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EXPRESSION OF MARKERS OF BONE TISSUE REMODELING IN BREAST CANCER AND PROSTATE CANCER CELLS *IN VITRO*

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The aim **of the study was to compare the expression of markers of bone remodeling *in vitro* in breast cancer (BCa) cells and prostate cancer (PCa) cells varying in their malignancy phenotype.** ***Materials and Methods*****: The study was performed on human BCa cells (MCF-7 and MDA-MB-231 lines) and PCa cells (LNCaP and DU-145 lines). Expression levels of bone tissue remodeling proteins (osteopontin (OPN), osteonectin (ON) and bone morphogenetic protein 7 (BMP-7) were determined immunocytochemically. The mRNA levels of bone tissue remodeling proteins OPN (*SPP1*), ON (*SPARC*), BMP-7 (*BMP7*)) and miRNA-10b, -27a, -29b, -145, -146a were assessed by quantitative reverse transcription polymerase chain reaction. To search for miRNAs involved in the regulation of target genes, miRNet v. 2.0 resource was used.** ***Results*****: We have shown that highly malignant MDA-MB-231 cells are characterized by significantly higher expression of OPN and ON on the background of decreased *SPARC* and *BMP7* mRNA expression. In highly malignant DU-145 cells, ON and *SPP1, SPARC*, and *BMP7* mRNA expression was significantly higher compared with low malignant LNCaP cells. MDA-MB-231 line was characterized by significantly higher expression of miRNA-10b, -27a, -29b, -145 and -146a. In DU-145 cells, significantly lower levels of expression of miRNAs-27a and -145 against the background of increasing levels of miRNAs-29b and -146a were recorded.** ***Conclusion*****: High malignancy phenotype of the BCa and PCa cells is characterized by high levels of expression of bone remodeling proteins, which may be caused by impaired regulation of their expression at the epigenetic level.**

Key Words**: breast cancer, prostate cancer, osteopontin, osteonectin, microRNA, malignancy phenotype.**

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Presently, the most common forms of hormone-dependent malignancies in women are breast cancer (BCa), and in men — prostate cancer (PCa) [1–3]. The main reason for the unsatisfactory results of BCa and PCa treatment is metastasis, as well as the lack of advanced algorithms for predicting the aggressiveness of the tumor process. According to modern literature, BCa and PCa metastasize to bone due to their ability to secrete bone remodeling proteins, which are normally expressed by osteogenic mesenchymal cells, bone cells and bone marrow [4–8]. The central role in these processes belongs to the ligand-receptor system RANK/RANKL/OPG, which is a key part of bone homeostasis, and directly regulates osteoclast differentiation and osteolysis. It is shown that the imbalance of homeostasis in this system underlies the tumor progression, in particular, the generalization of the process and the development of metastases [9–12]. Extracellular matrix proteins, including osteopontin (OPN), osteonectin (ON), and bone morphogenetic proteins (BMPs) of the BMP family, are equally important in the metastasis of the most common hormone-dependent neoplasms to bone [13–17]. According to some reports, the expression of OPN, ON and BMP-7 in BCa and PCa cells is accompanied by an aggressive course of the tumor process, correlates with metastatic potential and low survival rates [18–23].

In recent years, there have been a few reports that the development and progression of hormone-dependent tumors is accompanied by a change in the expression profile of microRNAs involved in the posttranscriptional regulation of bone remodeling gene expression [24–26]. However, there are no systematic developments in the study on the role of bone remodeling proteins in the formation of the malignancy degree of BCa and PCa. Information on the specificity and significance of disorders of epigenetic regulation of these proteins in the cancer progression is also quite contradictory and needs further study.

We aimed to conduct a comparative study of the expression status of markers of bone remodeling in BCa cells and PCa cells of different malignancy degree *in vitro.*

MATERIALS AND METHODS

Cell lines. The studies were performed *in vitro* on 2 human BCa cell lines (MCF-7 and MDA-MB-231) and 2 human PCa cell lines (LNCaP and DU-145). Cells were provided by the Bank of Cell Lines from Human and Animal Tissues at R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of National Academy of Sciences of Ukraine.

