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N. Lukianova *, T. Burda, O. Mushii, T. Zadvornyi, A. Pavlova, V. Chekhun

R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, the NAS of Ukraine, Kyiv, Ukraine

* Correspondence: Email: cytmolmarkers@gmail.com

INTEGRATED EXPRESSION PROFILE OF THE MMP–TIMP–miRNA AXIS IN BREAST CANCER CELL LINES OF DIFFERENT MOLECULAR SUBTYPES

Background. Invasion and metastasis of breast cancer (BC) critically depend on extracellular matrix (ECM) remodeling, regulated by matrix metalloproteinases (MMPs) and their endogenous tissue inhibitors (TIMPs). The dysregulation of the MMP-TIMP axis, together with the post-transcriptional control by microRNAs (miRNAs), contributes to the aggressive phenotype of BC. **Materials and Methods**. The expression of collagenases (MMP-1, MMP-8, MMP-13), gelatinases (MMP-2, MMP-9), TIMP1—4, and selected regulatory miRNAs (miR-34a-5p, miR-100-5p, miR-132-3p, miR-145-5p, miR-155-5p, miR-200b-5p) was analyzed by immunocytochemistry and real-time PCR in 4 human BC cell lines representing different molecular subtypes (MCF-7, T47D, MDA-MB-231, MDA-MB-468). **Results**. Distinct subtype-specific expression profiles were identified. At the mRNA level, the triple-negative BC cells showed the highest expression of collagenases (*MMP1, MMP8, MMP13*) and *MMP9*, whereas luminal BC cells of the MCF-7 line exhibited the maximal *MMP2* levels. At the protein level, collagenases predominated in the luminal BC cell lines (T47D, MCF-7), while gelatinases were most abundant in MDA-MB-231. *TIMP1* and *TIMP3* transcripts were the highest in T47D, *TIMP2* in MDA-MB-468 cells, while the *TIMP3* expression in MDA-MB-231 cells was absent. miRNA profiling revealed a generally higher expression of miR-34a-5p, miR-100-5p, miR-132-3p, and miR-145-5p in the triple-negative BC cell lines, whereas MCF-7 cells displayed the lowest levels except for miR-155-5p, the expression of which was maximal. The discrepancies between mRNA and protein levels suggest a miRNA-mediated post-transcriptional regulation, although not universally consistent across all MMPs. **Conclusions**. The study demonstrates that the MMP-TIMP-miRNA axis exhibits subtype-specific expression patterns in the BC cell lines. The observed heterogeneity highlights the importance of post-transcriptional regulation and suggests that integrated profiling of MMPs, TIMPs, and regulatory miRNAs may provide novel insights into the invasive potential of BC and identify candidate biomarkers for clinical validation.

Keywords: breast cancer, MMPs, TIMPs, microRNAs, extracellular matrix remodeling, invasion, metastasis.

Invasion and metastasis critically depend on the remodeling of the tumor microenvironment (TME), in particular, the enzymatic degradation of the extracellular matrix (ECM). Among proteases, matrix metalloproteinases (MMPs) are a key family of zinc-dependent endopeptidases capable of cleaving ECM components [1]. Beyond matrix turnover, MMPs regulate angiogenesis, inflammatory re-

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sponses, and intercellular signaling, thereby broadening their contribution to tumor biology [2, 3]. Under physiological conditions, MMPs activity is tightly controlled, but it becomes pathologically dysregulated during tumor growth. Accumulating evidence indicates that elevated MMPs expression and activity correlate with an aggressive disease course. In particular, excessive production of gelatinases MMP-2 and MMP-9 in breast cancer (BC) tissue is associated with a higher risk of metastasis and poorer patient survival, as demonstrated by meta-analysis [4]. Historically, MMPs were regarded as pro-oncogenic factors that facilitate invasion by degrading basement membranes [2]. In BC, the predominant effect of MMPs is the promotion of invasion and angiogenesis and the formation of distant metastases [5]. However, more recent studies have revealed a more complex functional landscape: some MMPs may also exert antitumor effects; for example, MMPs secreted by stromal cells can restrain tumor progression [2]. Consistent with this complexity, some trials of broad-spectrum MMPs inhibitors showed their limited efficacy and notable toxicity, prompting a shift toward more selective strategies and stricter target selection [6, 7].

Functional antagonists of MMPs are their endogenous tissue inhibitors (TIMPs). The TIMP family includes four proteins (TIMP-1, -2, -3, -4), which form complexes with active MMPs and block their proteolytic activity, thereby maintaining extracellular matrix homeostasis. Despite the shared ability to inhibit MMPs, individual TIMPs perform unique biological functions independent of MMPs. The role of TIMPs in carcinogenesis has proved to be ambiguous [8]. On the one hand, in model studies, increased TIMP levels consistently limit the invasive activity of tumor cells. In particular, TIMP-2 suppresses invasion in vitro and metastasis of MDA-MB-231 cells in vivo [9]. On the other hand, TIMP-2 is a necessary link in the activation of proMMP-2 [10]; and elevated TIMP-1 protein levels in tumor tissue and in the plasma/serum of patients with BC are associated with an unfavorable prognosis [11, 12]. Current data explain this controversy by a combination of MMP-dependent and MMP-independent effects of TIMPs, which include regulation of proliferation, apoptosis, migration, and angiogenesis, providing grounds for cautious interpretation of TIMP expression readouts in clinical practice [13].

