RESEARCH ARTICLE

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Plasma miR-141-3p as a Potential Diagnostic Biomarker for Early Breast Cancer and a Functional Regulator with Tumor-Suppressive Effects in Luminal A Breast Cancer Cells

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Abstract

Objective: Breast cancer (BC) remains the most common malignancy in women worldwide, highlighting the need for reliable non-invasive biomarkers for early detection. miR-141-3p, a member of the miR-200 family, has shown context-dependent roles in tumorigenesis, but its diagnostic specificity and clinical relevance in the plasma of patients with BC remain incompletely understood. Therefore, this study aimed to assess the diagnostic potential and functional role of *miR-141-3p* in BC. **Methods:** Plasma *miR-141-3p* levels were quantified in breast cancer patients and healthy controls using RT-qPCR. Diagnostic capacity was evaluated via receiver-operating characteristic (ROC) analysis. Associations between circulating *miR-141-3p* expression and BC risk were assessed using odds ratios (ORs). In vitro functional assays in MCF-7 cells (*miR-141-3p* overexpression) examined effects on proliferation, migration, and regulation of target genes. **Results:** Circulating miR-141-3p was significantly upregulated in BC patients. ROC analysis yielded an AUC of 0.877 (95% CI: 0.782–0.971), demonstrating good diagnostic performance. No statistically significant stage-dependent difference was observed, suggesting that circulating *miR-141-3p* dysregulation may not simply reflect tumour burden. In vitro, *miR-141-3p* overexpression suppressed MCF-7 cell proliferation and migration and reduced expression of EMT-associated markers. Furthermore, strong inverse correlations were observed between *miR-141-3p* levels and its predicted oncogenic targets. **Conclusion:** Circulating *miR-141-3p* shows promise as a non-invasive biomarker for BC detection and risk discrimination, although its specificity as a stand-alone marker may be limited. Its in vitro effects on proliferation, migration, and EMT markers indicate potential functional relevance. Further validation in larger cohorts and through in vivo studies is warranted.

Keywords: breast cancer, *miR-141-3p*, biomarker, proliferation, migration

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Introduction

Breast cancer (BC) is the most common malignancy in women around the world [1]. To improve patient survival, there is an urgent need to identify novel, minimally invasive biomarkers for early detection and disease management [2]. MicroRNAs (miRNAs/miRs) are recognized as potential biomarkers for various cancers, including BC. Emerging evidence has demonstrated that miRNAs play crucial roles in tumorigenesis and metastasis in BC [3]. Importantly, miRNAs are highly stable in peripheral blood, highlighting their promise as non-invasive biomarkers for cancer detection and prognosis [4].

Among these, *miR-141-3p*, a member of the miR-200 family, has been reported to regulate epithelial mesenchymal transition (EMT), metastasis, and tumor growth [5]. Studies across various malignancies suggest that *miR-141-3p* exhibits context-dependent effects, functioning either as an oncogene or a tumor suppressor depending on the cancer type and molecular context. For instance, miR-141 is upregulated and promotes cell proliferation in esophageal [6], prostate [7], nasopharyngeal [8], and cervical [9] cancers. In contrast, it is downregulated in colon [10], colorectal [11], and gastric [12] cancers, where its reduced expression is associated with the inhibition of tumor progression [13]. Recently, *miR-141-3p* has attracted growing attention for both diagnostic and functional complexity in BC. Elevated expression of *miR-141-3p* has been associated with tumor aggressiveness [14, 15] and chemoresistance [16], partly through regulation of the Keap1/Nrf2/GPX4 pathway and inhibition of ferroptosis. Conversely, reduced *miR-*

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141-3p levels have been linked to enhanced epithelial–mesenchymal transition and increased invasiveness [17]. Beyond tumor tissues, studies have also explored the extracellular fraction of *miR-141-3p*. Elevated plasma miR-141 has been reported in invasive and metastatic cases compared with benign lesions or controls [18, 19]. In contrast, a few studies have shown no significant change [20] or even reduced levels of *miR-141-3p* in patient plasma or serum, especially in early-stage or hormone receptor–positive subtypes [21]. These inconsistencies highlight the need to further investigate circulating *miR-141-3p* levels in breast cancer and to clarify its biological effects in relevant experimental models.

Therefore, this study aimed to investigate the expression and diagnostic value of plasma *miR-141-3p* in BC, with the hypothesis that its dysregulation may occur at early stages. In addition, we investigated its tumorigenic functions in vitro to clarify its biological role and highlight its potential as a biomarker for detection and clinical management of BC.

Materials and Methods

Study population

A total of 160 peripheral blood samples were collected, including 80 BC patients and 80 age-matched healthy controls. Blood samples from BC patients (mean age 44.05 ± 10.5 years; range 28–70 years) were obtained prior to any therapeutic intervention at Oncology Hospital, Ho Chi Minh City, Vietnam between August 2020 and December 2021. All breast cancer diagnoses were confirmed histopathologically according to the World Health Organization classification criteria, and tumor staging was based on the American Joint Committee on Cancer (AJCC) eighth edition guidelines. Blood samples were obtained from women with no history of malignancy. The participants had a mean age of 41.3 ± 10.5 years (range: 24–70 years). Only non-hemolyzed blood samples were included in the analysis. Written informed consent was obtained from all participants prior to sample collection. The study was approved by the Oncology Hospital, Ho Chi Minh City, Vietnam (ethical no: 113/BVUB-HĐĐĐ).