MCF-7 cells were grown in DMEM (Sigma, USA) supplemented with recombinant human insulin (0.01 mg/ml) and 10% fetal bovine serum (FBS). MDA-MB-231 cells were cultured in Leibovitz's L-15 medium (Sigma, USA) supplemented

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Abbreviations used: BCa – breast cancer; BMP – bone morphogenetic protein; FBS – fetal bovine serum; ON – osteonectin; OPN – osteopontin; PCa – prostate cancer; qRT-PCR – quantitative reverse transcription polymerase chain reaction.

with 10% FBS. DU-145 cells were maintained in DMEM (Invitrogen, C11995) supplemented with 10% fetal bovine serum (Gibco/Invitrogen, 10099-141), 100 U/ml penicillin G and 100 µg/ml streptomycin (Invitrogen, 15070-063). LNCaP cells were maintained in DMEM/F12 (Invitrogen, C11330) supplemented with 10% FBS, 100 U/ml penicillin G and 100 µg/ml streptomycin. All cultures were grown on glass cover slips in humidified atmosphere with 5% CO_2 at 37 °C. In all experiments, cells in log phase were subcultured for 24 h and the medium was replaced with fresh medium with or without reagents.

According to our earlier data, MCF-7 and LNCaP cell lines are of low malignancy degree, and MDA-MB-231 and DU-145 cells — of high malignancy degree [27–29].

Immunocytochemical methods. Cells for immunocytochemical studies were grown on glass cover slips, fixed in a cooled mixture of methanol:acetone (1:1) at –20 °C for 120 min and washed in PBS. The following primary monoclonal antibodies were used: anti-OPN (clone 7C5H12, Thermo Scientific, USA), anti-ON (clone ON1-1, Thermo Scientific, USA) and polyclonal antibodies anti-BMP-7 (Thermo Scientific, USA). The antibodies diluted in the blocking buffer were added to the cell specimens for 1 h followed by incubation with Ultra Vision LP Detection System (Lab Vision, Thermo Scientific, USA). After the washing, the immune reaction was visualized using DAB Quanto (Thermo Scientific, USA). When immunocytochemical reactions were completed, the cells were stained with hematoxylin by Mayer (Thermo Scientific, USA) for 10–15 s and placed in Aqua-Mount Mounting Medium (Thermo Scientific, USA). The results were evaluated in 3 visual fields by light microscopy (×100, oil immersion) using the classical H-Score method:

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

where S is "H-Score" index; N_{1+}, N_{2+} and N_{3+} are numbers of cells with low, medium or high levels of marker expression. The level of the studied markers expression was assigned as follows: low — from 0 to 100 H-Score points, medium — from 100 to 200 H-Score points, and high — from 200 to 300 H-Score points [30–31].

Total RNA isolation. Total RNA extraction was performed using RiboPrep RNA Isolation Kit (Amplisens, RF). Concentration of RNA was measured using NanoDrop 2000c Spectrophotometer (Thermo Scientific, USA). The purity of isolated RNA was by the ratio of OD at 260/280 nm. RNA was dissolved in TE buffer and stored at –20 °C.

Real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR). Quantitive PCR (qPCR) was performed on a QuantStudio 5 Dx Real-Time PCR System (ThermoScientific, USA) with a commercial Maxima SYBR Green/ROX Master Mix (2 X) PCR kit (ThermoScientific, USA) according to the manufacturer's protocol. To determine miRNA expression, we used a single stem-loop primer for synthesis of cDNA and universal reverse primer 5'-GTGCAGGGTCCGAGGT-3', according to the methods of stem-loop miRNA RT-qPCR [32]. The polymerase from Reverta-L (Amplisens, RF) kit was used.

Sequences of primers were obtained by using the resource genomics.dote.hu:8080/mirnadesigntool and synthesized by Metabion, Germany (Table 1).

Table 1. Primer sequences used for assessing the expression of miRNAs in the cancer cells

miRNA	Stem-loop primer	Forward primer
miR-10b	5'-GTTGGCTCTGGTGCAGG GTCCGAGGTA TTCGCAC-CAGAGCCAAC TGAAGT-3'	5'-GGCCAGAGGTTGTAAC-GTT-3'
miR-27a	5'-GTTGGCTCTGGTGCAGG GTCCGAGGTA TTCGCACCAGAGCCAAC CTGGGG-3'	5'-TTGCTGAGGAG-CAGGGCT-3'
miR-29b	5'-GTTGGCTCTGGTGCAGG GTCCGAGGTA TTCGCACCAGAGCCAAC CTCCTA-3'	5'-GGCTTCTGGAAGCTG-GTTT-3'
miR-145	5'-GTTGGCTCTGGTGCAGG GTCCGAGGTA TTCGCACCAGAGCCAAC AACCAT-3'	5'-GTTTCACCTTGTCCT-CACG-3'
miR-146a	5'-GTTGGCTCTGGTGCAGG GTCCGAGGTA TTCGCACCAGAGCCAAC ACGATG-3'	5'-GTCCGATGTGTATCCT-CAG-3'

