miRNA-mediated regulation of extracellular matrix dynamics across breast cancer subtypes

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Abstract

Introduction: Interactions between extracellular signals and the extracellular matrix (ECM) influence cellular phenotype and molecular functions, affecting proliferation, differentiation, adhesion, apoptosis, and migration. Deregulation of ECM remodeling contributes to the development of diseases, including breast cancer. The study was aimed to identify microRNAs (miRNAs) that may regulate the activity of genes involved in ECM remodeling and focal adhesion across five breast cancer subtypes in Polish women.
Material and methods: The study enrolled patients representing five breast cancer subtypes: 130 luminal A, 100 HER2-negative luminal B, 96 HER2-positive luminal B, 36 non-luminal HER2-positive, 43 triple-negative breast cancer (TNBC) cases. Cancer tissue samples were collected during surgery along with healthy tissue margins (control group). The expression profiles of genes associated with ECM remodeling and focal adhesion were evaluated with mRNA microarrays and reverse transcription quantitative polymerase chain reaction (RT-qPCR). Protein expression was assessed using enzyme-linked immunosorbent assay (ELISA). miRNA detection and target prediction were performed using miRNA microarrays.
Results: Overexpression of COL1A1, FN1, ITGB1, and THBS1 may be associated with reduced levels of miR-129, miR-432, miR-124, and miR-384, respectively. Decreased COL6A6 expression may result from increased activity of miR-1246. Additionally, the study revealed increased levels of COL1A2, COMP, and SPP1 with reduced activity of RELN, across all five breast cancer subtypes.
Conclusions: This is the first study to comprehensively analyze miRNA-mediated regulation of ECM-related genes across five breast cancer subtypes

in a Polish cohort. Overexpression of COL1A1, FN1, and ITGB1 is linked to reduced levels of specific miRNAs, while decreased COL6A6 expression is associated with increased miR-1246 activity.

Key words: breast cancer, extracellular matrix, micro RNA.

Introduction

Breast cancer is the most commonly diagnosed cancer in women and the leading cause of cancer-related deaths globally [1]. According to the National Cancer Registry in Poland, breast cancer accounted for 23.55% of all cancers among women in 2022 and was the second leading cause of cancer-related deaths. Notably, it accounted for 27.6% of both cancer cases and deaths among young women (aged 20–44) [2].

Breast cancer diagnosis involves the classification based on the presence or absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) [3]. Luminal A is the most frequently diagnosed subtype, characterized by the presence of ER and PR, with HER2 being absent. It is considered non-aggressive and has a good prognosis [4]. Luminal B subtype is ER-positive and can be either HER2-negative or HER2-positive. It is associated with a worse prognosis due to its higher proliferative potential than luminal A [5]. In the case of the non-luminal HER2-positive subtype, HER2 is expressed, while ER and PR are absent on the cell surface [6]. Triple negative breast cancer (TNBC) is defined by the absence of ER, PR, and HER2, and is considered the most aggressive subtype [7].

The main part of solid tumors is composed of cancer cells and the tumor microenvironment, which includes cancer-associated fibroblasts, immune cells, adipocytes, endothelial cells, and the extracellular matrix (ECM) [8]. Interactions of extracellular signals and the ECM influence cellular phenotype and molecular functions, affecting cell proliferation, differentiation, adhesion, apoptosis, and migration [9]. These interactions are primarily mediated by integrins under both physiological and pathological conditions [10]. The ECM mainly contains glycoproteins, proteoglycans, matricellular proteins, including secreted phosphoprotein 1 (SPP1) and thrombospondin (THBS), as well as structural proteins such as collagen (COL), laminins (LAM), and tenascin (TN) [9, 11]. Remodeling of the ECM basement membrane is required for cancer cells migration into the surrounding stroma and for tumor formation [12]. Depending on the extent of ECM protein degradation, local invasion or intravasation into the vascular system may occur, leading to migration to distant sites and the formation of metastases [13].

The ECM also undergoes dynamic remodeling in breast cancer, contributing to tumor progression [14]. Type I collagen, encoded by *COL1A1* and *COL1A2*, is the most abundant and plays a major role in maintaining tissue integrity. Its accumulation stiffens the tumor stroma and facilitates invasion and metastasis [15]. Cancer-associated fibroblasts (CAFs) are known to mediate collagen remodeling, promoting cancer cell migration and progression [16].

Studies indicate that type I collagen stiffens the ECM, which causes the stroma of cancer tissue to appear stiffer than that of healthy tissue [17]. Acerbi *et al*. demonstrated that collagen deposition, linearization, and thickening are associated with the development and progression of breast cancer. They found that stromal stiffness and its heterogeneity were greater in aggressive breast cancer subtypes compared to less aggressive luminal A and B subtypes [18]. Cao *et al.* further highlighted that stiffer breast cancer tissue enhances cancer cell growth and survival, while also activating migration-related signaling pathways [19]. Interestingly, the ECM also plays a role in promoting resistance to breast cancer treatments, including endocrine-targeted therapy, chemotherapy, and radiation. Therefore, a deeper understanding of ECM structure and regulation may provide novel therapeutic targets for breast cancer [20]. Moreover, microRNAs (miRNAs) play a significant role in cancer by regulating the expression of target genes, adding another layer of complexity to tumor biology [21].

The aim of this study was to identify miRNAs that may potentially regulate the activity of genes involved in ECM remodeling and focal adhesion across five subtypes of breast cancer in Polish women.

Material and methods

Patients

The study enrolled 405 patients with various breast cancer subtypes. 130 samples were qualified as luminal A subtype, 100 samples as HER2-negative luminal B, 96 samples as HER2-positive luminal B, 36 samples as non-luminal HER2-positive, 43 samples as triple-negative breast cancer (TNBC). The control group consisted of samples of healthy tissue margins collected during the surgery. Tissue samples were differentiated into neoplastic and non-neoplastic by pathological evaluation. All patients in the study were classified as T1N0M0. Patient characteristics are listed in Table I.