Peripheral blood (2 mL) was collected into EDTA-K2 anticoagulant tubes and immediately processed using a two-step centrifugation protocol (3000 ×g for 20 min and 16,000 ×g for 10 min at 4°C) to minimize cellular contamination. Plasma samples were carefully separated, visually inspected to exclude hemolyzed specimens, and stored at -80°C until RNA extraction.

Cell lines and cell culture

Human breast cancer cell lines (MCF-7) (passages 5) was obtained from the Laboratory of Department of Physiology & Animal Biotechnology, Faculty of Biology - Biotechnology, University of Science, VNU-HCM, Vietnam, and maintained under standard culture conditions. MCF-7 was cultured in high-glucose Dulbecco's Modified Eagle Medium (DMEM; Gibco, Thermo Fisher Scientific, USA). All media were supplemented with 10% fetal bovine serum (FBS; Gibco) and 1% penicillin–streptomycin (Gibco). Cells were cultured at 37°C in a humidified atmosphere containing 5% CO_2.

Transient transfection

The sequences of *miR-141-3p* mimic were as follows: 5'-UAACACUGUCUGGUAAAGAUGG-3'/5'-AUCUUUACCAGACAGUGUUANN-3'(MedChemExpress_MCE). Reverse transfection was performed using Lipofectamine™ RNAiMAX Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Briefly, MCF-7 cells were transfected with *miR-141-3p* mimics at a final concentration of 50 nM into 6-well plates with a density of 6×105 cells per well. Cells were incubated at 37 °C in a humidified incubator containing 5% CO_2 and harvested at 48 hours post-transfection for subsequent analyses. Two control groups were included: untransfected cells (blank controls) and cells treated with Lipofectamine only, without mimic (mock controls), to account for nonspecific effects of the transfection reagent.

RNA extraction and quantitative RT-PCR

Total RNA and small RNA from plasma and cultured cells were extracted using TRIzol™ Reagent (Invitrogen, Carlsbad, CA, USA). For plasma samples, an additional small RNA recovery step was included to enhance the yield of small RNA fractions [22]. Reverse transcription was performed using the SensiFAST cDNA Synthesis kit (BIOLINE, England) according to the manufacturer's instructions. The reaction mixture was incubated at 16 °C for 10 min, 42 °C for 15 min and 85 °C for 5 min in a thermal cycle. Quantitative real-time PCR (qPCR) was performed using the SensiFAST HRM Kit (BIOLINE, England) in a 20 µL reaction mixture comprising 2 µL cDNA, 10 µL 2X SYBR master Mix, 0.4 µL of each forward and reverse primer (200 nM final concentration), and nuclease-free water. Amplification was carried out on the LineGene 9660 Real-Time PCR System (Bioer, China) under the following cycling conditions: initial denaturation at 95 °C for 2 minutes, followed by 40 cycles of 95°C for 5 seconds and 60 °C for 10 seconds, followed by melting curve analysis.

miR-141-3p expression was quantified using specific primers (Stem-loop RT primer: 5′- GTCGTATCCAGTGCAGGGTC CGAGGTATTCGCACTGGATA CGACCCATCT -3′; forward primer: 5′- GGCGTAACACTGTCTGGTAA -3′; reverse primer: 5′- GCAGGGTCCGAGGTATTC -3′) synthesized by Integrated DNA Technologies (IDT, USA). MiR-16-5p, a commonly used reference miRNA in plasma-based studies, was selected as the endogenous control [23], while RNU6B (U6) served as the internal control for cell line experiments. Relative expression levels of *miR-141-3p* were calculated using the $2^{-\Delta\Delta Ct}$ method, where ΔCt = Ct_*miR-141-3p* − Ct_miR-16 (for plasma) or Ct_U6 (for cells).

For mRNA expression analysis, the expression levels of *HMGB1*, *KLF12*, *EGFR*, *p21*, Vimentin, and *VEGF* were quantified using gene-specific primers (Supplementary Table 1). *GAPDH* was used as the internal control. mRNA expression was quantified using the $2^{-\Delta\Delta Ct}$

method. All experiments were performed in triplicate.

Cell proliferation and migration assays

Cell proliferation was evaluated using the Cell Counting Kit-8 (CCK-8; Elabscience, USA). Briefly, 7.5×10^3 cells were seeded into 96-well plate, and cell viability was determined according to the manufacturer's instructions by measuring absorbance at 450 nm at 24, 48, and 72 h post-transfection. Cell migration was evaluated using a scratch assay, in which cells were grown to confluence in 6-well plates and a wound was created in the monolayer using a sterile 100 µL pipette tip. The wound width was recorded under a microscope at 0 and 12 h post-scratch. The wound area was quantified using ImageJ software, and migration rates were calculated as the percentage of wound closure relative to the initial scratch width. All experiments were independently performed in triplicate.