The expression of miRNAs was determined using the $2^{-\Delta CT}$ method relative to RNU48 miRNA used as a reference gene (endogenous control). Primer sequences for RNU48 were taken from www.ncbi.nlm.nih.gov and synthesized by Metabion, Germany: RT-primer: 5'-CTCTGACC-3', forward 5'-AGTGATGATGACCCCAGGTAACTC-3', reverse 5'-CTGCGGTGATGGCATCAG-3'. Table 2 presents primer sequences used for mRNA detection.

Table 2. Primer sequences used for assessing the expression of mRNAs in tumor tissue

Protein	Gene	Forward primer	Reverse Primer
B-Actin	*ACTB*	5'-TGTTACCAACTG GGACGACA-3'	5'-GGGGTGTTGA AGGTCTCAAA-3'
OPN	*SPP1*	5'-CGAGGTGATAGT GTG-GTTTATGG 3'	5'-GCACCATTCA ACTCCTCGCTTTC-3'
ON	*SPARC*	5'-TGCCTGATGAGA CAGAGGTGGT-3'	5'-CTTCGGTTTCC TCTG-CACCATC-3'
BMP-7	*BMP7*	5'-CCAGGAGCACTTG GGCAG-3'	5'-GCCACCATGA AGGGCTGC-3'

Single stranded cDNA for mRNA detection was synthesized from 100 ng of total RNA using Reverta-L (Amplisens, RF) for reverse transcription by manufacturer instructions.

ΔCT (delta threshold cycle) values were calculated automatically by QuantStudio 5 Dx Real-Time PCR System (ThermoScientific, USA). All values of miRNA expression are presented as $2^{-\Delta CT}$ [27, 33].

Bioinformatics analysis. To search for miRNAs involved in the regulation of target genes use the resource miRNet v. 2.0. (https://www.mirnet.ca/miRNet/home.xhtml), which combines information

from 14 different databases. Analysis of possible target genes and the role of the studied miRNAs depending on the histological type of tumors was performed on the following databases miRTarBase v8.0, Kyoto Encyclopedia of Genes and Genomes, and DisGeNET [34].

Statistical analysis was performed using GraphPad Prism v. 8.00 software (GraphPad Software Inc., USA). All data were obtained in triplicates. The values were expressed as means ± standard deviation. Student's *t*-test was used to evaluate the significance of the differences between groups. A value of $p < 0.05$ was accepted as statistically significant.

RESULTS AND DISCUSSION

The analysis of the immunocytochemical expression profile of bone remodeling markers has shown the significant increase in OPN and ON (2.4-fold ($p < 0.05$) and 1.5-fold ($p < 0.05$) respectively) expression in BCa cells of high malignant phenotype (Table 3). In case of PCa cells, DU-145 cells of highly-malignant phenotype demostrated 1.3-fold increased ($p < 0.05$) ON expression level compared to low-malignant LNCaP cells. It should be noted that the expression of BMP-7 in the cells of all studied BCa and PCa cell lines was high (H-score more than 200 points) and did not depend on whether they are of high or low malignancy phenotype.

Table 3. Expression of bone remodeling markers and their mRNAs human BCa and PCa cells

	BCa		PCa	
	MCF-7	MDA-MB-231	LNCaP	DU-145
	Protein expression level, H-Score			
OPN	83.3 ± 6.3	198.3 ± 6.3*	155.0 ± 12.6	158.3 ± 8.3
ON	165.3 ± 7.3	242.5 ± 9.0*	175.0 ± 6.2	231.6 ± 4.6*
BMP-7	247.0 ± 3.6	238.0 ± 6.3	219.0 ± 5.3	254.0 ± 13.5
	mRNA expression level, a.u.			
SPP1	1.26 ± 0.15	1.24 ± 0.10	0.38 ± 0.08	0.85 ± 0.09*
SPARC	48.5 ± 2.30	12.3 ± 1.25*	0.43 ± 0.05	1.43 ± 0.18*
BMP7	9.4 ± 1.66	1.24 ± 0.21*	8.82 ± 1.32	72.6 ± 5.31*

Note: * – the difference is significant compared with the corresponding index in the cell line of low malignancy ($p < 0.05$).