Total ribonucleic acid (RNA) extraction

TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA; cat. no. 15596026) was used

Table I. Characteristics of patients included in the study

Subtype	Grade			Age		BMI [kg/m^2]
	G1	G2	G3	< 50 years	> 50 years	
Luminal A	23 (18%)	48 (37%)	59 (45%)	43 (33%)	87 (67%)	30.78 ±2.76
HER2-negative luminal B	31 (31%)	57 (57%)	12 (12%)	32 (32%)	68 (68%)	30.18 ±4.56
HER2-positive luminal B	23 (24%)	57 (59%)	16 (17%)	19 (20%)	77 (80%)	32.09 ±6.19
Non-luminal HER2-positive	9 (25%)	12 (33%)	15 (42%)	9 (25%)	27 (75%)	33.18 ±5.67
TNBC	14 (32%)	21 (49%)	8 (19%)	10 (23%)	33 (77%)	34.67 ±2.98

HER2 – human epidermal growth factor receptor 2, TNBC – triple-negative breast cancer, BMI – body mass index

to obtain total RNA. The RNeasy mini kit (QIAGEN, Hilden, Germany; cat. no. 74104) and DNase I (Fermentas International Inc., Burlington, ON, Canada; cat. no. 18047019) were used to purify the obtained extracts. To evaluate the quality and quantity of the extracts, electrophoresis was performed in a 1% agarose gel and absorbance was measured.

mRNA microarrays

Microarray analysis was performed using the HG-U133A 2_0 arrays (Affymetrix, Santa Clara, CA, USA) and the GeneChip™ 3′IVT PLUS kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA; cat. no. 902416). The Kyoto encyclopedia of genes and genomes (KEGG) pathway map was used to generate a gene list. From ECM-receptor interaction (hsa04512) and focal adhesion (hsa04510), overlapping genes were selected. A list of 65 genes was obtained, which corresponded to 187 mRNAs on the microarray.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

The next step was to validate the determined expression profile using RT-qPCR with the SensiFast SYBR No-ROX One-Step Kit (Bioline, London, UK). Nine genes whose activity significantly changed in each breast cancer subtype were selected for analysis: collagen type I alpha 1 (*COL1A1*), collagen type I alpha 2 (*COL1A2*), collagen type VI alpha 6 (*COL6A6*), cartilage oligomeric matrix protein (*COMP*), fibronectin 1 (*FN1*), integrin beta-1 (*ITGB1*), reelin (*RELN*), secreted phosphoprotein 1 (*SPP1*), throm-

Table II. RT-qPCR primers

mRNA	RT-qPCR primers (5′-3′)	Melting temperature [°C]
COL1A1	Forward: GAGGGCCAAGACGAAGACATC	58
	Reverse: CAGATCACGTCATCGCACAAC	58
COL1A2	Forward: CCTGGTGCTAAAGGAGAAAGAGG	59
	Reverse: ATCACCACGACTTCCAGCAGGA	60
COL6A6	Forward: GGATCGTTCGCAACATCTGTACC	59
	Reverse: GCTGACCTTCAAGCAAAGTCTGC	61
COMP	Forward: GGAGATGCTTGTGACAGCGATC	60
	Reverse: TGAGTCCTCCTGGGCACTGTTA	59
FN1	Forward: ACAACACCGAGGTGACTGAGAC	58
	Reverse: GGACACAACGATGCTTCCTGAG	59
ITGB1	Forward: GGATTCTCCAGAAGGTGGTTTCG	58
	Reverse: TGCCACCAAGTTTCCCATCTCC	58
RELN	Forward: GTCTACCTTCCACTCTCCACCA	57
	Reverse: GTCCAGCATCACAAATCCCTCG	59
SPP1	Forward: CGAGGTGATAGTGTGGTTTATGG	57
	Reverse: GCACCATTCAACTCCTCGCTTTC	60
THBS1	Forward: GCTGGAAATGTGGTGCTTGTCC	59
	Reverse: CTCCATTGTGGTTGAAGCAGGC	59
ACTB	Forward: TCACCCACACTGTGCCCATCTACGA	63
	Reverse: CAGCGGAACCGCTCATTGCCAATGG	66

COL1A1 – collagen type I alpha 1, COL1A2 – collagen type I alpha 2, COL6A6 – collagen type VI alpha 6, COMP – cartilage oligomeric matrix protein, FN1 – fibronectin 1, ITGB1 – integrin beta-1, RELN – reelin, SPP1 – secreted phosphoprotein 1, THBS1 – thrombospondin 1, ACTB – β-actin.

bospondin 1 (*THBS1*) (Table II). Calculations were performed with the $2^{-\Delta\Delta Ct}$ method. β-actin (*ACTB*) was used as an endogenous control [22].

Enzyme-linked immunosorbent assay (ELISA)

Protein expression profile was assessed with ELISA (Abbexa, Cambridge, UK) using the following kits: COL1A1 kit (MyBioSource, Inc., San Diego, CA, USA; cat. no. MBS703198), COL1A2 kit (MyBioSource, Inc., San Diego, CA, USA; cat. no. MBS036858), COL6A6 kit (MyBioSource, Inc., San Diego, CA, USA; cat. no. MBS7269131), COMP kit (MyBioSource, Inc., San Diego, CA, USA; cat. no. MBS765927), FN1 kit (MyBioSource, Inc., San Diego, CA, USA; cat. no. MBS765285), ITGB1 kit (MyBioSource, Inc., San Diego, CA, USA; cat. no. MBS761086), RELN kit (MyBioSource, Inc., San Diego, CA, USA; cat. no. MBS904954), SPP1 kit (MyBioSource, Inc., San Diego, CA, USA; cat. no. MBS2880407), and THBS1 kit (MyBioSource, Inc., San Diego, CA, USA; cat. no. MBS701627).

miRNA profiling and prediction

Microarrays miRNA 2.0 (Affymetrix, Santa Clara, CA, USA), FlashTag Biotin HSR RNA Labeling Kit (Affymetrix, Santa Clara, CA, USA), Hybridization Wash and Stain Kit (Affymetrix, Santa Clara, CA, USA) were used to identify miRNAs differentiating breast cancer from the control. The miRDB tool (http://mirdb.org) was used to predict which miRNAs could regulate the expression of *COL1A1*, *COL1A2*, *COL6A6*, *COMP*, *FN1*, *ITGB1*, *RELN*, *SPP1*, and *THBS1*. To increase the reliability of predictions, a target score of ≥ 80 was set [23].

Statistical analysis

The results of microarray experiments were analyzed using Transcriptome Analysis Console (Thermo Fisher Scientific, Waltham, MA, USA). One-way analysis of variance (ANOVA) and Tukey's post hoc test were carried out ($p < 0.05$; FC > 2 or FC < -2). The RT-qPCR and ELISA results were analyzed using Statistica 13.3 (StatSoft, Krakow, Poland). Data distribution was evaluated using the Shapiro-Wilk test. The lack of normal distribution allowed the use of Kruskal-Wallis and Dunn's tests.

Based on approximately 19,620 women diagnosed with breast cancer in Poland in 2019 [24], assuming a 95% confidence level and a 5% margin of error, the recommended group size was 377, as calculated using the sampling calculator [25].

Overall survival (OS) analysis for each breast cancer subtype was estimated using the Kaplan-Meier plotter (http://kmplot.com/; accessed: June 27, 2024) [26, 27]. The follow-up threshold was 60 months.