Statistical analysis

Statistical analyses were performed using GraphPad Prism version 9.0 and R version 4.5.1. Data are presented as mean ± SD unless otherwise indicated. Differences between two groups and multiple groups were assessed using the Mann–Whitney U test, and Kruskal–Wallis H test, respectively. Effect sizes were calculated using Hedges' g and interpreted qualitatively. Receiver operating characteristic (ROC) curve analyses were performed using Youden's index to determine the optimal cutoff point, and 95% CIs for sensitivity and specificity were calculated at the selected threshold. The association between circulating *miR-141-3p* expression and breast cancer risk was assessed using odds ratios (ORs). Pearson correlation was used to identify the correlation between the expression of *miR-141-3p* and target genes of *miR-141-3p*. Hedges' g effect sizes were estimated, and interpreted as follows: g < 0.2, very small; 0.2–0.5, small; 0.5–0.8, medium; and > 0.8, large. A p-value < 0.05 was considered statistically significant. P-values of less than 0.05, less than 0.01, less than 0.001, and less than 0.001 were marked by *, **, ***, and **** respectively.

Results

Upregulation of miR-141-3p in breast cancer plasma

The expression of plasma *miR-141-3p* was relatively quantified in 80 BC patients and 80 healthy controls. *miR-141-3p* levels were significantly higher in the BC group ($2^{-\Delta Ct}$: 0.041 ± 0.063) than in controls ($2^{-\Delta Ct}$: 0.006 ± 0.004), corresponding to a 4.52-fold increase ($2^{-\Delta\Delta Ct} = 4.52$; 95% CI: 1.87–10.94; P < 0.0001, Mann–Whitney U test; Table 1; Figure 1a). The OR value for the association between elevated *miR-141-3p* and BC was 11.667 (95% CI: 1.227–110.953; P = 0.014).

To evaluate the diagnostic potential of *miR-141-3p* in BC, ROC curve analysis was performed. Plasma *miR-141-3p* discriminated BC from controls with an AUC of 0.877 (95% CI: 0.782–0.971) (Figure 1c), indicating very good discriminative performance. Using the optimal cutoff value of 0.0092 relative units, the sensitivity and specificity were both 79.2% (95% CI: 59.5–90.8). For comparison, alternative thresholds produced a sensitivity of 83.3% (95% CI: 64.1–93.3) and specificity of 75.0% (95% CI: 55.1–88.0) at 0.0081 relative units, and a

Table 1. Clinical Characteristics and Plasma *miR-141-3p* Expression in Breast Cancer

Characteristics		N	Fold change ($2^{-\Delta Ct}$) (Mean ± SD)	p-value
Group				<0.0001[a]
	Normal group	80	0.006 ± 0.004	
	Breast cancer group	80	0.041 ± 0.063	
TNM stage				0.112[b]
	0-I	13	0.030 ± 0.022	
	II	13	0.041 ± 0.054	
	III	54	0.011 ± 0.007	

ΔCt = Ct_miR141-3p − Ct_miR-16-5p; a Mann-whitney U test; b Kruskal-Wallis H test

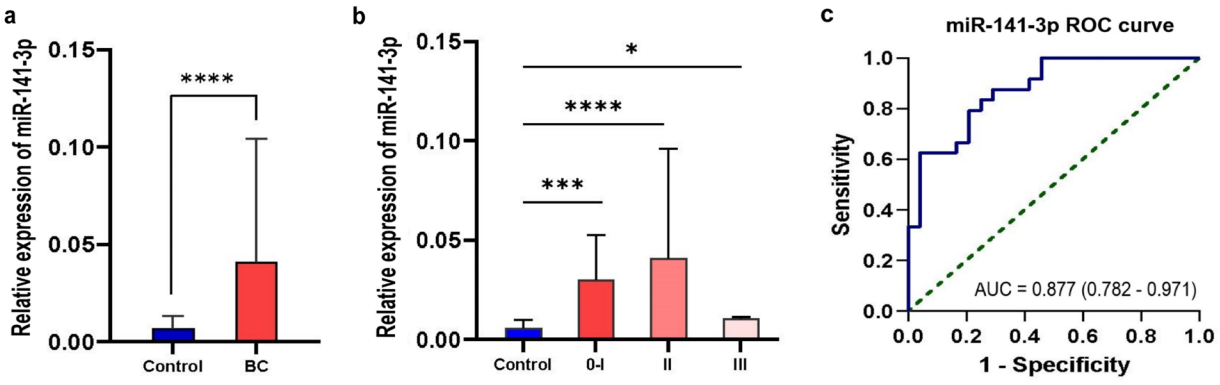

Figure 1. Comparison of *miR-141-3p* Expression Levels. (a) *miR-141-3p* levels in plasma from BC patients and healthy controls. (b) *miR-141-3p* levels in BC patient plasma stratified by TNM stage. (c) Receiver operating characteristic (ROC) curve for *miR-141-3p* in BC.