The «MMP-TIMP» ratio is regulated with the participation of miRNAs, which provide post-transcriptional control of the expression of these genes. In the context of BC, numerous miRNAs have been identified as such that modulate migration, invasion, and metastasis, including through effects on the expression of MMPs and TIMPs [14]. The experimental data available to date confirm the association of miRNA dysregulation with an aggressive tumor phenotype and poorer patient survival, underscoring the promise of their use as diagnostic and prognostic markers of BC [14, 15].

In this context, integrated profiling that combines quantitative determination of MMP and TIMP gene expression with parallel analysis of miRNAs in BC cells or tissue is appropriate, since these molecules provide post-transcriptional control and often explain discrepancies between mRNA and protein levels. Such an approach will make it possible to more accurately assess the contribution of the MMP-TIMP-miRNA axis to the formation of the invasive phenotype of malignantly transformed cells.

Given the above, the aim of our study was to characterize the expression profiles of MMPs, TIMPs, and miRNAs involved in their regulation in BC cells of different molecular subtypes under in vitro conditions.

Materials and Methods

Cell lines. Experimental in vitro studies were performed using 4 human BC cell lines of different molecular subtypes (MCF-7, T47D, MDA-MB-231, MDA-MB-468), obtained from the Cell Bank of Human and Animal Tissues of the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, the NAS of Ukraine.

Cells were cultured at 37 °C in a humidified atmosphere with 5% CO_2 in DMEM with high glucose and L-glutamine (Sigma, USA) supplemented with 10% FBS (BioWest, USA). Cells were passaged twice weekly at a seeding density of $2—4\times10^4$ cells per cm^2 of surface area, when the monolayer reached ~80% confluence.

Immunocytochemistry. For immunocytochemical analysis, cells were grown on coverslips. Fixation was performed in a methanol/acetone solution (1:1) for 2 h at −20 °C.

The monoclonal antibodies to MMP-1 (clone 6A5, Invitrogen, USA), MMP-2 (clone 2C1-1D12,

Thermo Scientific, USA), MMP-8 (clone C8, Thermo Scientific, USA), MMP-9 (clone MA5-15886, Thermo Scientific, USA), and MMP-13 (clone VIIIA2, Invitrogen, USA) were used at manufacturer-recommended dilutions. Incubation with specific antibodies was carried out for 1 h. Visualization was performed using the Master Polymer Plus Detection System (Biocare Medical, Canada). After completion of the reaction, slides were rinsed with water, counterstained with hematoxylin, and mounted in Paramount Aqueous Mounting Medium (Thermo Fisher Scientific, USA).

Expression of the studied markers was assessed by counting immunopositive cells using a PrimoStar microscope (Zeiss, Germany) at ×400 magnification. Semiquantitative evaluation employed the H-score method [16].

Real-time PCR. To analyze the expression of MMP, TIMP, and miRNA genes, real-time polymerase chain reaction was used. Total RNA was isolated from cells using the Quick-DNA Miniprep Kit (Zymo Research, USA) according to the manufacturer's instructions. RNA concentration and purity were assessed spectrophotometrically on a NanoDrop 2000c (Thermo Scientific, USA). The samples were stored in a Tris-EDTA buffer at −20 °C. The reverse transcription was performed according to a modified protocol for the LunaScript® RT SuperMix Kit (New England Biolabs, USA), and quantitative amplification used the standard protocol for the Luna® Universal qPCR Master Mix (New England Biolabs, USA). The primer sequences for miRNA detection were designed using http://genomics.dote.hu:8080/mirnadesigntool/; for mRNAs of the studied genes – using NCBI Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast). The primers were synthesized by Metabion (Germany). The relative expression levels were determined by the $2^{-\Delta Ct}$ method [17], using RNU48 miRNA or β-actin mRNA as endogenous reference controls.

Statistical analysis. Statistical processing was performed using GraphPad Prism v.8.0 (GraphPad Software Inc., USA). For comparisons between two groups, Student's *t* test (when data met normality) or the Mann — Whitney *U* test (when normality was violated) was applied. For comparisons among three or more groups, ANOVA was used. Data are presented as M ± m (mean ± standard error of the mean) or as percentages. Differences were considered significant at $p \leq 0.05$.

Results

Analysis of MMP gene expression at the mRNA and protein levels in human BC cell lines of different molecular subtypes. We analyzed members of the MMP family belonging to the collagenases (MMP-1, MMP-8, MMP-13) and gelatinases (MMP-2, MMP-9). Expression was assessed at the mRNA level (Fig. 2) and at the protein level (Fig. 1 and Fig. 3) to identify the differences among the cell lines of the distinct BC subtypes.

MMP-1 (collagenase-1). The *MMP1* mRNA level varied substantially across the BC cell lines examined (Fig. 2, *a*). The highest *MMP1* mRNA levels were detected in the triple-negative MDA-MB-231 and MDA-MB-468 cells, whereas the hormone-sensitive T47D and MCF-7 cells showed significantly lower values, by 2.0- to 6.6-fold. In contrast, at the protein level, the highest MMP-1 expression was recorded in T47D (224.9 ± ± 11.3 H-score points) and MCF-7 cells (182.0 ± ± 13.4 H-score points), while MDA-MB-231 cells exhibited low values (34.7 ± 3.1 H-score points), and MDA-MB-468 cells showed moderate expression (109.5 ± 4.7 H-score points) (Fig. 3, *a*).