We have also found the significant changes in the mRNA level of the studied bone remodeling markers in BCa and PCa cells. As shown in Table 3, low-malignant MCF-7 cells were characterized by 3.94-fold higher ($p < 0.05$) and 7.86-fold higher ($p < 0.05$) levels of *SPARC* mRNA and *BMP7* mRNA, respectively, compared with highly-malignant MDA-MB-231 cells. We found no significant difference in *SPP1* mRNA in these BCa cells. In contrast, highly malignant DU-145 cells were characterized by significantly higher mRNA levels of all three bone remodeling compared to LNCaP cells. In particular, the expression level of *SPP1* mRNA was 2.2-fold higher ($p < 0.05$), *SPARC* mRNA 3.32-fold higher ($p < 0.05$), and *BMP7* mRNA 8.23-fold higher ($p < 0.05$) in DU-145 cells compared to LNCaP cells.

Thus, the high malignancy phenotype in BCa cells is characterized by an increase in OPN and ON expression levels while *SPARC* and *BMP7* mRNA expression is down-regulated. The increase in ON expression at the level of protein is also demonstrated in PCa cells of high malignancy phenotype.

Given the identified disorders of bone remodeling protein and gene expression, we analyzed miRNAs involved in the regulation of OPN and ON expression. Our *in silico* analysis using the miRNet v. 2.0 resource identified 104 miRNAs (Fig. 1) that potentially could target mRNAs of bone remodeling genes under study. In particular, 18 miRNAs are involved in the regulation of *SPP1* expression, and *SPARC* mRNA is a target of 78 miRNAs. At the same time, 8 miRNAs can affect the expression of both *SPP1* and *SPARC* genes (Fig. 1, *a*). In addition, another 62 miRNAs may indirectly affect the expression of *SPP1* and *SPARC* through the regulation of 14 transcription factors (Fig. 1, *b*). Based on the data of *in silico* analysis, we selected miRNAs-10b, -27a, -29b, -145 and -146a to study the epigenetic mechanisms regulating *SPP1* and *SPARC* expression in the BCa and PCa cells.

Analysis of the expression of these miRNAs has shown that MDA-MB-231 cells were characterized by significantly higher levels of all studied miRNAs (Fig. 2). In particular, the expression level of miRNA-10b in MDA-MB-231 cells was higher by 2.6 times ($p < 0.05$), microRNA-27a — 3.8 times ($p < 0.05$), miRNA-29b — 1.9 times ($p < 0.05$), miRNA-145 — 3.0 times ($p < 0.05$), miRNA-146a — 6.0 times ($p < 0.05$) compared to MCF-7 cells (Fig. 2). In contrast, no significant difference in miRNA-10b expression was detected between the PCa cell lines. In DU-145 cells, the expression levels of miRNA-27a and miRNA-145 were 1.8 ($p < 0.05$) and 5.4 ($p < 0.05$) times lower, and miRNA-29b and miRNA-146a — 2.1 ($p < 0.05$) and 2.5 ($p < 0.05$) times higher compared to LNCaP cells (Fig. 2).

According to our previous data [27–29], highly malignant BCa and PCa cells are characterized by low adhesive properties (high levels of N-cadherin expression against the background of reduced E-cadherin expression), high proliferative and invasive activity, and high expression of oncogenic miRNAs. The accumulated results of numerous experimental studies to date suggest an important role of the proteins of bone matrix remodeling in acquisition of malignancy phenotype in BCa and PCa. It has been shown that OPN, ON, and BMP-7 can promote tumor progression by regulating cell adhesion, migration, and invasion. In particular, colorectal cancer cells cultured in the presence of exogenous OPN increase their migratory and invasive activity while apoptosis and autophagy are inhibited via activating the p38 MAPK signaling pathway [35]. Chang *et al*. [36] demonstrated that inhibition of OPN expression in lung cancer cells leads to activation of Beclin1-induced autophagy. The ability of OPN to regulate invasion processes in BCa and PCa cells acting as both a paracrine