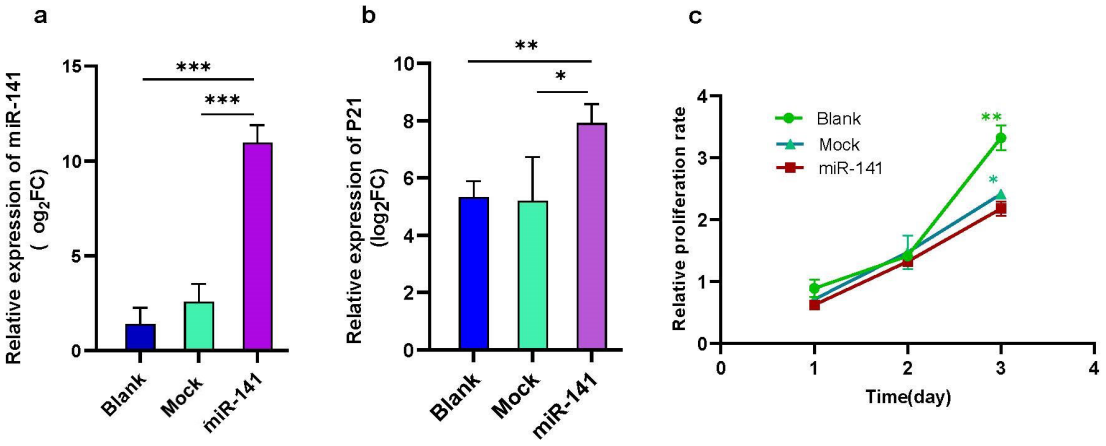

Figure 2. Overexpression of *miR-141-3p* Suppressed Cell Proliferation. (a) Validation of *miR-141-3p* overexpression in transfected MCF-7 cells. (b) Increased p21 expression following *miR-141-3p* overexpression. (c) CCK-8 assay showing reduced proliferative capacity of MCF-7 cells upon *miR-141-3p* overexpression compared with blank and mock controls at 3 days post-transfection.

sensitivity of 62.5% (95% CI: 42.7–78.8) with specificity of 95.8% (95% CI: 79.8–99.8) at 0.014 relative units. These findings suggest good discriminative performance, although the trade-off between sensitivity and specificity varied across thresholds.

When stratified by TNM stage, no statistically significant differences in circulating *miR-141-3p* were observed across stages (P = 0.112, Kruskal–Wallis H test; Table 1). Exploratory subgroup analysis showed that stage 0–I patients ($2^{-\Delta Ct}$: 0.030 ± 0.022) displayed a 4.65-fold increase relative to controls (95% CI: 1.76–12.3; P = 0.0003, Mann–Whitney U test; Figure 1b). The fold changes for stage II and stage III were 5.15-fold (95% CI: 2.51–10.59) and 2.24-fold (95% CI: 1.27–3.95), respectively.

MiR-141–3p inhibits BC cell proliferation

To assess the effect of *miR-141-3p* on cell proliferation, we first examined the expression of *p21*, a well-established marker of cell cycle inhibition. Overexpression of *miR-141-3p* in MCF-7 cells via mimic transfection markedly increased *p21* expression compared with blank ($P < 0.01$, $g = 3.36$) and mock controls ($P < 0.05$, $g = 1.83$; Figure 2a–b), suggesting enhanced cell cycle inhibition. Consistently, CCK-8 assay demonstrated that *miR-141-3p* overexpression significantly reduced the proliferative capacity of MCF-7 cells relative to blank ($P < 0.01$, $g = 5.56$) and mock groups ($P < 0.05$, $g = 2.22$) after three days of transfection (Figure 2c). Collectively, these results demonstrate that *miR-141-3p* functions as a negative regulator of BC cell proliferation.

MiR-141–3p inhibits BC cell migration

To evaluate the effect of *miR-141-3p* on cell migration, a wound-healing assay was performed. As shown in Figure 3, the wound closure rate in the *miR-141-3p* mimic

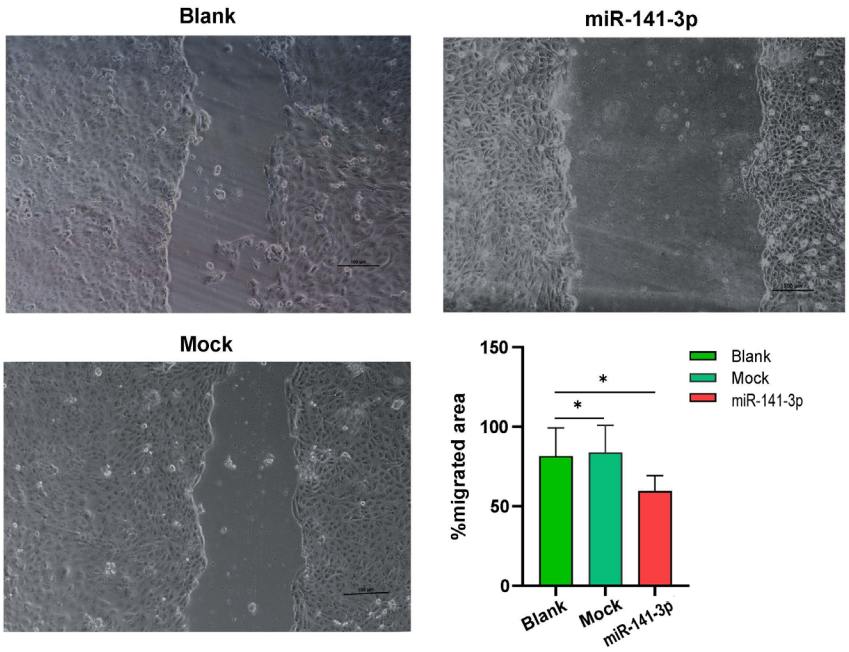

Figure 3. Overexpression of *miR-141-3p* Suppressed Migration of MCF-7 cells at 12 hours Post-Scratch