Results

Gene expression profile assessed with mRNA microarrays

Among 187 mRNAs corresponding to 65 genes associated with the ECM remodeling and focal adhesion, one-way ANOVA revealed that 37 mRNAs significantly changed their expression in breast cancer compared to the control ($p < 0.05$; FC > 2 or < - 2). Tukey's post-hoc test showed that 14 mRNAs significantly changed their expression in luminal A subtype, 20 mRNAs in HER2-negative luminal B subtype, 22 mRNAs in HER2-positive luminal B subtype, 32 mRNAs in non-luminal HER2-positive subtype, 34 mRNAs in TNBC. Figure 1 shows a Venn diagram of characteristic and common genes.

The analysis showed that overexpression of *LAMA4* and *THBS2* significantly differentiated TNBC from the control. For the remaining subtypes, no characteristic genes meeting the conditions adopted in the study were recorded. *IBSP* overexpression was observed in luminal A and B subtypes. In addition, high levels of *LAMC2* with reduced activity of *ITGA7*, *TNXA*, *TNXB* were observed in all subtypes except luminal A. Interestingly, significant overexpression was also noted for *VWF* in HER2-positive luminal B, non-luminal HER2-positive and TNBC. High levels of *COL6A1*, *COL6A2*, *COL6A3*, *ITGB4*, *ITGB6*, *LAMB1*, *TNC* common to non-luminal HER2-positive and TNBC were also reported. Furthermore, 9 genes significantly changed expression regardless of breast cancer subtype: *COL1A1*, *COL1A2*, *COL6A6*, *COMP*, *FN1*, *ITGB1*, *RELN*, *SPP1*, *THBS1* (Table III).

Regardless of the cancer subtype, significant overexpression of *COL1A1*, *COL1A2*, *COMP*, *FN1*, *ITGB1*, *SPP1*, *THBS1* with reduced activity of *COL6A6* and *RELN* was reported in the study.

COL1A1, COL1A2, COL6A6, COMP, FN1, ITGB1, RELN, SPP1, THBS1 expression evaluated with RT-qPCR and ELISA

RT-qPCR was then used to assess the expression profile of *COL1A1*, *COL1A2*, *COL6A6*, *COMP*, *FN1*, *ITGB1*, *RELN*, *SPP1*, and *THBS1* differentiating breast cancer regardless of its subtype (Figure 2).

The expression profile determined by RT-qPCR corresponded to the microarray results. The next step included assessing the concentration of the studied genes at the protein level (Table IV).

The level of COL1A1, COL1A2, COMP, FN1, ITGB1, SPP1, and THBS1 proteins was significantly increased in all breast cancer subtypes compared to the control group, which was consistent with the microarray and RT-qPCR analysis. COL6A6 protein reached a level below detection in all cancer samples, whereas the protein concentration of RELN was also decreased and possible to detect only in luminal A cancer.

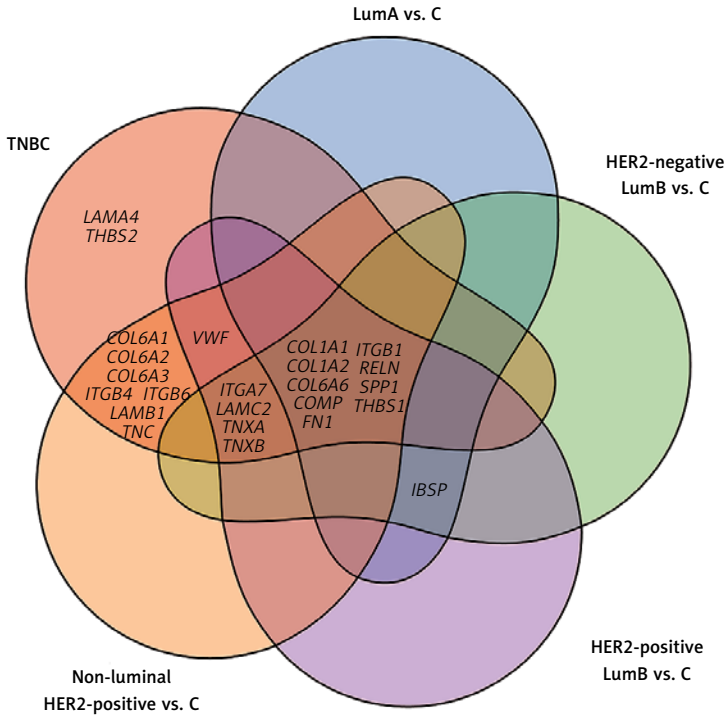

Figure 1. Venn diagram showing differentially expressed mRNAs ($p < 0.05$; FC > 2 or < -2) associated with ECM remodeling and focal adhesion across five breast cancer subtypes compared to control samples determined using mRNA microarray

LumA – luminal A, LumB – luminal B, HER2 – human epidermal growth factor receptor 2, TNBC – triple-negative breast cancer, C – control, COL1A1 – collagen type I alpha 1, COL1A2 – collagen type I alpha 2, COL6A1 – collagen type VI alpha 1, COL6A2 – collagen type VI alpha 2, COL6A3 – collagen type VI alpha 3, COL6A6 – collagen type VI alpha 6, COMP – cartilage oligomeric matrix protein, FN1 – fibronectin 1, IBSP – integrin binding sialoprotein, ITGA7 – integrin alpha-7, ITGB1 – integrin beta-1, ITGB4 – integrin beta-4, ITGB6 – integrin beta-6, LAMB1 – laminin subunit beta 1, LAMC2 – laminin subunit gamma 2, RELN – reelin, SPP1 – secreted phosphoprotein 1, THBS1 – thrombospondin 1, TNC – tenascin c, TNXA – tenascin XA, tenascin XB, VWF – von Willebrand factor.

