watkins G, Douglas-Jones A, Bryce R, et al. Increased levels of SPARC (osteonectin) in human breast cancer tissues and its association with clinical outcomes. *Prostaglandins Leukot Essent Fatty Acids*. 2005;72(4):267-272. https://doi.org/10.1016/j.plefa.2004.12.003
- 30. Shi S, Ma HY, Han XY, et al. Prognostic significance of SPARC expression in breast cancer: a meta-analysis and bio-informatics analysis. *BioMed Res Int.* 2022;8600419. https://doi.org/10.1155/2022/8600419
- 31. Alcaraz LB, Mallavialle A, Mollevi C, et al. SPARC in cancer-associated fibroblasts is an independent poor prognostic factor in non-metastatic triple-negative breast cancer and exhibits pro-tumor activity. *Int J Cancer*. 2023;152(6):1243-1258. https://doi.org/10.1002/ijc.34345
- 32. Azim HA Jr, Singhal S, Ignatiadis M, et al. Association between SPARC mRNA expression, prognosis and response to neoadjuvant chemotherapy in early breast cancer: a pooled in-silico analysis. *PloS One*. 2013;8(4):e62451. https://doi.org/10.1371/journal.pone.0062451

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### ЕКСПРЕСІЯ ГЕНІВ *SPP1* ТА *SPARC* У ПУХЛИННІЙ ТКАНИНІ ХВОРИХ НА РАК МОЛОЧНОЇ ЗАЛОЗИ

Стан питання. Рак молочної залози (РМЗ) є однією із найпоширеніших онкопатологій у жінок як в Україні, так і у світі, що визначає необхідність пошуку нових діагностичних та прогностичних маркерів. У цьому аспекті актуальним є вивчення матрицелюлярних протеїнів, зокрема остеопонтину (OPN) та остеонектину (ON). Мета роботи. Дослідити показники експресії SPP1 та SPARC на рівні мРНК та білка у тканині РМЗ та встановити їх зв'язок з основними клініко-патологічними характеристиками пухлинного процесу і показниками виживаності хворих. Матеріали та методи. Робота базується на аналізі результатів обстеження та лікування 60 хворих на РМЗ ІІ—ІІІ стадії та 15 пацієнток із фіброаденомами молочної залози, які знаходились на лікуванні

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у Київському міському клінічному онкологічному центрі протягом 2015—2019 рр. Рівні мРНК SPP1 та SPARC визначали методом ПЛР в реальному часі. Дослідження експресії SPP1 та SPARC на рівні білка проводили імуногістохімічним методом. **Результати.** Встановлено, що тканина злоякісних новоутворень молочної залози характеризується у 3,5 (p < 0,05) та в 7,4 (p < 0,05) разів меншими рівнями відповідно мРНК SPP1 та SPARC у порівнянні з тканиною доброякісних новоутворень. Показано, що показники експресії OPN та ON у тканині PM3 є відповідно в 1,6 (p < 0,05) та 5,6 (p < 0,05) разів більшими порівняно із тканиною фіброаденом. Аналіз зв'язку між експресією SPP1 та SPARC на рівні білка та мРНК у тканині PM3 та основними клініко-патологічними характеристиками пухлинного процесу встановив існування залежності з наявністю метастазів в реґіонарних лімфатичних вузлах, ступенем диференціювання та молекулярним підтипом новоутворень. Виявлено, що високі рівні експресії SPP1 та SPARC у тканині PM3 для оцінки агресивності перебігу пухлинного процесу та оптимізації тактики лікування хворих. **Ключові слова:** рак молочної залози, остеопонтин, остеонектин, прогнозування перебігу, виживаність.