MMP-8 (collagenase-2). The highest *MMP8* mRNA expression was observed in MDA-MB-231 cells, exceeding the corresponding values in T47D and MCF-7 cells by 25.0-fold ($p < 0.05$) and 7.5-fold ($p < 0.05$), respectively (Fig. 2, *c*). At the same time, the mRNA levels of collagenase-2 in MDA-MB-468 cells were also more than 2-fold ($p < 0.05$) higher than in the luminal subtypes. The highest MMP-8 protein level was recorded in T47D cells (273.0 ± 15.6 H-score points), which was 19% higher than in MDA-MB-468 ($p < 0.05$) and 1.5-fold higher than in MCF-7 ($p < 0.05$). In MDA-MB-231 cells, MMP-8 protein expression was the lowest (Fig. 3, *c*), despite the highest transcriptional activity of this gene.

MMP-13 (collagenase-3). The highest *MMP13* mRNA levels were detected in MDA-MB-231 cells, exceeding those in T47D by more than 20-fold ($p < 0.05$), in MDA-MB-468 by 15.2-fold ($p < 0.05$), and in MCF-7 by 7.4-fold ($p < 0.05$) (Fig. 2, *e*). In contrast, at the protein level, the pattern was reversed (Fig. 3, *e*): high MMP-13 expression was

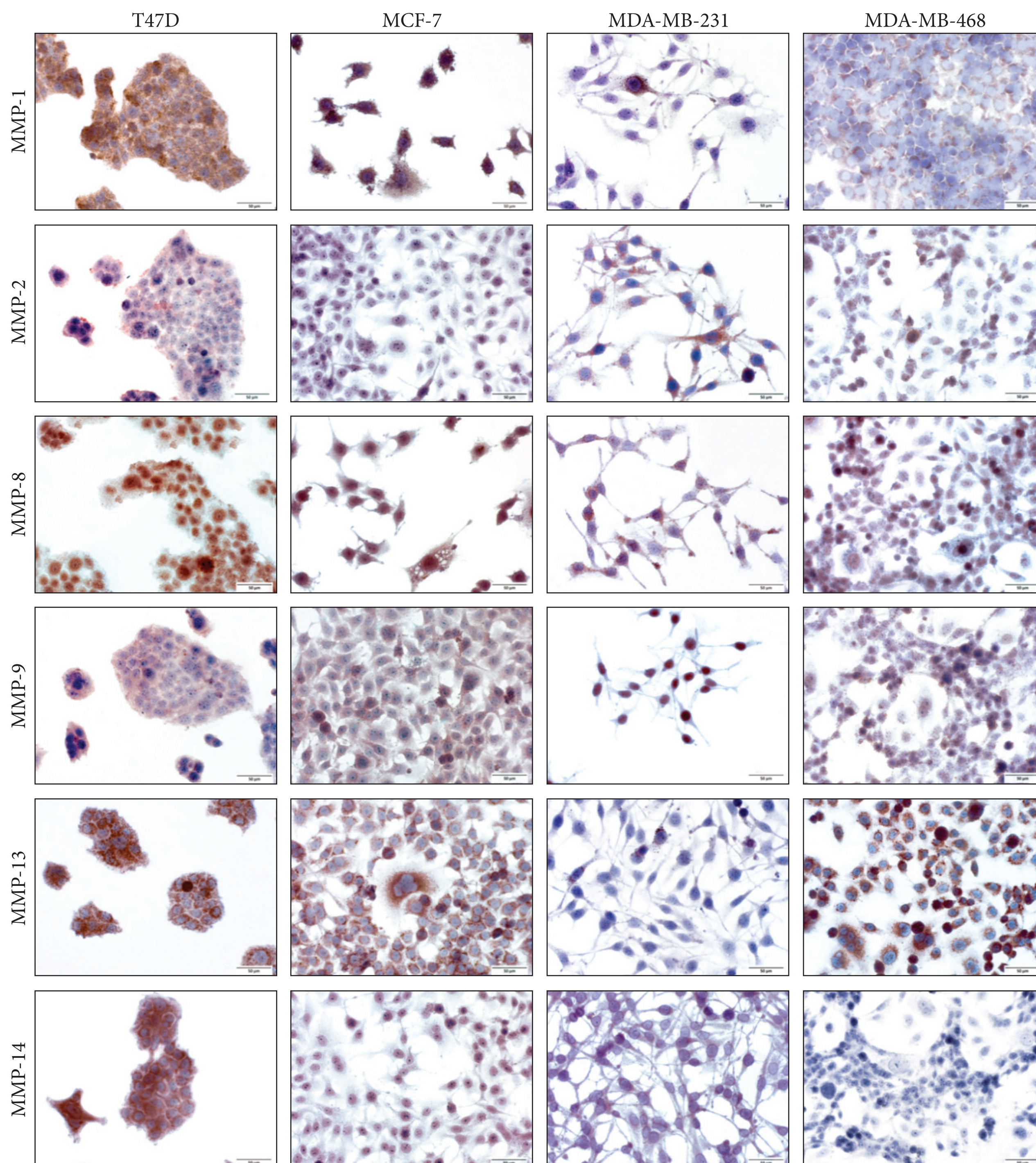

Fig. 1. Features of MMP expression at the protein level in cells of human BC lines of different molecular subtypes. Immunocytochemical method; Mayer's hematoxylin counterstain; ×400

characteristic of luminal A T47D cells (239.6 ± ± 10.0 H-score points) and luminal B MCF-7 cells (206.0 ± 11.8 H-score points). In the triple-negative MDA-MB-231 and MDA-MB-468 cells, this indicator was significantly lower and amounted to 8.0 ± ± 2.5 and 178.6 ± 7.4 H-score points, respectively.