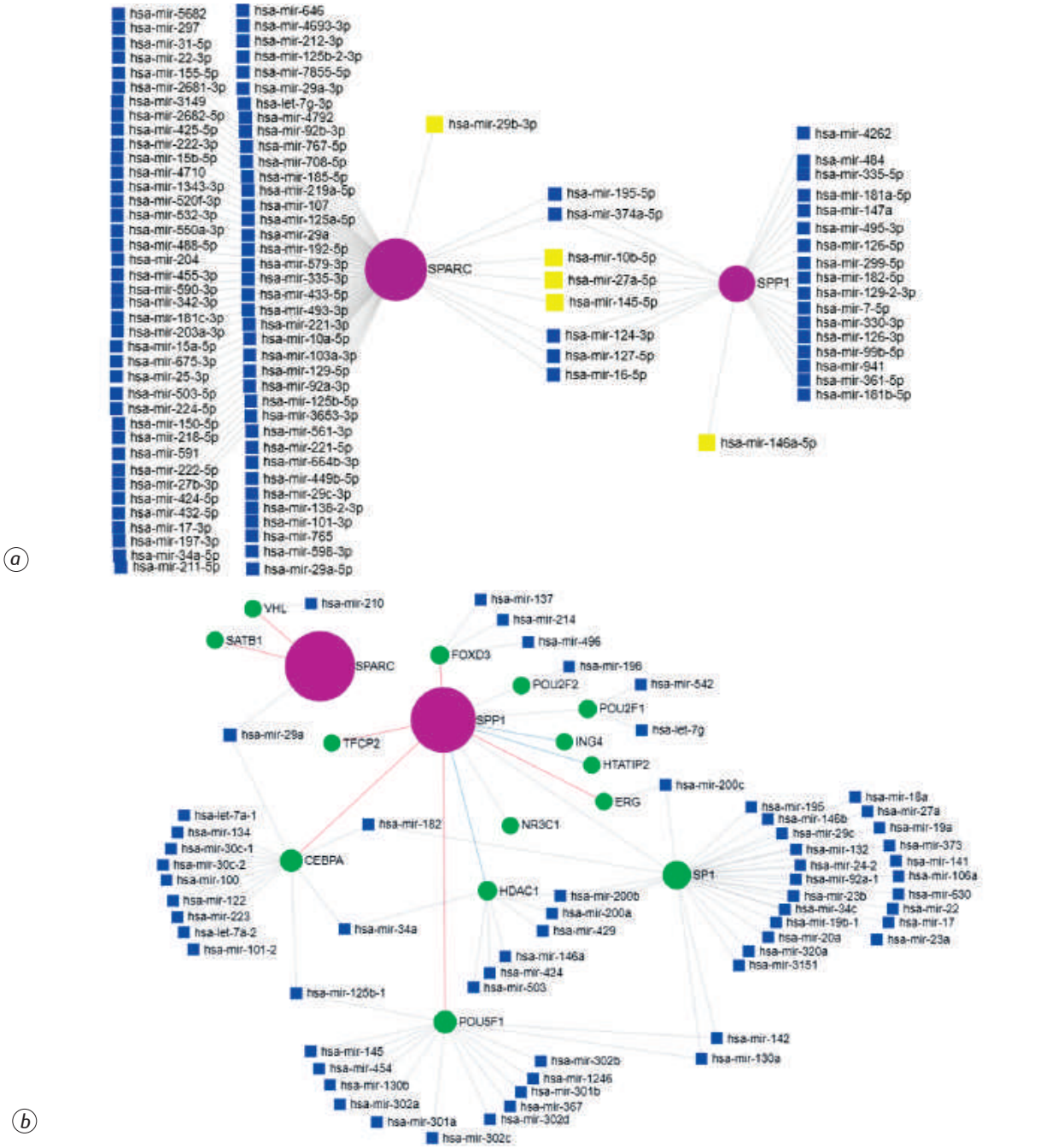

Fig. 1. Network of microRNA interactions (*a* — direct; *b* — indirect) involved in the regulation of OPN and ON expression. Pink circles indicate target genes, green — transcription factors, squares — microRNAs that regulate them. The microRNAs whose expression levels were selected for analysis in our work were highlighted in yellow

and autocrine mediator of tumor progression was also shown [37, 38].

Experiments with vector systems showed that ON expression did not affect the proliferation of MDA-MB-231 cells, apoptosis, migration, aggregation or cleavage of collagen by IV proteases, but their *in vitro* invasion through matrigel and matrigel colony formation decreased [39]. At the same time, Gilles *et al*. [40] have demonstrated that ON is able to induce MMP-2 activation in MDA-MB-231 and BT549 cells, but not in MCF-7 cells. High levels of ON expression were characteristic of PCa cell lines derived from PCa metastases [20]. It has also been shown that the culture of human PCa cells with