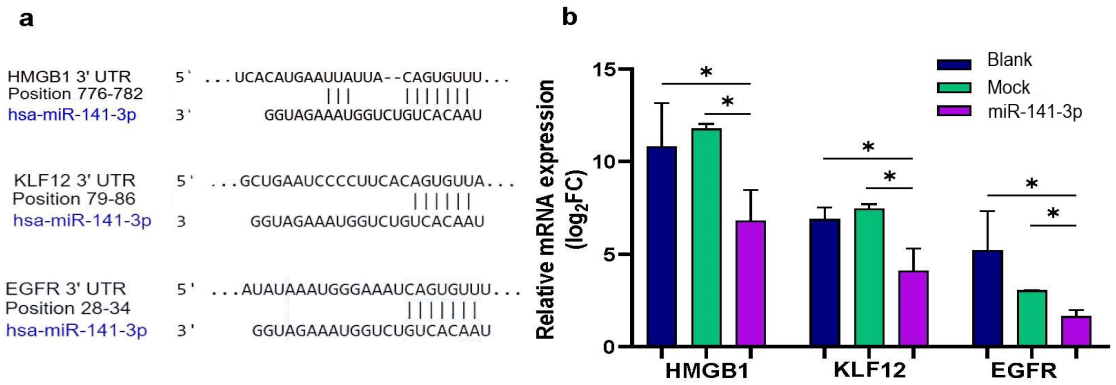

Figure 4. Predicted Binding Sites of *miR-141-3p* and Its Regulatory Effects on *HMGB1, KLF12,* and *EGFR* in MCF-7 cells. (a) Sequence alignment showing *miR-141-3p* binding sites within the 3′-UTRs of *HMGB1, KLF12,* and *EGFR*. (b) *miR-141-3p* overexpression reduced *HMGB1, KLF12,* and *EGFR* expression in MCF-7.

