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Elevated MRPS23 expression facilitates aggressive phenotypes in breast cancer cells

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Abstract

Mitochondrial ribosomal protein S23 (MRPS23), encoded by a nuclear gene, is a well-known driver of proliferation in cancer. It participates in mitochondrial protein translation, and its expression association has been explored in many types of cancer. However, MRPS23 expression associations are rarely reported in breast cancer (BC). In this study, we explored the MRPS23 expression in BC cells compared with the non-tumoral breast cells. Overexpression and knockdown analysis of MRPS23 were performed in BC cells. Transfection efficiency was evaluated by western blot and qRT-PCR analysis. The role of MRPS23 in the malignant biological behaviors of BC cells was investigated using *in-vitro* experiments. Our results demonstrate that MRPS23 was aberrantly overexpressed at both the transcript and protein levels in BC cells. Additional findings reveal that deficiency of MRPS23 is associated with a decrease in cell proliferation/viability and compromised cell migration/invasion in BC cells. Relative to the sh-Ctrl group, the expression levels of cadherin, SNAI 1, and TWIST 1 decreased in the MRPS23 knockdown BC cells. We further found a significant decrease in the expression levels of Cyclin D1, Axin 2, LEF1, NKD1, and Survivin in MRPS23 knockdown cells. In conclusion, we found an association between MRPS23 knockdown and the metastasis ability of BC cells. These findings reveal that MRPS23 significantly decreases the migration and invasion of BC, thus inhibiting BC progression. We confirmed for the first time that MRPS23 expression determines the metastasis features of BC. Hence, the findings justify the key role of this protein in BC progression; therefore, it may be a potential therapeutic target for BC therapy.

Keywords: MRPS23, Knockdown, Overexpression, Cancer progression, BC

1. Introduction

In terms of female malignancies, breast cancer (BC) is one of the most common and rapidly expanding global health concerns [1]. Recent estimates state that 2.3 million people are diagnosed with BC annually and that 450,000 people die from the disease [1,2]. Among the primary risk factors that contribute to the development of BC include age, common genetic mutations in the BC predisposition genes (*BRCA1* and *BRCA2*), nulliparity, early menarche, first pregnancy in older age (> 30 years), late age at menopause, use of oral contraceptives, and having a personal or family history of BC or related medical conditions [3–6]. Despite the improved treatment, increased diagnosis, and other implantations in mammographic screening, its burden is increasing worldwide, especially in high-income countries [7,8].

To deal with this leading cause of death in women, working on the identification of new genetic drivers of proliferation could be important for prognostication and the development of new targeted treatments. One such cancer driver gene that has garnered increasing attention is Mitochondrial Ribosomal Protein S23 (MRPS23), a 22-kDa protein encoded by a gene located at 17q22–23. Through high-throughput genomic analyses, Gatza and colleagues identified *MRPS23* as a key gene involved in promoting cancer cell proliferation. The protein plays a central role in mitochondrial function, particularly by regulating the mitochondrial ribosome. This regulation is

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critical for maintaining mitochondrial protein synthesis; its disruption causes metabolic reprogramming in cancer cells, leading to enhanced survival, proliferation, and resistance to apoptosis [9]. Several studies have established a connection between MRPS23 expression and various cancers, including colorectal cancer, uterine leiomyoma, hepatocellular carcinoma (HCC), and glioma [9-16]. These findings suggest that MRPS23 plays a significant role in driving tumor growth and metastasis across different tumor types. For instance, in cervical cancer, Lyng et al. (2006) first demonstrated an association between MRPS23 and metastasis, showing that MRPS23 is upregulated in cervical tumors exhibiting increased recurrence and lymph node metastasis [17]. This heightened expression correlates with rapid tumor growth and enhanced invasive capacity, underscoring MRPS23's pivotal role in promoting aggressive cancer phenotypes [16-18]. Similarly, in HCC, increased expression of MRPS23 has been linked to larger tumor size, more advanced tumor stages, and poorer overall survival outcomes [19]. However, despite the clear association of MRPS23 with tumor aggressiveness in HCC, knockdown studies have not demonstrated a significant reduction in the metastatic potential of HCC cells, unlike its more substantial impact on metastasis in cervical cancer. This suggests that, in HCC, MRPS23 may be a downstream marker of aggressive tumor behavior rather than a direct driver of metastasis [19]. Beyond its role in proliferation, MRPS23 has been implicated in regulating mitochondrial pathway-based apoptosis through interactions with key regulatory proteins such as p21, p53, and cytochrome C [20-22]. These pathways are integral to maintaining cellular homeostasis by controlling cell cycle progression and programmed cell death. The p53 tumor suppressor pathway, in particular, is crucial for inducing apoptosis in response to cellular stress or DNA damage, while p21 acts as a cell cycle inhibitor. By modulating these pathways, MRPS23 may contribute to the evasion of apoptosis, thereby allowing cancer cells to continue proliferating unchecked. Studies have also suggested that MRPS23 may influence these pathways in a context-dependent manner, further regulating key cellular events such as cell death and survival [23-28]. Published literature highlights the detrimental impact of MRPS23 overexpression in various cancers, linking it to increased cell proliferation, enhanced tumor invasiveness, and metastasis. However, despite its well-established role in cancers, reports on MRPS23 expression in BC remain limited. This gap in knowledge suggests the need for further investigation into the role of MRPS23 in BC progression.