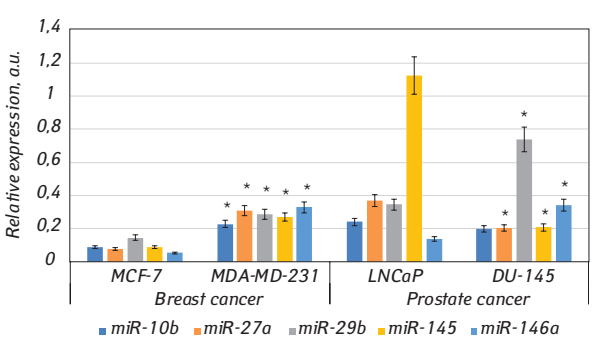

Fig. 2. Expression of miRNAs involved in the regulation of bone remodeling protein expression in BCa and PCa cells of various degrees of malignancy. *$p < 0.05$ as compared with the corresponding index in the low malignant cells ($p < 0.05$)

exogenous ON leads to increased activity of MMP-2, which indicates its possible involvement in the invasion and metastasis not only via hematogenous but also lymphogenous way [41].

Inhibition of BMP-7 expression in human BCa cells was found to reduce cell proliferation by arrest at the G_1 phase of cell cycle, and the addition of exogenous BMP-7 protected cells from apoptosis. In addition, BMP-7 also increases migratory and invasive activity of the cells [42]. Contrary to these data, Ying *et al.* [43] reported that BMP-7 is able to inhibit TGFβ1-activated genes of epithelial-mesenchymal transition in human BCa cells resulting in inhibition of their invasive activity.

In general, today there is no single view on the role of BMP-7 in the development of PCa. The inhibition of apoptosis via Smad and c-Jun NH2-terminal kinase pathways has been shown in human PCa cells cultured in the presence of exogenous BMP-7 [44]. However, another group of scientists demonstrated that exogenous BMP-7 dose-dependently inhibits beta-induced activation of Smad3/4 nuclear complexes via ALK5 and induced E-cadherin expression, which in turn may lead to the decreased invasive activity [45].

In view of the above, the role of these proteins in the acquisition of malignant phenotype in BCa and PCa cells remains unclear. Our study showed that high expression of OPN and ON is a characteristic feature of highly malignant BCa and PCa cells. It should be noted that BMP-7 expression level is high in all studied cell lines regardless whether they are high or low malignant.

An important role in the regulation of gene expression is played by microRNAs — small noncoding RNAs, which by binding to the 3′-noncoding region of mRNA of the target gene ensure its degradation [46]. Normally, miRNAs are involved in many vital processes such as proliferation, differentiation, apoptosis, and others. Numerous studies in recent years have shown that the development of malignant neoplasms is accompanied by a change in the ratio of specified miRNAs. Moreover, the same miRNA can be both oncogenic and oncosuppressive depending on its target genes, as well as the histological origin of the cells in which it is expressed. Using the miRNet v. 2.0. resource and miRTarBase v8.0, KEGG and DisGeNET databases we have analyzed predicted target genes of the studied miRNAs involved either only in BCa or PCa, or in both pathologies (Fig. 3).

Given the data of bioinformatics analysis of target genes of the studied microRNAs, as well as available literature data (Table 4), in BCa cells miRNA-10b, miRNA-27a, miRNA-29b, and miRNA-146a have oncogenic properties, which is in line with their significantly higher expression in the cells of the highly malignant phenotype. In contrast, in BCa cells miRNA-145 has oncosuppressive properties; in particular, its increased expression is associated with inhibition of cell growth and activation of apoptosis. In PCa cells, the studied miRNAs exert oncosuppressive properties, which is in keeping with the significantly lower levels of miRNA-27a and miRNA-145 in PCa cells of high malignancy phenotype. Thus, the features of the expression profile of miRNAs involved in the regulation of the expression of bone remodeling proteins may indicate their contribution to the malignancy phenotype of BCa and PCa.