Table III. List of mRNAs representing ECM remodeling and focal adhesion-related genes differentiating breast cancer from the control group regardless of its subtype determined with RT-qPCR ($p < 0.05$; FC > 2 or <-2)

ID	mRNA	Fold change				
		LumA vs. C	HER2- negative LumB vs. C	HER2- positive LumB vs. C	HER2-positive vs. C	TNBC vs. C
202310_s_at	*COL1A1*	3.41	3.84	5.11	6.9	7.51
202311_s_at		3.77	4.12	5.38	5.68	6.56
217430_x_at		2.98	3.55	4.89	5.17	6.11
202404_s_at	*COL1A2*	3.83	4.06	5.09	5.63	7.74
230867_at	*COL6A6*	–4.67	–6.06	–6.35	–7.43	–8.72
205713_s_at	*COMP*	2.23	2.88	3.06	5.5	8.39
1558199_at	*FN1*	2.59	3.11	4.01	4.96	6.68
210495_x_at		3.08	4.58	5.6	7.1	8.73
211719_x_at		3.1	4.76	6.08	7.01	7.62
212464_s_at		3.26	4.01	5.31	6.17	6.79
1553678_a_at	*ITGB1*	5.71	6.33	6.78	9.12	10.73
205923_at	*RELN*	–2.47	–3.14	–4.04	–4.99	–5.11
209875_s_at	*SPP1*	3.99	5.09	6.43	7.67	9.11
201108_s_at	*THBS1*	2.84	3.51	4.23	5.93	7.08
201109_s_at		2.55	3.13	4.02	5.1	6.71
201110_s_at		3.64	4.96	5.27	5.86	6.24

ID – number of the probe, LumA – luminal A, LumB – luminal B, HER2 – human epidermal growth factor receptor 2, TNBC – triple-negative breast cancer, C – control, COL1A1 – collagen type I alpha 1, COL1A2 – collagen type I alpha 2, COL6A6 – collagen type VI alpha 6, COMP – cartilage oligomeric matrix protein, FN1 – fibronectin 1, ITGB1 – integrin beta-1, RELN – reelin, SPP1 – secreted phosphoprotein 1, THBS1 – thrombospondin 1.

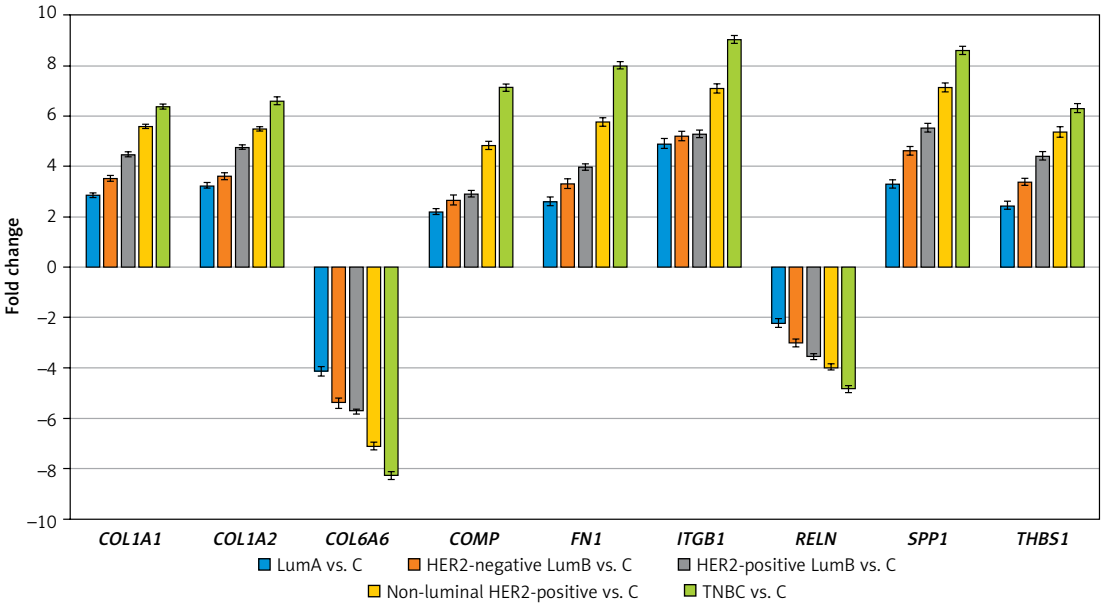

Figure 2. Expression profile of *COL1A1*, *COL1A2*, *COL6A6*, *COMP*, *FN1*, *ITGB1*, *RELN*, *SPP1*, and *THBS1* determined by RT-qPCR. Data is presented as mean ± standard deviation

LumA – luminal A, LumB – luminal B, HER2 – human epidermal growth factor receptor 2, TNBC – triple-negative breast cancer, C – control, COL1A1 – collagen type I alpha 1, COL1A2 – collagen type I alpha 2, COL6A6 – collagen type VI alpha 6, COMP – cartilage oligomeric matrix protein, FN1 – fibronectin 1, ITGB1 – integrin beta-1, RELN – reelin, SPP1 – secreted phosphoprotein 1, THBS1 – thrombospondin 1.

Table IV. Concentration of COL1A1, COL1A2, COL6A6, COMP, FN1, ITGB1, RELN, SPP1, THBS1 proteins in breast cancer subtypes and the control group determined by ELISA ($p < 0.05$)

Protein [ng/ml]	Control	LumA	HER2-negative LumB	HER2-positive LumB	HER2-positive	TNBC
COL1A1	3.7 ±0.31	7.91 ±0.19*	11.98 ±0.27*	14.42 ±0.27*	19.38 ±0.34*	24.33 ±0.31*
COL1A2	7.19 ±0.19	17.41 ±0.17*	21.04 ±0.21*	25.32 ±0.26*	31.49 ±0.34*	35.49 ±0.23*
COL6A6	2.05 ±0.2	Below detection threshold*	Below detection threshold*	Below detection threshold*	Below detection threshold*	Below detection threshold*
COMP	3.08 ±0.15	6.61 ± 0.2*	6.86 ±0.19*	7.14 ±0.18*	10.71 ±0.25*	16.64 ±0.16*
FN1	6.23 ±0.13	10.93 ±0.22*	11.19 ±0.24*	12.01 ±0.19*	16.2 ±0.24*	25.19 ±0.19*
ITGB1	0.07 ±0.01	0.21 ±0.01*	0.26 ±0.01*	0.27 ± 0.01*	0.4 ±0.01*	0.52 ±0.01*
RELN	1.52 ±0.11	0.65 ±0.1*	Below detection threshold*	Below detection threshold*	Below detection threshold*	Below detection threshold*
SPP1	0.84 ±0.08	1.87 ±0.09*	3.01 ±0.18*	3.43 ±0.17*	4.44 ±0.19*	6.13 ±0.17*
THBS1	7.42 ±0.18	12.12 ±0.13*	16.61 ±0.19*	18.64 ±0.16*	24.04 ±0.16*	27.82 ±0.21*

*LumA – luminal A, LumB – luminal B, HER2 – human epidermal growth factor receptor 2, TNBC – triple-negative breast cancer, C – control, COL1A1 – collagen type I alpha 1, COL1A2 – collagen type I alpha 2, COL6A6 – collagen type VI alpha 6, COMP – cartilage oligomeric matrix protein, FN1 – fibronectin 1, ITGB1 – integrin beta-1, RELN – reelin, SPP1 – secreted phosphoprotein 1, THBS1 – thrombospondin 1. * p < 0.05 vs. control.*