MMP-2 (gelatinase A). High *MMP2* mRNA expression was recorded in MCF-7 cells, whereas in T47D, MDA-MB-231, and MDA-MB-468 cells, the corresponding values were lower by 13.0-fold ($p < 0.05$), 6.0-fold ($p < 0.05$), and 1.8-fold ($p < 0.05$), respectively (Fig. 2, *b*). The highest MMP-2 protein levels were observed in MDA-MB-231 cells (169.0 ± 7.2 H-score points), which were nearly 24-fold ($p < 0.05$) higher than in MCF-7, 2.2-fold ($p < 0.05$) higher than in

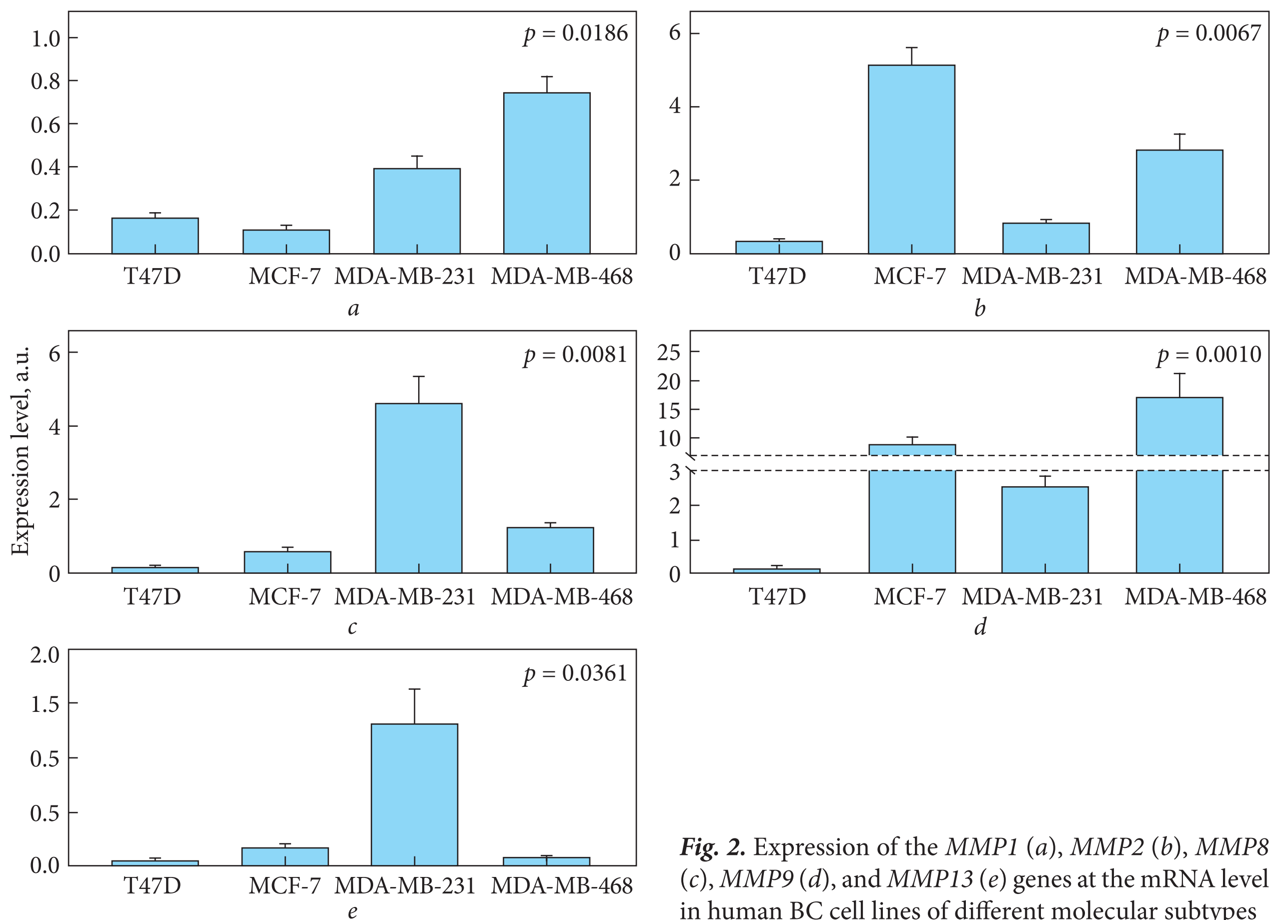

Fig. 2. Expression of the *MMP1* (*a*), *MMP2* (*b*), *MMP8* (*c*), *MMP9* (*d*), and *MMP13* (*e*) genes at the mRNA level in human BC cell lines of different molecular subtypes

MDA-MB-468, and 1.8-fold ($p < 0.05$) higher than in T47D (Fig. 3, *b*).

MMP-9 (gelatinase B). The highest *MMP9* mRNA expression was noted in MDA-MB-468 cells, 17.39 ± 3.90 a.u., which was nearly twice ($p < 0.05$) the value for MCF-7 and 6.7-fold ($p < 0.05$) that for MDA-MB-231 (Fig. 2, *d*). The greatest MMP-9 protein level was detected in MDA-MB-231 cells (237.0 ± 14.8 H-score points), approximately 5-fold ($p < 0.05$) higher than in MCF-7 (Fig. 3, *d*). In T47D cells, gelatinase B protein expression was 87.1 ± 5.6 H-score points despite the lowest mRNA level. In MDA-MB-468 cells, MMP-9 protein expression equaled 67.0 ± ± 6.6 H-score points, even though this line showed high *MMP-9* mRNA expression.