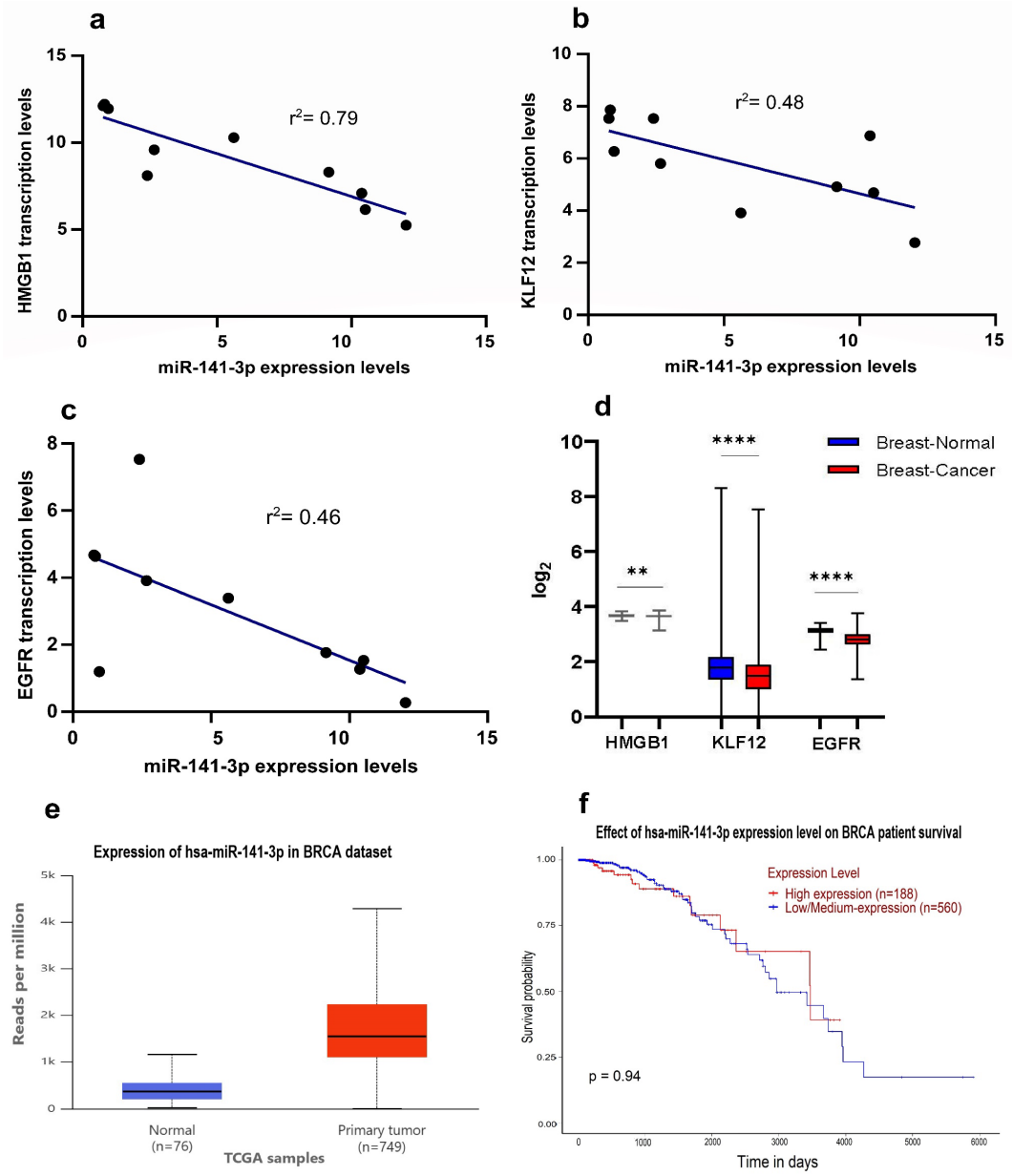

Figure 5. Expression correlation of *miR-141-3p* and its target genes in breast cancer. (a-c) Pearson correlation analysis showed significant negative correlations between *miR-141-3p* and *HMGB1, KLF-12*, and *EGFR* in MCF-7 cells. (d) The expression levels of *HMGB1, KLF12*, and *EGFR* were significantly reduced in BC tissues compared with normal tissues based on the *GENT2* database. (e) *miR-141-3p* expression was markedly upregulated in BC tissues according to TCGA data. (f) Kaplan–Meier survival analysis showed no significant association between *miR-141-3p* expression and overall survival of BC patients.

group (59.45%) was significantly reduced compared with the blank group (81.60%; $P < 0.05$, $g = 1.24$) and the mock control group (83.77%; $P < 0.05$, $g = 1.39$) at 12 hours post-scratch. These results demonstrate that *miR-141-3p* overexpression suppresses breast cancer cell migration, supporting its role as a negative regulator of this process.

To explore whether the inhibitory effect of *miR-141-3p* on migration is related to EMT-associated changes, we assessed mesenchymal marker expression. As shown in Supplementary Figure S1, overexpression of *miR-141-3p* markedly decreased *VEGF* mRNA levels compared with both the blank ($P < 0.05$, $g = 2.66$) and mock control groups ($P < 0.05$, $g = 2.96$). Similarly, vimentin mRNA expression was significantly reduced in the *miR-141-3p* mimic group relative to the blank ($P < 0.05$, $g = 2.09$) and mock control groups ($P < 0.05$, $g = 2.06$). These findings suggest that the suppressive effect of *miR-141-3p* on migration may be partially mediated through the downregulation of mesenchymal features associated with EMT.

miR-141-3p negatively regulates HMGB1, KLF12, and EGFR in MCF-7 cells

Computational prediction using the TargetScan algorithm revealed that the 3'-UTR regions of *HMGB1*, *KLF12*, and *EGFR* contain conserved complementary sites matching the seed sequence of *miR-141-3p* (Figure 4a). To verify whether *miR-141-3p* regulates these predicted targets, MCF-7 cells were transfected with a *miR-141-3p* mimic, and gene expression levels were measured 48 hours post-transfection. As shown in Figure 4b, overexpression of *miR-141-3p* significantly reduced the expression of *HMGB1*, *KLF12*, and *EGFR* compared with control cells, supporting a negative association between *miR-141-3p* overexpression and the mRNA levels of these predicted target genes.

To explore the regulatory relationship between *miR-141-3p* and its potential target genes in MCF-7 cells, Pearson correlation analysis was performed using their relative mRNA expression levels. The analysis revealed significant inverse correlations between *miR-141-3p* and all three genes, showing a very strong negative correlation with *HMGB1* ($r = –0.89$) and strong negative correlations with *KLF12* ($r = –0.69$) and *EGFR* ($r = –0.68$) (Figure 5a–c). These results indicate that *miR-141-3p* may act as a negative regulator of these oncogenic genes in breast cancer cells.

To further examine their expression patterns in breast cancer tissues, data from the GENT2 database were analyzed. The results showed that *HMGB1*, *KLF12*, and *EGFR* were significantly downregulated in breast cancer tissues compared with normal controls, with $\log_2$ fold changes of –0.061, –0.679, and –1.544, respectively (Figure 5d). Consistently, TCGA data revealed that *miR-141-3p* expression was markedly upregulated in breast cancer tissues (Figure 5e). However, Kaplan–Meier survival analysis demonstrated no significant association between *miR-141-3p* expression and overall survival of breast cancer patients ($P = 0.94$) (Figure 5f).