Table V. Expression of miRNAs determined by miRNA microarray ($p < 0.05$; FC > 2 or < –2) and their potential mRNA targets identified using mirDB tool (target score ≥ 80)

mRNA	miRNA	Target score	Fold change				
			LumA vs. C	HER2-negative LumB vs. C	HER2-positive LumB vs. C	HER2-positive vs. C	TNBC vs. C
COL1A1	miR-129	90	–2.04	–2.22	–2.25	–2.98	–3.31
COL6A6	miR-1246	87	2.11	2.48	2.56	3.44	6.79
FN1	miR-432	82	–2.02	–2.18	–2.63	–2.82	–3.13
ITGB1	miR-124	100	–2.36	–2.73	–2.71	–3.66	–4.14
THBS1	miR-384	86	–2.19	–2.62	–3.03	–3.87	–4.55

LumA – luminal A, LumB – luminal B, HER2 – human epidermal growth factor receptor 2, TNBC – triple-negative breast cancer, C – control, COL1A1 – collagen type I alpha 1, COL6A6 – collagen type VI alpha 6, FN1 – fibronectin 1, ITGB1 – integrin beta-1, THBS1 – thrombospondin 1.

miRNA target prediction

The last step of the study was to verify whether miRNAs differentiating breast cancer from the control could be involved in the regulation of the expression of *COL1A1*, *COL1A2*, *COL6A6*, *COMP*, *FN1*, *ITGB1*, *RELN*, *SPP1*, and *THBS1* (Table V).

The analysis showed that the expression of *COL1A2*, *COMP*, *RELN*, and *SPP1* is probably not regulated by miRNAs identified by microarrays and prediction criteria. Overexpression of *COL1A1*, *FN1*, *ITGB1*, and *THBS1* may be associated with reduced levels of miR-129, miR-432, miR-124, and miR-384, respectively. The analysis also showed that decreased *COL6A6* expression may be a consequence of increased activity of miR-1246.

Overall survival (OS) analysis

Overall survival analysis was carried out for 9 genes selected in the study: of *COL1A1*, *COL1A2*, *COL6A6*, *COMP*, *FN1*, *ITGB1*, *RELN*, *SPP1*, and *THBS1*. For each cancer subtype, only graphs with $p < 0.05$ were presented (Figure 3–7).

In luminal A cancer, worse survival was associated with downregulated *COL1A1*, *COL6A6*, and overexpression of FN1 (Figure 3). In HER2-negative luminal B cancer, reduced levels of *COL6A6* and *SPP1* negatively impacted overall survival (Figure 4). In HER2-positive luminal B cancer, decreased *COL1A2* activity and overexpressed *ITGB1* were associated with worse prognosis (Figure 5). The analysis also revealed a negative impact of *COL1A2*, *COMP*, *ITGB1*, and *RELN* overexpression on overall survival in non-luminal HER2-positive cancer (Figure 6). In TNBC, a high level of *COMP* was linked to worse overall survival. In addition, decreased activity of *SPP1* may also negatively affect the prognosis, although caution should be exercised due to the p-value close to 0.05 (Figure 7).

Discussion

In our study, we analyzed the expression levels of ECM remodeling and focal adhesion-related genes across 5 molecular subtypes of breast cancer: luminal A, HER2-negative luminal B,

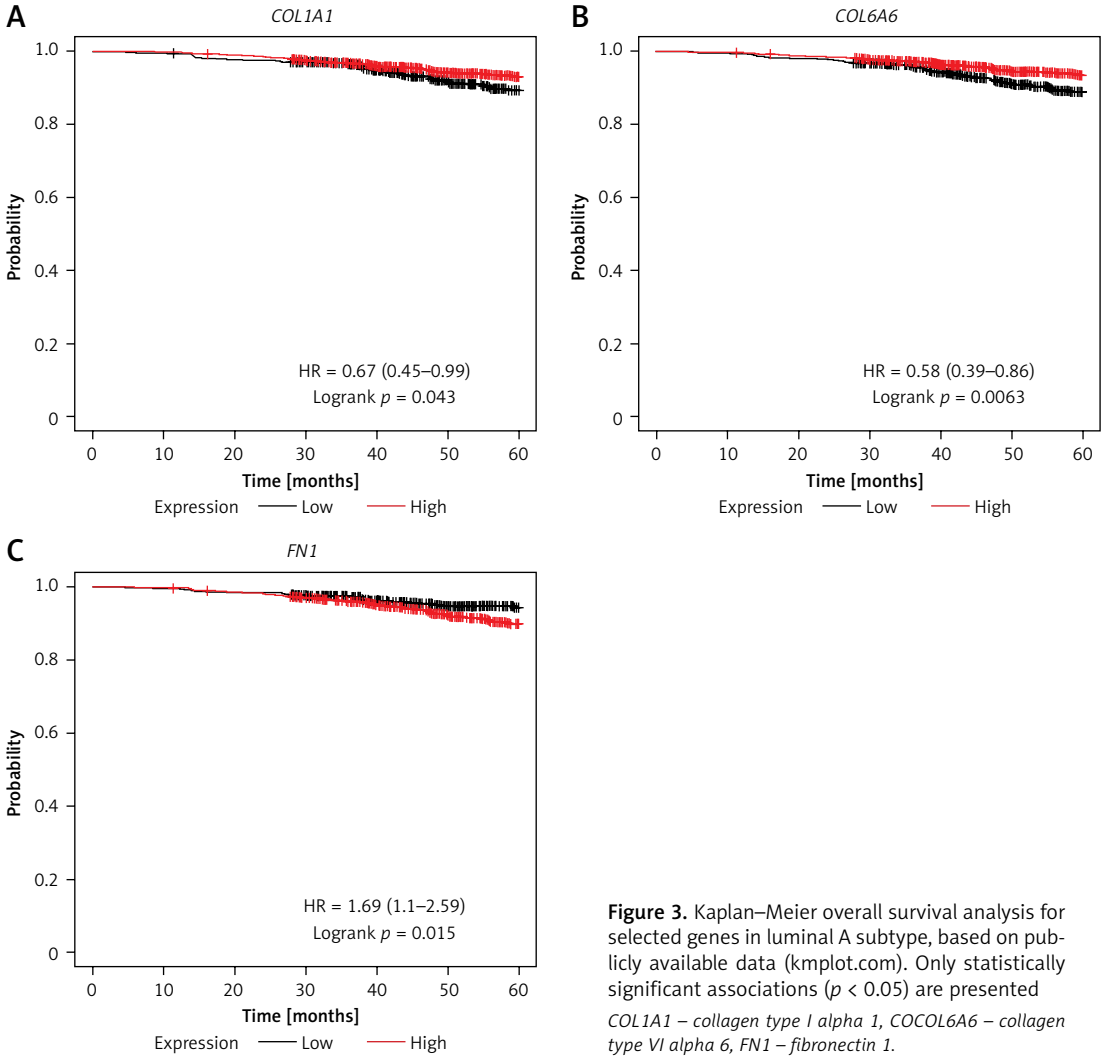

Figure 3. Kaplan–Meier overall survival analysis for selected genes in luminal A subtype, based on publicly available data (kmplot.com). Only statistically significant associations ($p < 0.05$) are presented

COL1A1 – collagen type I alpha 1, COCOL6A6 – collagen type VI alpha 6, FN1 – fibronectin 1.