Analysis of TIMP gene expression at the mRNA level in human BC cell lines of different molecular subtypes. It was found that the *TIMP1* mRNA level was the highest in T47D cells, exceeding the corresponding values in MCF-7 by 4.3-fold ($p < 0.05$), in MDA-MB-231 by 2.3-fold ($p < 0.05$), and in MDA-MB-468 by 1.8-fold ($p < 0.05$) (Fig. 4, *a*). The highest *TIMP2* mRNA expression was detected in MDA-MB-468 cells (0.0918 ± ± 0.0178 a.u.), which was approximately 2.3-fold ($p < 0.05$) and 1.8-fold ($p < 0.05$) higher than that in T47D and MDA-MB-231 cells, respectively, and 5.4% ($p < 0.05$) higher than in MCF-7 cells (Fig. 4, *b*). In T47D cells, the *TIMP3* mRNA level exceeded that in MCF-7 and MDA-MB-468 cells by 3.2-fold ($p < 0.05$) and 2.9-fold ($p < 0.05$), respectively. *TIMP3* transcripts were not detected in MDA-MB-231 cells (Fig. 4, *c*). The highest *TIMP4* mRNA expression was detected in MDA-MB-468 cells (93.0 ± 6.5 a.u.), which was 1.6-fold ($p < 0.05$) higher than in MCF-7 (58.9 ± 3.8 a.u.), and markedly exceeded the corresponding values in MDA-MB-231 (2.98 ± 0.19 a.u., 31-fold, $p < 0.05$) and T47D cells (0.063 ± 0.036 a.u., ~1470-fold, $p < 0.05$) (Fig. 4, *d*).

Analysis of miRNA expression in human BC cell lines of different molecular subtypes. We selected miRNAs involved in the regulation of MMP gene expression using the miRTarBase resource [18]. The identified miRNAs exhibit specificity toward individual MMP genes, providing a post-transcriptional control of their expression (Table).

Fig. 3. Expression of the *MMP1* (*a*), *MMP2* (*b*), *MMP8* (*c*), *MMP9* (*d*), and *MMP13* (*e*) genes at the protein level in human BC cell lines of different molecular subtypes

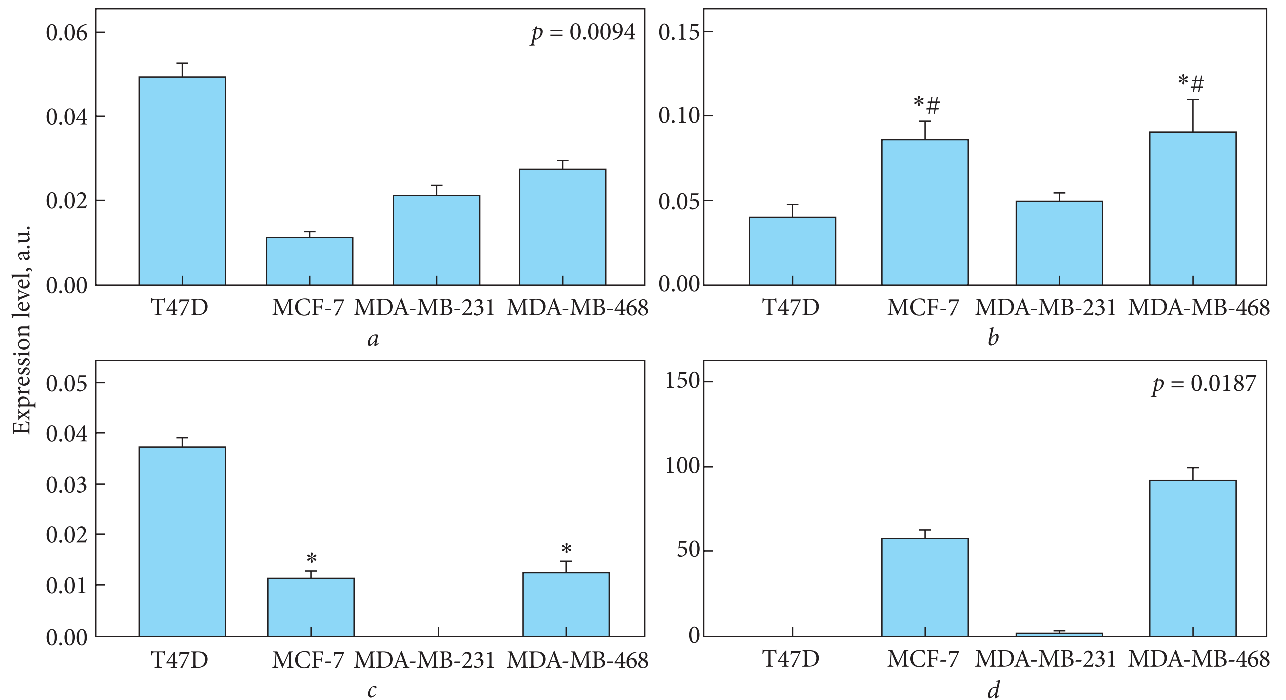

Fig. 4. Expression of the *TIMP1* (*a*), *TIMP2* (*b*), *TIMP3* (*c*), and *TIMP4* (*d*) genes at the mRNA level in human BC cell lines of different molecular subtypes. * $p < 0.05$, compared to T47D cells; # $p < 0.05$, compared to MDA-MB-231 cells

A comparative analysis (Fig. 5) showed that the highest expression levels of hsa-miR-34a-5p, hsa-miR-100-5p, hsa-miR-132-3p, and hsa-miR-145-5p were observed in triple-negative BC MDA-MB-231 cells. Luminal A BC T47D cells are characterized by high expression of hsa-miR-34a-5p, hsa-miR-132-3p, and hsa-miR-145-5p, whereas luminal B BC MCF-7 cells display the lowest basal levels of the four miRNAs examined. Notably, MCF-7 cells showed the highest expression of hsa-miR-155-5p. In MDA-MB-468 cells, most analyzed miRNAs exhibited predominantly moderate expression; however, these cells also expressed the highest levels of hsa-miR-200b-5p among the BC lines studied.