Discussion

Accumulating evidence indicates that miRNAs exhibit aberrant expression across a wide range of human cancers. As critical post-transcriptional regulators, miRNAs modulate tumorigenesis by controlling cell proliferation, apoptosis, and EMT through diverse transcriptional networks [24]. Within this regulatory landscape, the miR-200 family has been recognized as a pivotal suppressor of EMT, thereby restraining cancer invasion and metastasis [25]. Among its members, *miR-141-3p* frequently displays context-dependent expression and functions across cancer types [7, 8, 11, 26]. Based on this background, the present study investigated the expression pattern, diagnostic potential, and biological function of *miR-141-3p* in breast cancer.

Our data demonstrated that plasma *miR-141-3p* levels were significantly elevated in patients with BC compared with healthy controls, suggesting its potential as a circulating biomarker. This observation aligns with previous reports reporting altered *miR-141-3p* expression in several malignancies, including prostate [28], colorectal, and ovarian cancers [29], as well as nasopharyngeal carcinoma in the Vietnamese population [26], thereby extending its clinical relevance to breast cancer. The observed association between elevated circulating *miR-141-3p* and breast cancer risk, supported by the OR of 11.667 (95% CI: 1.227–110.953), reinforces its potential relevance as a disease-associated biomarker.

The diagnostic evaluation demonstrated that plasma *miR-141-3p* possessed very good discriminative ability, with an AUC of 0.877 and high sensitivity and specificity (79.2%) at optimal threshold. However, variability in specificity across different cutoffs suggests that *miR-141-3p* alone may be insufficient as a stand-alone diagnostic marker. Instead, it may be more informative when combined with other biomarkers. Previous studies have reported that CA15-3, a conventional serum marker for BC, exhibits lower diagnostic performance [27]; however, a direct head-to-head comparison was not conducted in the present cohort. Despite the promising AUC value, the wide confidence interval of the odds ratio and the variability in sensitivity and specificity across different thresholds indicate that plasma *miR-141-3p* should be interpreted cautiously as a diagnostic biomarker. Given that *miR-141-3p* dysregulation has also been reported in other malignancies, its diagnostic utility may be enhanced when evaluated as part of a multi-marker panel rather than as a stand-alone indicator.

Although plasma *miR-141-3p* levels did not differ significantly across TNM stages, an exploratory pattern was observed in which stage II patients exhibited the highest expression (5.15-fold), followed by stage 0–I (4.65-fold) and stage III (2.24-fold). Although no statistically significant stage-dependent difference was observed, the exploratory fold-change pattern suggests that circulating *miR-141-3p* dysregulation may occur across different stages of disease. Such patterns have also been reported for other circulating miRNAs, particularly those influenced by tumor microenvironment dynamics rather than tumor burden alone [28]. Importantly,

because molecular subtype information (*ER/PR/HER2* status), tumour grade, and lymph node involvement were not available for the present cohort, subtype-specific or prognostically relevant differences in *miR-141-3p* expression could not be evaluated. This is a notable limitation, as *miR-141-3p* expression may vary across luminal, *HER2*-enriched, and triple-negative BC subtypes, and the absence of such stratification may obscure biologically meaningful associations. Future studies should prioritise the collection of comprehensive molecular and pathological data to enable subtype-specific analyses.

Functionally, we demonstrated that *miR-141-3p* acts as a negative regulator of both cell proliferation and migration in MCF-7 cells. Overexpression of *miR-141-3p* significantly suppressed cell growth, as measured by the CCK-8 assay, and markedly impaired the wound healing capacity of MCF-7 cells. These findings are consistent with the tumor-suppressive roles of *miR-141-3p* reported in other cancer models [11, 29, 30] and further support its inhibitory effect on breast cancer progression.

Moreover, the inhibitory effect of *miR-141-3p* on cell migration was accompanied by modulation of EMT-related markers. In particular, vimentin, a mesenchymal marker [31], and *VEGF*, which is implicated in vascular remodeling and has also been linked to EMT regulation [32], were both downregulated. These findings imply that *miR-141-3p* might attenuate migratory potential, at least partially, through modulation of EMT; however, the underlying regulatory mechanisms remain to be clarified.

At the molecular level, inverse correlations were observed between *miR-141-3p* and its predicted targets (*HMGB1*, *KLF12*, and *EGFR*) in MCF-7 cells, implying that *miR-141-3p* may act as a post-transcriptional suppressor of these oncogenic genes in breast cancer. Each of these targets has been implicated in oncogenic signaling in various cancers, including breast cancer: *HMGB1* activates NF-κB, *PI3K/AKT*, and *MAPK* cascades to promote cell survival and metastasis [33, 34]; *KLF12* regulates Wnt/β-catenin signaling and transcriptional programs that facilitate EMT [35]; and *EGFR* serves as a major upstream activator of *PI3K/AKT*, MAPK, and β-catenin pathways, driving proliferation and invasion in breast cancer [36, 37]. Therefore, the observed negative is consistent with a model in which *miR-141-3p* may exert tumour-suppressive effects, at least in part, through modulation of *PI3K/AKT*, MAPK, and Wnt/β-catenin-related signalling (Figure S2). The observed inverse relationships are consistent with a potential tumor-suppressive role of *miR-141-3p* through modulation of these oncogenic pathways, although further functional studies are required to confirm causality.