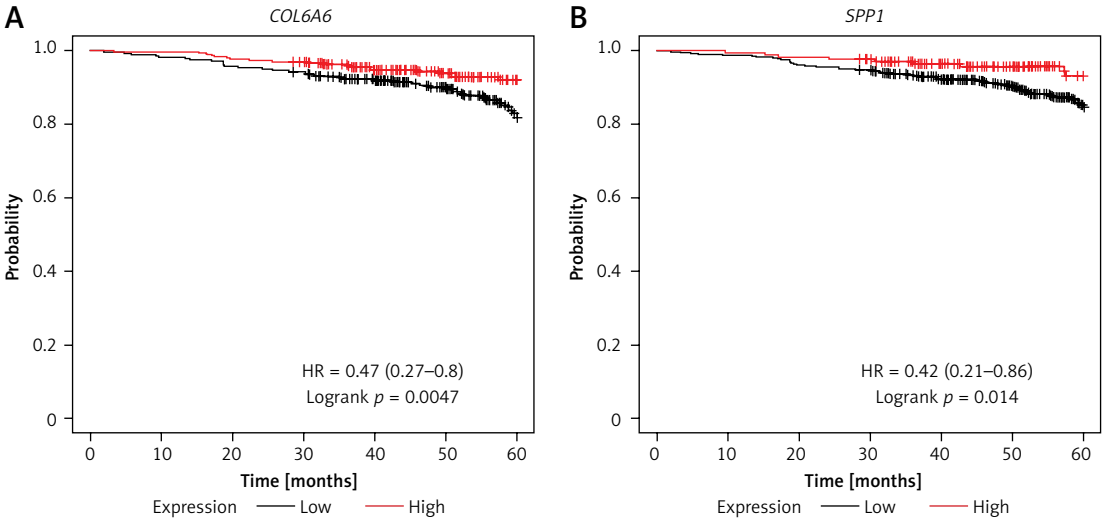

Figure 4. Kaplan–Meier overall survival analysis for selected genes in HER2-negative luminal B subtype, based on publicly available data (kmplot.com). Only statistically significant associations ($p < 0.05$) are presented

COL6A6 – collagen type VI alpha 6, SPP1 – secreted phosphoprotein 1.

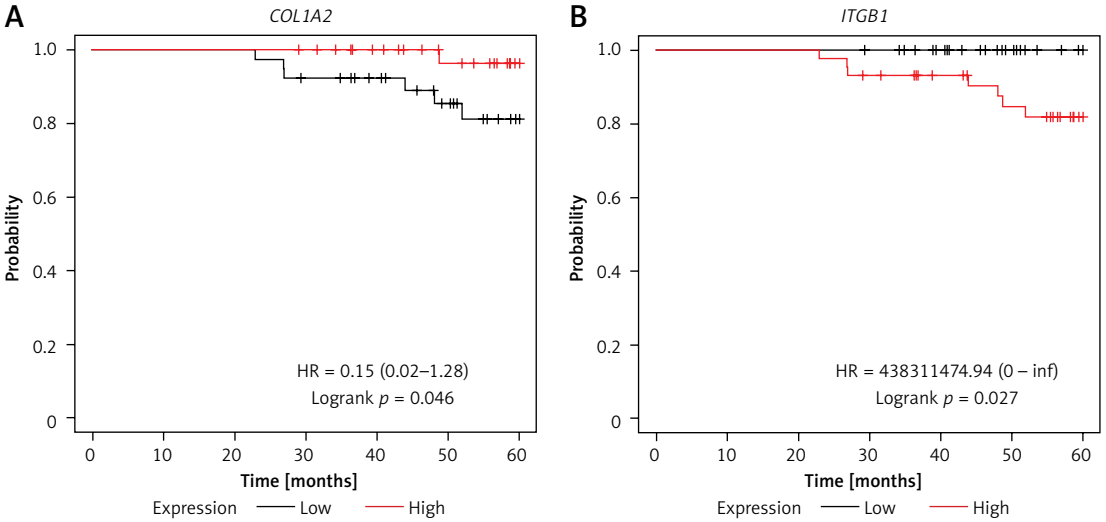

Figure 5. Kaplan–Meier overall survival analysis for selected genes in HER2-positive luminal B subtype, based on publicly available data (kmplot.com). Only statistically significant associations ($p < 0.05$) are presented

COL1A2 – collagen type I alpha 2, ITGB1 – integrin beta-1.

HER2-positive luminal B, non-luminal HER2-positive, and TNBC. We identified 9 genes that were consistently dysregulated regardless of the subtype. These findings were confirmed at both the mRNA and protein levels. Overexpression was observed for *COL1A1*, *COL1A2*, *COMP*, *FN1*, *ITGB1*, *SPP1*, and *THBS1*, while *COL6A6* and *RELN* were downregulated. Further miRNA target predictions revealed potential regulators contributing to these changes.

Recent studies have highlighted the diagnostic and prognostic relevance of miRNA–mRNA interactions in breast cancer. For instance, Kong *et al.* demonstrated that circPLK1 promotes TNBC progression by sponging miR-296-5p, thereby reducing its tumor-suppressive activity [28]. Similarly, Wu *et al.* identified a novel circKIF4A–miR-637–*STAT3* axis that facilitates brain metastasis in TNBC, emphasizing the clinical importance of miRNA-mediated regulatory networks [29]. Lu *et al.* further reported that miR-214-3p suppresses breast cancer cell proliferation and improves the tumor immune microenvironment by downregulating *B7H3*, revealing its dual role as a tumor suppressor and immune modulator [30]. These findings emphasize the emerging importance of noncoding RNA networks in breast cancer progression and treatment resistance. While earlier studies have primarily investigated individual miRNA–mRNA pairs, our integrated analysis across transcriptomic, proteomic, and predictive miRNA layers offers a broader, subtype-specific perspective on ECM remodeling in breast cancer.