Analysis of the obtained data indicated that a characteristic feature of triple-negative BC MDA-MB-231 and MDA-MB-468 cells is a high level of mRNA expression of collagenases, as well as the highest *MMP9* level in MDA-MB-468. In contrast, luminal B BC MCF-7 cells exhibited the highest *MMP2* mRNA levels. At the protein level, the opposite pattern was observed: a high collagenase expression in T47D and MCF-7 cells, whereas gelatinases predominated in MDA-MB-231 cells. Regarding the inhibitors of the MMP activity, the highest mRNA levels of *TIMP1* and *TIMP3* were found in T47D cells, whereas *TIMP2* mRNA predominated in the MDA-MB-468 line, and *TIMP3* transcripts were not detected in MDA-MB-231 cells. Notably, *TIMP4* expression was also maximal in MDA-MB-468 cells, while being markedly lower in MCF-7 and nearly absent in T47D and MDA-MB-231. Certain differences were also recorded in the miRNA expression profiles across BC molecular subtypes. In particular, triple-negative BC cells generally showed a higher expression of most of the analyzed miRNAs compared with luminal subtypes, especially MCF-7 cells. However, it should be noted that MCF-7 cells were characterized by the highest expression of hsa-miR-155-5p.

Features of the regulation of MMP gene expression by miRNAs

miRNA	MMP1	MMP2	MMP8	MMP9	MMP13
hsa-miR-34a-5p	+	+		+	
hsa-miR-100-5p					+
hsa-miR-132-3p					+
hsa-miR-145-5p	+	+		+	
hsa-miR-155-5p	+				
hsa-miR-200b-5p		+	+		

Discussion

The metastatic dissemination remains the leading cause of death from BC, with distant lesions accounting for most fatalities despite advances in systemic therapy [19, 20]. A key prerequisite for metastasis is ECM remodeling, in which MMPs serve as central mediators, as supported by numerous experimental data and clinical observations [2, 21]. Given the crucial role of MMPs in the metastatic cascade, it is pertinent to analyze the expression profiles of gelatinases and collagenases at two levels, mRNA and protein, depending on the molecular subtype of BC.

In our in vitro study on human BC cell lines, a heterogeneous pattern of MMP expression was established. A generalized profile of MMPs expression and their regulatory molecules in BC cells of different molecular subtypes is presented in Fig. 6. Overall, we demonstrated that a characteristic feature of luminal A subtype BC cells is a reduced mRNA expression, accompanied by elevated protein levels of collagenases, complemented by active expression of TIMPs and microRNAs involved in their regulation. At the same time, luminal B subtype cells are characterized by high mRNA expression of gelatinases, although at the protein level, collagenases still predominate. Basal subtype cells, in turn, show a high expression of both collagenases and gelatinases.

Moreover, in a broad panel of BC lines, the increased expression of several MMPs (MMP-1/-2/-3/-8/-9/-10/-11/-13) has been described as such that correlates with invasive activity [22]. The likely reasons for the imbalance we recorded are post-transcriptional mechanisms, namely the translational inhibition by specific miRNAs, variation in translational efficiency, and differences in protein stability, together with subtype-specific secretion of MMPs. In particular, for MMP-2, an inhibitory effect of miR-20a on translation has been demonstrated and, conversely, its activation by nucleolin [23].

As known, TIMPs are key modulators of the proteolytic balance, primarily regulating MMP activity; however, they can also exert MMP-inde-