The contrasting expression patterns of *miR-141-3p* (upregulated in TCGA) and its predicted targets *HMGB1*, *KLF12*, and *EGFR* (downregulated in GENT2) suggest a potential inverse regulatory relationship. These observations are correlative and do not establish causality. While *HMGB1* and *EGFR* have been reported to be upregulated and associated with poor prognosis in certain breast cancer subtypes or patient cohorts [38, 39], their reduced mRNA levels in GENT2 may reflect cohort composition, tumor heterogeneity, or post-transcriptional regulation. Subtype-specific analyses from TCGA further indicated that *miR-141-3p* expression was elevated in luminal and triple-negative breast cancers (TNBC), whereas *HMGB1*, *KLF12*, and *EGFR* levels in these subtypes were not consistently lower than in *HER2*-enriched tumors (Figure S3), suggesting context-dependent regulation rather than a uniform compensatory mechanism. Therefore, these findings should be considered hypothesis-generating rather than confirmatory. The absence of a significant correlation between *miR-141-3p* expression and overall survival in TCGA suggests that additional molecular or clinical factors may influence its prognostic relevance. Future studies should investigate whether *miR-141-3p* directly modulates these pathways in vivo and to clarify its functional relevance across distinct molecular subtypes of BC.

In MCF-7 cells, *miR-141-3p* showed inhibitory effects on proliferation and migration and was inversely associated with *HMGB1*, *EGFR*, and *KLF12*. At first glance, the upregulation of *miR-141-3p* in tumor tissues and patient plasma appears contradictory to its tumor-suppressive effects observed in MCF-7 cells. Circulating miRNAs reflect contributions from tumor cells, stromal components, immune cells, and systemic responses, and are influenced by selective secretion and exosomal loading mechanisms [40-42]. Therefore, elevated plasma levels may not directly mirror intracellular functional activity. Moreover, our functional assays were limited to a single luminal A cell line, whereas previous studies indicate that *miR-141-3p* can have both oncogenic and tumor-suppressive roles depending on breast cancer subtype and experimental context. Collectively, these results support a context-dependent model, in which *miR-141-3p* inhibits proliferation and migration in luminal A cells, while its overall tissue and plasma elevation may reflect compensatory mechanisms and cellular heterogeneity rather than a direct correlation with tumor growth. However, this mechanistic interpretation remains speculative, and dedicated in vivo and multi-subtype studies are needed to resolve this discrepancy and clarify the precise cellular sources of elevated circulating *miR-141-3p* in BC.

Several limitations should be acknowledged. First, the sample size was relatively modest, and the distribution across disease stages was uneven, which may reduce the robustness of the findings. Moreover, molecular subtype data (*ER/PR/HER2* status), tumour grade, and lymph node involvement were not available, precluding subtype-specific analyses and limiting the clinical generalisability of the results. Nevertheless, all patient samples were collected prior to therapeutic intervention, reducing the likelihood that the observed circulating *miR-141-3p* levels were influenced by treatment-related effects. Second, functional experiments were conducted exclusively in MCF-7 cells, representing a luminal A breast cancer model. Given the molecular heterogeneity of breast cancer, these in vitro findings should be interpreted with caution when extrapolated to heterogeneous clinical samples. Subtype-specific functional studies and in vivo validation

will be required to clarify the biological relevance of *miR-141-3p* across different breast cancer contexts. Third, protein-level analyses (Western blot/IHC) and luciferase reporter assays were not conducted, leaving the direct regulatory relationship between *miR-141-3p* and targets to be further validated. Taken together, these considerations underscore the largely correlative nature of the present findings and highlight the need for integrative mechanistic studies linking circulating *miR-141-3p* dynamics with tumor-intrinsic function.

In conclusion, our findings suggest that circulating *miR-141-3p* may serve as a promising, albeit non-specific, biomarker for breast cancer detection when interpreted in appropriate clinical contexts. Its inhibitory effects on proliferation and migration observed in vitro support a potential functional role that appears to be highly context- and subtype-dependent. Further validation in larger, well-characterized cohorts and in vivo models is necessary to clarify its diagnostic and biological significance.

Author Contribution Statement

Duong TTC, Nguyen HT, Nguyen TTN, Nguyen NT and Huynh LH conceived and designed the study; Duong TTC, Nguyen NT and Huynh LH performed experiments, collected and processed the data; Duong TTC, Nguyen HT, Nguyen TTN, Nguyen NT and Huynh LH contributed to data analysis, interpretation, and critical discussion of the results; Duong TTC prepared the original draft of the manuscript; Nguyen HT, Nguyen TTN, Huynh LH, and Nguyen NT critically revised and edited the manuscript for important intellectual content; Nguyen HT supervised the research process and acquired project funding.

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General

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Ethical Declaration

The study was conducted in accordance with the Declaration of Helsinki and approved by the Oncology Hospital, Ho Chi Minh City, Vietnam (ethical no: 113/BVUB-HĐĐĐ/2020). Written informed consent was obtained from all participants. Cell line was maintained under standard culture conditions.

Approval

The study was approved by the Oncology Hospital, Ho Chi Minh City, Vietnam (ethical no: 113/BVUB-HĐĐĐ/2020).

Conflict of Interest

The authors declare no conflict of interest.

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