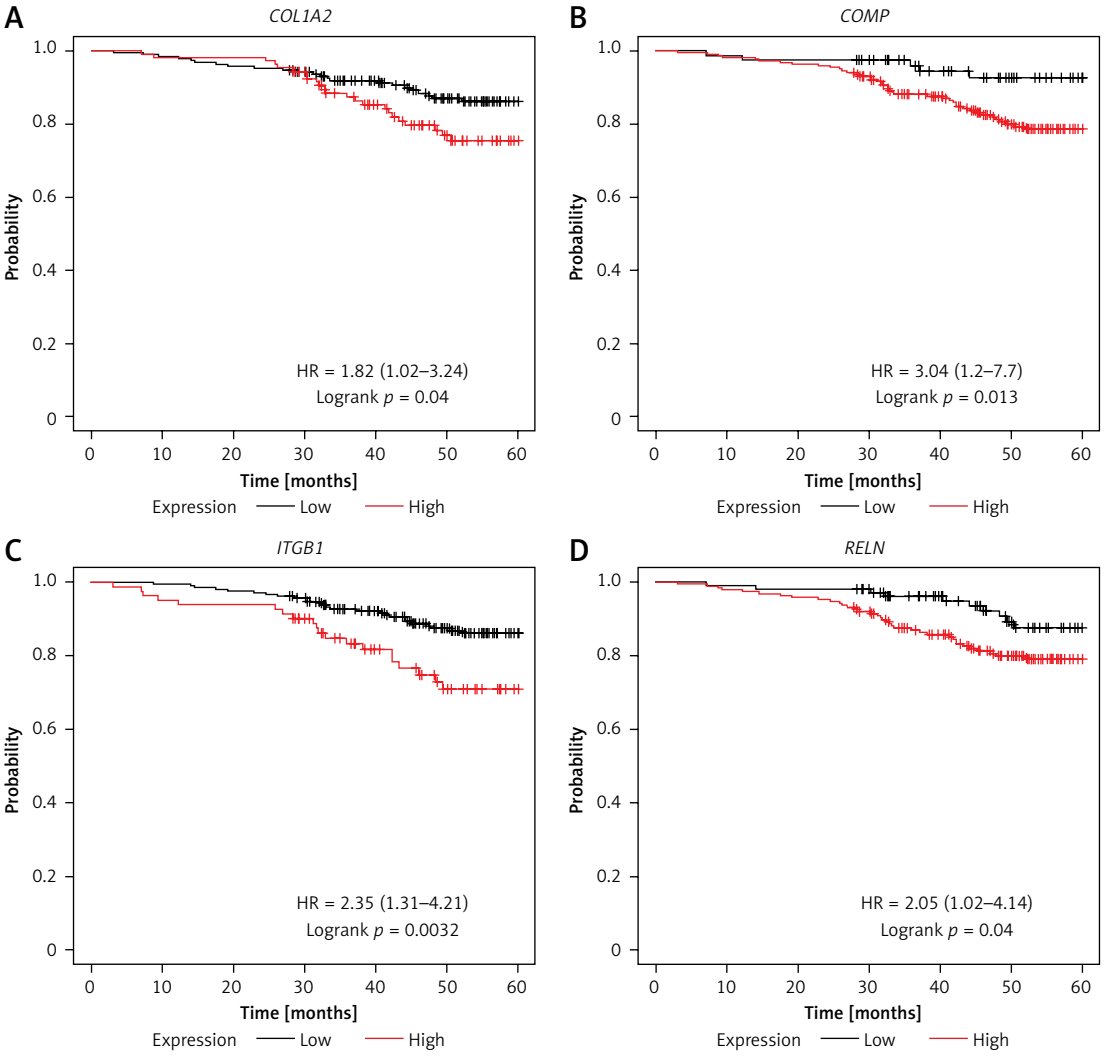

Figure 6. Kaplan–Meier overall survival analysis for selected genes in non-luminal HER2-positive subtype, based on publicly available data (kmplot.com). Only statistically significant associations ($p < 0.05$) are presented

COL1A2 – collagen type I alpha 2, COMP – cartilage oligomeric matrix protein, ITGB1 – integrin beta-1, RELN – reelin.

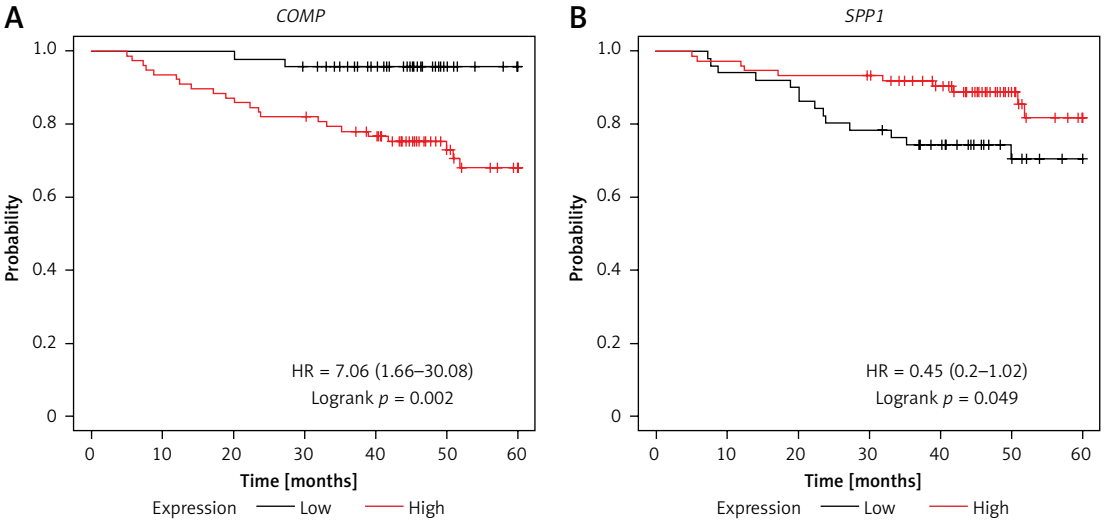

Figure 7. Kaplan–Meier overall survival analysis for selected genes in triple-negative breast cancer, based on publicly available data (kmplot.com). Only statistically significant associations ($p < 0.05$) are presented

COMP – cartilage oligomeric matrix protein, SPP1 – secreted phosphoprotein 1.