In conclusion, we found that the BCa and PCa cells of high malignancy are characterized by high levels of expression of bone remodeling proteins (OPN and ON in BCa, and ON in PCa), which may suggest the deregulation of their expression at the epigenetic level. In line with this, we found significantly higher expression of miRNAs-10b, -27a, -29b, -145 and -146a in MDA-MB-231. In highly malignant DU-145 cells, significantly lower levels of expression of miRNAs-27a and -145 against the background of increasing levels of miRNAs-29b and -146a were revealed. The obtained data may create

Table 4. The main biological effects of miRNAs involved in the regulation of bone remodeling proteins in human BCa and PCa cells

	Target genes associated with BCa/or PCa	BCa	PCa
		Biological functions	
MicroRNA-10b	*CREB1, PIKDCD, CDKN1A, PTEN, AKT1, CDK2, BRCA1, TP53 PIK3CA*	Activation of cell migration, invasion and metastasis [47]	Inhibition of proliferation and invasion [48]
MicroRNA-27a	*MXI1, AKT1, APEX, PEBP1, SFRP1, EGFR*	Induction of cell migration and epithelial-mesenchymal transition [49]	Inhibition of proliferation and migration, activation of apoptosis [50]
MicroRNA-29b	*PIK3CG, AKT2, PDGFA, MDM2, AKT3, PDGFRA, PDFFRB, PDGFC, PDGFB, PTEN, ID3, CDK6, ESR1, NOTCH2, IFNG, DNMT3B, NCOA3, INSIG1, STAT3, COL5A1, DNMT3A, MKI67, ANGPTL4, FOS, DNMT1 BCL2, PIK3R1*	Activation of cell migration, invasion and metastasis, as well as avoidance of apoptosis [51]	Inhibition of proliferation and activation of apoptosis [52]
MicroRNA-145	*IRS1, PODXL, CDKN1A, NRAS, BRAF, MYC, BNIP3, HIF1A, MSH3, VEDFA, IGFR1, PDGFD, MDM2, E2F3, MUC4, SERPINE1, CDK6, CDK4, DUSP6, COL5A1, EGFR, ESR1*	Inhibition of cell growth and induction of apoptosis [53]	Activation of apoptosis [54]
MicroRNA-146a	*HSPA1A, ICAM1, TGFB1, BGLAP, NFKB1, CDK1A, PLAUR, SOS, RARB, FOS, SFRP1, NOTCH2, BRCA1, BRCA2, MIF, EGFR, PTGS2, CCND1, CXCL12, CXCL8, IL6*	Promotion of proliferation and invasion [55]	Inhibition of cell proliferation, invasion and metastasis [56]