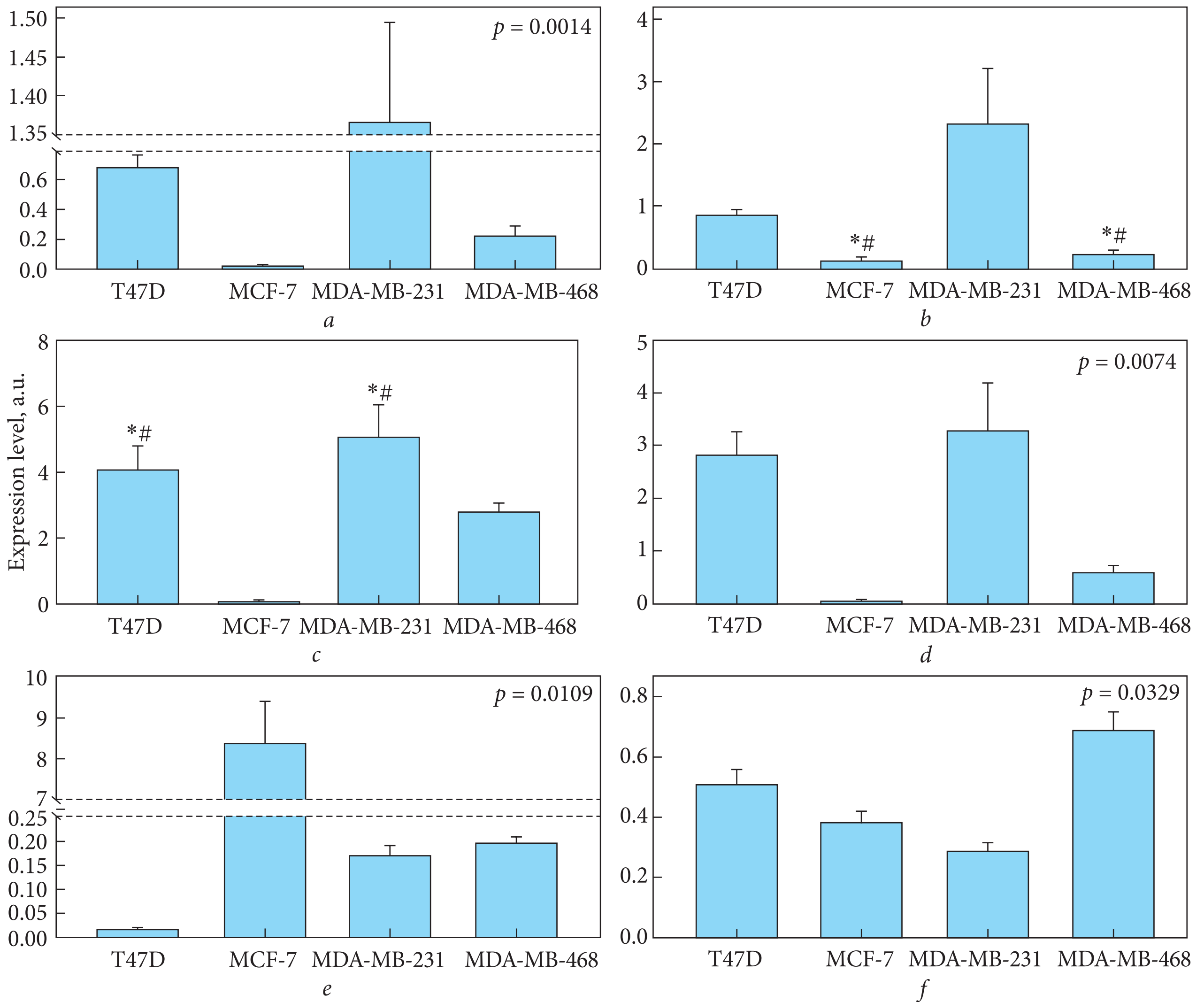

Fig. 5. Expression of the miRNAs hsa-miR-34a-5p (*a*), hsa-miR-100-5p (*b*), hsa-miR-132-3p (*c*), hsa-miR-145-5p (*d*), hsa-miR-155-5p (*e*), and hsa-miR-200b-5p (*f*) in human BC cell lines of different molecular subtypes. In (*b*), * $p < 0.05$, compared to T47D cells; # $p < 0.05$, compared to MDA-MB-231 cells. In (*c*), * $p < 0.05$, compared to MCF-7 cells; # $p <$ < 0.05, compared to MDA-MB-468 cells

pendent control over proliferation, apoptosis, and cell signaling. Their contribution to BC progression is dual: by inhibiting MMPs, TIMPs can limit invasion, while individual family members are capable of activating proliferative and antiapoptotic signals [8, 24]. Therefore, we decided to characterize TIMP mRNA levels in BC cell lines to define their subtype-specific expression profiles, providing an additional layer of the regulatory background.

Given the established discrepancy between MMP mRNA and protein levels in the models examined, we next considered the expression profiles of relevant miRNAs as a possible post-transcriptional regulatory factor explaining these differences. In MDA-MB-231 cells, we recorded the highest levels of several miRNAs (miR-34a-5p, miR-100-5p, miR-132-3p, miR-145-5p); T47D was characterized by high expression of miR-132-3p, miR-145-5p, and miR-200b-5p, whereas MCF-7 cells displayed minimal levels of most of these miRNAs, except miR-155-5p. In MDA-MB-468 cells, the highest levels of miR-200b-5p were observed. Comparison with the literature indicates divergent trends.

According to miRNA-mediated post-transcriptional control of MMP genes may partly account for the observed discrepancies between mRNA and protein levels. Given the specificities (Table 1), the most consistent link is seen for MMP-13: in MDA-MB-231 cells, elevated *MMP-13* mRNA coincides with minimal protein expression against the back-

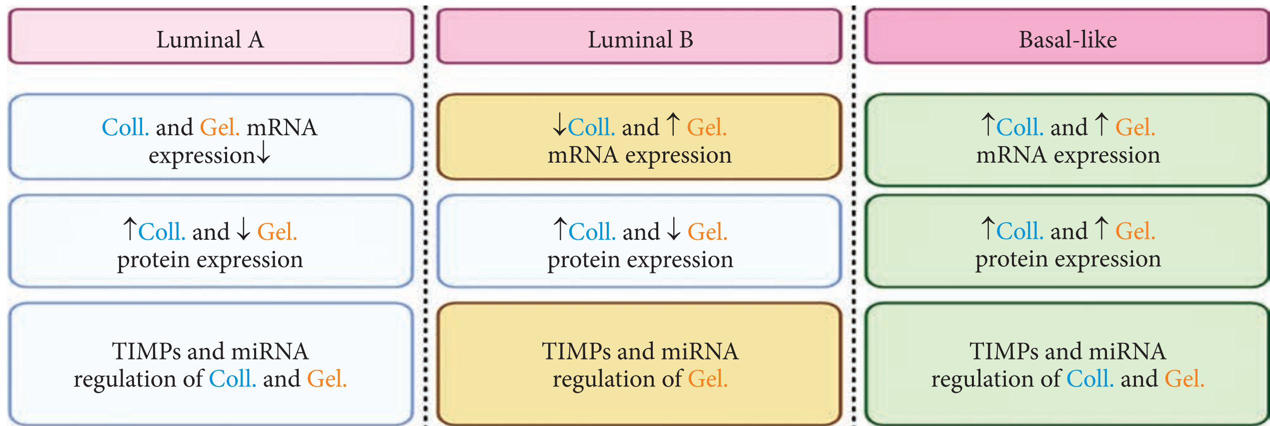

Fig. 6. Expression profile of collagenases (Coll.), gelatinases (Gel.), and their regulatory molecules (TIMPs and miRNAs) in BC cell lines of different molecular subtypes