COL1A1 and *COL1A2*, which encode the main chains of type I collagen, were consistently overexpressed across all subtypes in our study. Type I collagen accumulation, largely mediated by CAFs, increases ECM stiffness, thereby promoting cancer cell invasion and metastasis [16]. Liu *et al.* reported elevated *COL1A1* levels in breast cancer, particularly in ER-positive tumors, and linked this to poor survival outcomes [31]. Ma *et al.* demonstrated that downregulation of *COL1A1* inhibits tumor growth by inhibiting CAFs activation and ECM remodeling in the tumor microenvironment [32]. Yang *et al.* reported elevated *COL1A2* expression across all breast cancer subtypes and linked it to poor overall survival and relapse-free survival in HER2-positive patients [33]. Our results support these findings and further demonstrate that *COL1A2* overexpression is significantly associated with reduced overall survival in non-luminal HER2-positive patients. In silico analysis also suggests that downregulation of miR-129 may contribute to the observed *COL1A1* upregulation, while no miRNAs meeting our criteria were identified as regulators of *COL1A2*. Previous studies confirm reduced miR-129 levels in breast cancer and its tumor-suppressive role, including the inhibition of proliferation and chemoresistance [34–37]. However, Setijono *et al.* described miR-129 overexpression in TNBC, proposing a possible context-dependent oncogenic function [38].

We also observed downregulation of *COL6A6* and *RELN* in all breast cancer subtypes. Low *COL6A6* has been associated with reduced overall survival in luminal A and HER2-negative luminal B cancers. Known as a tumor suppressor, *COL6A6* has been reported to inhibit growth and metastasis in other cancer types [39, 40], and its loss has been described in breast cancer cohorts [41, 42]. Yeh *et al.* identified both *COL6A6* and *RELN* as downregulated in breast cancer [42], consistent with our observations across all subtypes. Our analysis showed that miR-1246 overexpression may contribute to *COL6A6* downregulation. Multiple studies highlight miR-1246 as a biomarker of breast cancer [43] and a promoter of a drug resistance and invasiveness [44], though conflicting evidence suggests a suppressive role in TNBC metastasis via epithelial-mesenchymal transition (EMT) [45]. Notably, miR-1246 derived from TNBC exosomes can activate stromal fibroblasts, enhancing invasion and migration [46].

COMP and *SPP1* were overexpressed across all subtypes and have been linked to breast cancer aggressiveness. Hanitrarimalala *et al.* showed that *COMP*, a member of the thrombospondin family, has been implicated in promoting breast cancer stemness and invasiveness [47]. It is considered a potential predictive marker of metastasis, particularly in ER+ and HER2-positive tumors [48, 49]. In our cohort, high *COMP* expression was associated with reduced survival in non-luminal HER2-positive and TNBC patients. *SPP1* is similarly implicated in invasion and metastasis [50, 51]. Interestingly, reduced *SPP1* levels were associated with poorer survival in HER2-negative luminal B and marginally in TNBC, suggesting a dual role that may depend on tumor context. Our findings extend previous reports by demonstrating that *COMP* overexpression is not only relevant in ER+ or HER2+ tumors but also carries prognostic significance in TNBC. Neither gene was predicted to be regulated by the miRNAs selected in our analysis.

miRNA predictions for *FN1*, *ITGB1*, and *THBS1* suggest that their overexpression may result from the downregulation of miR-432, miR-124, miR-384, respectively. *FN1* is involved in host defense, adhesion, proliferation, wound healing, and metastasis [52], with elevated expression linked to poor prognosis [53]. Notably, *FN1* correlates with immune cell infiltration and immune checkpoint activity, offering potential for immunotherapy stratification [54]. LINC02381 and miR-1271 interactions [55], as well as miR-200b suppression in doxorubicin-resistant breast cancer cells [56], have been implicated in *FN* regulation. In our analysis, we found a potential link between *FN1* and miR-432, whose low activity promotes proliferation, invasion, and migration of breast cancer cells [57].

ITGB1, which connects ECM to the cytoskeleton and mediates signal transduction, plays a central role in metastasis and therapy resistance [58, 59]. Rana *et al.* identified WAVE2/miR-29/*ITGB1* signaling axis, critical for tumor growth regulation and metastasis in TNBC. It was also confirmed that increased *ITGB1* expression is associated with worse survival [60]. Our analysis showed that *ITGB1* overexpression negatively affected overall survival in luminal and non-luminal HER2-positive subtypes. We also found potential regulation by miR-124, whose loss has been widely linked to increased proliferation and chemoresistance [61–63].

THBS1 presents a dual role in cancer. While it inhibits angiogenesis and may suppress tumor growth, its expression in stromal cells may facilitate metastasis [64]. Li *et al.* reported that overexpression of serum *THBS1* in HER2-positive patients was linked to brain metastases [65]. Furthermore, *THBS1* inhibition could interfere with metastasis and improve the efficacy of TNBC immunotherapy [66]. Our findings suggest that miR-384 downregulation may contribute to *THBS1* overexpression. Low miR-384 levels promote the proliferation and invasion of breast cancer cells [67, 68].

Taken together, our findings highlight a consistent ECM-related signature across all breast cancer subtypes, with subtype-specific survival

associations and potential miRNA regulators. In summary, our findings complement and expand the existing literature by identifying both shared and subtype-specific ECM gene dysregulation patterns, linking them with predicted miRNA regulators and clinical outcomes. These insights may support the development of personalized therapeutic strategies, particularly for aggressive or treatment-resistant subtypes.

The limitation of our study may be the different group sizes, with the smallest number of non-luminal HER-positive and TNBC patients. The material collected for the study comes from Polish women, which may potentially reduce the applicability of the presented results.

In conclusion, this study comprehensively characterized the expression of ECM remodeling and focal adhesion-related genes across five molecular subtypes of breast cancer in Polish patients. Nine genes (*COL1A1*, *COL1A2*, *COMP*, *FN1*, *ITGB1*, *SPP1*, *THBS1*, *COL6A6*, *RELN*) were consistently dysregulated regardless of subtype, with expression patterns correlating with patient survival. Bioinformatic miRNA prediction identified five miRNAs (miR-129, miR-1246, miR-432, miR-124, miR-384) potentially responsible for modulating these genes.

The observed changes highlight a common ECM-related signature in breast cancer that intensifies with tumor aggressiveness. These findings provide new insights into the molecular landscape of breast cancer and suggest that targeting ECM–miRNA interactions could offer novel therapeutic opportunities, particularly in subtypes characterized by poor prognosis or treatment resistance.

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Ethical approval

Ethical considerations were paramount throughout this study, adhering to the principles outlined in the 2013 Declaration of Helsinki concerning human experimentation. Approval was obtained from the Bioethical Committee of the Regional Medical Chamber in Krakow, under reference number 81/KBL/OIL/2023, dated 10 March 2023.

Conflict of interest

The authors declare no conflict of interest.

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