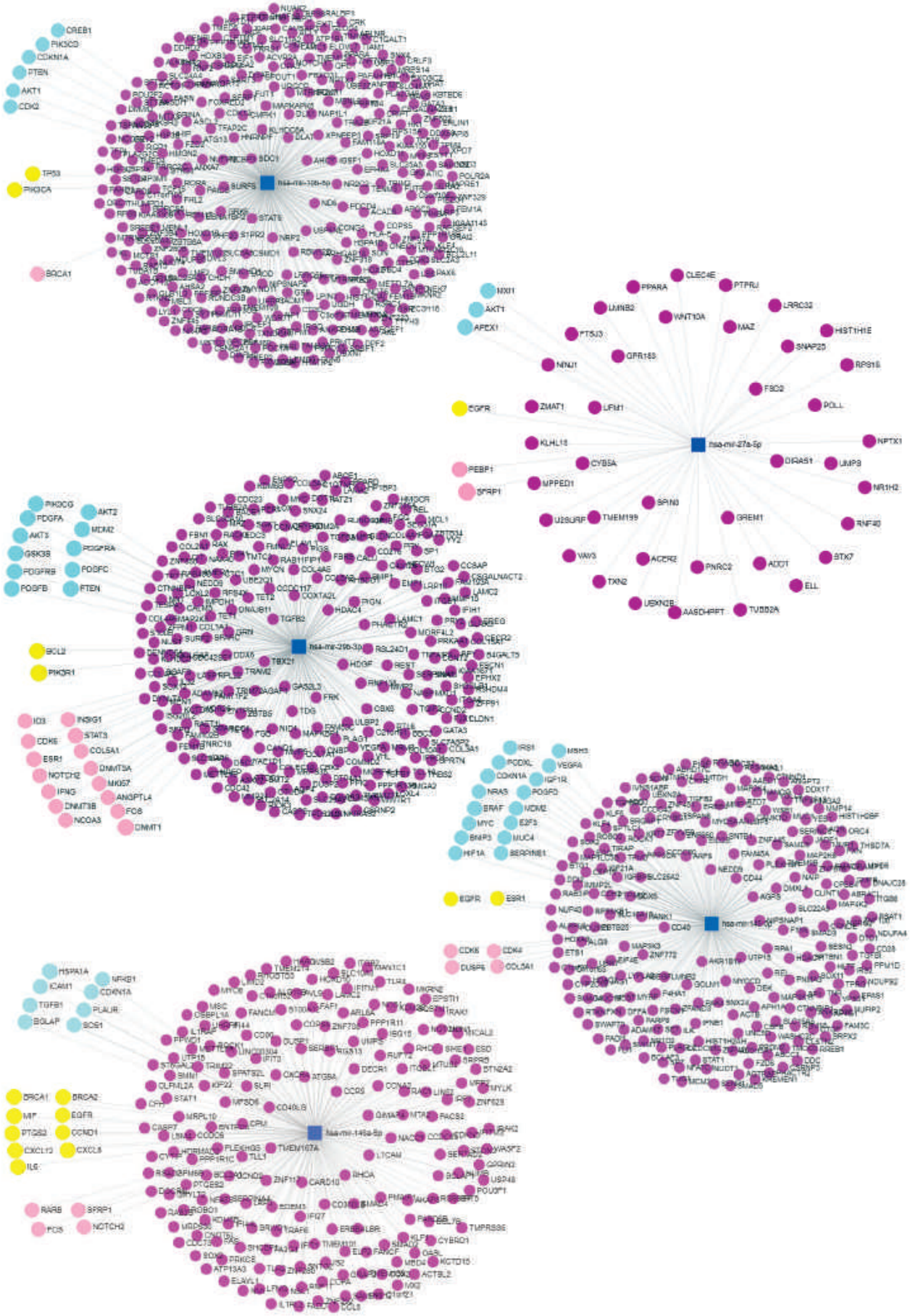

Fig. 3. Predicted target genes of the studied microRNAs involved in the regulation of OPN and ON expression. Circles indicate target genes, squares — microRNAs that regulate them. Genes whose expression disorders are characteristic of PCa are highlighted in blue, of BCa — in pink, of both — in yellow

the basis for the development of new prognostic criteria for BCa and PCa patients, which require further study in clinical setting.

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ЕКСПРЕСІЯ МАРКЕРІВ РЕМОДЕЛЮВАННЯ КІСТКОВОЇ ТКАНИНИ У КЛІТИНАХ РАКУ МОЛОЧНОЇ ЗАЛОЗИ ТА РАКУ ПЕРЕДМІХУРОВОЇ ЗАЛОЗИ *IN VITRO*

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Мета: Провести порівняльне дослідження експресії маркерів ремоделювання кісткової тканини у клітинах раку молочної залози (РМЗ) та раку передміхурової залози (РПЗ) різного ступеня злоякісності в системі *in vitro*. ***Матеріали та методи***: Дослідження проводили на клітинах РМЗ людини (лінії MCF-7 та MDA-MB-231) та клітинах РПЗ (лінії LNCaP та DU-145) різного ступеня злоякісності. Рівні експресії білків ремоделювання кісткової тканини (остеопонтин (osteopontin — OPN), остеонектин (osteonectin — ON) і кістковий морфогенетичний протеїн 7 (Bone morphogenetic protein 7 — BMP-7) визначали з використанням імуноцитохімічного аналізу. Рівні мРНК OPN (*SPP1*), ON (*SPARC*), BMP-7 (*BMP7*) та мікроРНК-10b, -27a, -29b, -145, -146a оцінювали за допомогою кількісної полімеразної ланцюгової реакції. Для пошуку мікроРНК, залучених до регуляції цільових генів, використовували ресурс miRNet v. 2.0. ***Результати***: Встановлено, що клітини високого ступеня злоякісності лінії MDA-MB-231 РМЗ людини характеризуються значно вищими рівнями експресії OPN та ON на тлі зниження експресії мРНК *SPARC* та *BMP7*. У клітинах високого ступеня злоякісності DU-145 експресія мРНК *ON, SPP1*, *SPARC* та *BMP7* була значно вищою порівняно з клітинами LNCaP. Лінія MDA-MB-231 характеризувалася значно вищою експресією мікроРНК-10b, -27a, -29b, -145 та -146a. У клітинах DU-145 зафіксовано достовірно нижчі рівні експресії мікроРНК-27a і -145 на тлі підвищення рівня мікроРНК-29b і -146a. ***Висновок:*** Встановлено, що формування високого ступеня злоякісності у клітинах РМЗ та РПЗ асоціюється з високим рівнем експресії білків ремоделювання кісткової тканини, що може бути зумовлено порушенням регуляції їх експресії на епігенетичному рівні.

Ключові слова: рак молочної залози, рак передміхурової залози, BMP-7 — кістковий морфогенетичний протеїн, остеопонтин, остеонектин.