ground of an enriched pool of specific miRNAs, whereas in luminal BC cells, relatively lower mRNA levels are associated with high H-scores amid a lower representation of such miRNAs. For MMP-1, a similar — but not universal — profile is observed: in MDA-MB-231, higher levels of miRNAs specific to MMP1 accompany reduced protein expression, whereas the opposite is seen in T47D/MCF-7. The miR-200b-5p profile does not provide a convincing explanation for interline differences in MMP-8. miRNAs specific to *MMP2* do not show the expected inverse relationship with protein expression, and miRNAs specific to *MMP9* do not yield a unified explanation, since in triple-negative BC cells, an inverse relationship between protein expression and miR-200b-5p is apparent, which, however, is not characteristic of T47D. Thus, miRNAs may contribute to the gap between mRNA and protein expressions for certain MMPs but do not exhaust the observed differences. Additional regulatory mechanisms are likely involved, which were not assessed in this study.

Promising directions for further research include determining the proteolytic activity of MMPs and the inhibitory capacity of TIMPs with clear separation of intracellular and secreted fractions, thereby allowing protein levels to be related to the actual proteolytic potential. It is also appropriate to account for microenvironmental influences by employing 3D cultures and co-cultures with stromal and immune cells under controlled mechanical and metabolic conditions. A dedicated component should be the causal assessment of miRNA roles: systematic experiments for priority miRNAs followed by measurement of MMP transcript and protein levels, as well as the secreted and active enzyme fractions. It is advisable to expand the target panel to include additional MMPs and relevant miRNAs, in parallel with quantitative profiling of TIMPs at the protein level, to directly assess the enzyme–inhibitor balance. The final step should be clinical validation on tumor material of different BC subtypes, with quantitative profiling of MMP/TIMP/miRNA, spatial localization, and correlation with clinical data in order to determine the reproducibility and potential prognostic relevance of the identified patterns.

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Н. Лук'янова, Т. Бурда, О. Мушій,
Т. Задворний, А. Павлова, В. Чехун

Інститут експериментальної патології, онкології
та радіобіології імені Р.Є. Кавецького, НАН України, Київ, Україна

ІНТЕГРОВАНИЙ ПРОФІЛЬ ЕКСПРЕСІЇ ОСІ ММР-TIMP-МІКРОРНК
У КЛІТИНАХ РАКУ МОЛОЧНОЇ ЗАЛОЗИ РІЗНОГО МОЛЕКУЛЯРНОГО ПІДТИПУ

Вступ. Інвазія та метастазування раку молочної залози (РМЗ) критично залежать від ремоделювання позаклітинного матриксу (ECM), яке регулюється матриксними металопротеїназами (MMPs) та їх ендогенними тканинними інгібіторами (TIMPs). Дисрегуляція осі MMP—TIMP разом із посттранскрипційним контролем з боку мікроРНК сприяє агресивному фенотипу РМЗ. **Матеріали та методи.** Експресію колагеназ (MMP-1, MMP-8, MMP-13), желатиназ (MMP-2, MMP-9), TIMP1—4 та вибраних регуляторних мікроРНК (miR-34a-5p, miR-100-5p, miR-132-3p, miR-145-5p, miR-155-5p, miR-200b-5p) проаналізовано в чотирьох лініях клітин РМЗ людини, що представляють різні молекулярні підтипи (MCF-7, T47D, MDA-MB-231, MDA-MB-468). Експресію оцінювали методом імуноцитохімії та ПЛР у реальному часі з подальшою статистичною обробкою. **Результати.** Виявлено чіткі підтип-специфічні профілі експресії. На рівні мРНК клітини тричі-негативного РМЗ продемонстрували найвищу експресію колагеназ (*MMP1, MMP8, MMP13*) та *MMP9*, тоді як люмінальні клітини MCF-7 характеризувалися максимальним рівнем *MMP2*. На білковому рівні колагенази переважали в люмінальних лініях (T47D, MCF-7), тоді як желатинази були найбільш поширені в MDA-MB-231. Транскрипти *TIMP1* і *TIMP3* були найвищими в T47D, *TIMP2* — в MDA-MB-468, тоді як експресія *TIMP3* була відсутня в MDA-MB-231. Профілювання мікроРНК показало загалом вищу експресію у тричі-негативних лініях (особливо miR-34a-5p, miR-100-5p, miR-132-3p, miR-145-5p), тоді як MCF-7 продемонстрували найнижчі рівні, за винятком miR-155-5p, яка була максимальною. Невідповідність між рівнями мРНК та білка свідчить про посттранскрипційну регуляцію за участю мікроРНК, хоча це не було універсально послідовним для всіх MMPs. **Висновки.** Дослідження показало, що вісь MMP — TIMP— мікроРНК характеризується підтип-специфічними патернами експресії у клітинних лініях РМЗ. Виявлена гетерогенність підкреслює важливість посттранскрипційної регуляції та свідчить, що інтегроване профілювання MMPs, TIMPs і регуляторних мікроРНК може забезпечити нове розуміння інвазивного потенціалу та визначити біомаркери-кандидати для клінічної валідації.

Ключові слова: рак молочної залози, MMPs, TIMPs, мікроРНК, ремоделювання позаклітинного матриксу, інвазія, метастазування.