In the present study, we investigated the MRPS23 expression in BC cells compared with the normal breast cells. The study explored the relationship between suppressed MRPS23 and the migration of BC cells to verify how MRPS23 expression determines BC progression. We are anticipating that the outcome of our current investigations about MRPS23's role in BC progression will lead the way to design new therapeutic approaches for BC diagnosis and treatment; however, we suggest further *in-vivo* and *in-vitro* pre-clinical trials and in-depth exploration.

2. Material Methods

2.1. Cell culture

BC cell lines, including MCF-10, MDA-MB-468, MDA-MB-453, MDA-MB-231, and MCF-7, were procured from the Henan International Joint Laboratory for Nuclear Protein Regulation. These cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM; Thermo Fisher Scientific, MA, USA), supplemented with 10% fetal bovine serum (FBS; PAN-Seratech, Aidenbach, Germany), and 1% (v/v) penicillin-streptomycin solution (Solarbio Science, Beijing, China) to ensure optimal growth conditions. Cultures were maintained in a humidified incubator at 37°C with 5% CO2 to mimic physiological conditions. The authenticity and purity of the cell lines were verified based on their morphological characteristics, growth patterns, and comparison with reference information provided by the supplier. All cell lines were regularly monitored for mycoplasma contamination using a PCR-based detection kit, and mycoplasma-free cells were used in the experiments. This standardized environment supports the proliferation and viability of the cell lines for subsequent experimental assays.

2.2. Lentivirus Vector generations of anti-MRPS23 shRNA vectors

To suppress the expression of MRPS23, we designed and generated short hairpin RNA (shRNA) constructs specifically targeting the MRPS23 gene (NCBI accession number NM_001108289.1). These shRNA sequences were cloned into a lentiviral (LV) expression vector for efficient delivery into BC cells. Both overexpression and knockdown vectors were co-transfected alongside the LV backbone plasmid into BC cells using Lipofectamine 3000 reagent, ensuring efficient transfection. The shRNA constructs were labeled with a green fluorescent protein (GFP) reporter gene to enable visualization and tracking of transfected cells. The vectors were designated based on the specific sequences they carried: sh-MRPS23 for the MRPS23-targeting shRNA and sh-Ctrl for the non-targeting control sequence. An average viral titer of at least 5.0 × 10^8 TU/ml was achieved, ensuring efficient transduction of target cells. This lentiviral system allowed stable knockdown and overexpression of MRPS23 in BC cells, facilitating subsequent functional analyses related to cell migration, invasion, and proliferation.

2.3. Cell Migration and Invasion Assays

2.3.1. Wound Healing Assay

The scratch wound-healing motility assay was performed to evaluate the migration ability of transfected BC cells. Transfected BC cells were seeded at $5x10^5$ cells/well in 6 wells plate and incubated at 37°C and 5% CO_2. After the cells reached 80-90% confluence, the cells were then scratched by a sterile micropipette tip, washed twice, and the migration rate was viewed under the light microscope at 0, 12, and 24 hours. Image J software was used to measure the distance, and the following formula was applied to measure the migration rate: migration rate (%) = [(A0-A1)/A0] ×100, where A0 and A1 are the widths at 0 and 24 hours, respectively.

2.3.2. Transwell Assay

A transwell assay was performed to assess the invasion capability of transfected cells. A total of 50,000 cells were seeded into the upper chambers of 8μm-pore Boyden transwell (6.5 mm, Costar, Corning, NY, USA), either uncoated or Matrigel-coated. Matrigel stock solution was di-

luted 1:2 with sterile, ice-cold deionized water and mixed thoroughly. In a biosafety hood, 50µL of the diluted Matrigel was carefully added to the membrane of the transwell insert and allowed the Matrigel for 30 minutes to solidify. The upper chambers contained 200µl of serum-free media, while the lower chambers were filled with 600µl of media supplemented with 20% FBS. After 24 hours of incubation, non-migrated cells were removed with a cotton swab. The migrated cells were fixed in 75% ethanol, stained with 0.1% crystal violet for 25 minutes, and rinsed with PBS. The stained cells were then observed under an inverted light microscope (Carl Zeiss, Thornwood, NY, USA).

2.4. Western blot assays

Transfected MDA-MB-231 cells were seeded in 10 cm culture dishes and cultured for 24 hours. Cells were detached from culture dishes by trypsinization with 0.25% Trypsin–EDTA and then transferred to centrifuge tubes, followed by centrifugation at 1500 rpm for 8 minutes. Cells were washed three times with ice-cold PBS and then lysed in RIPA buffer (Beyotime Biotechnology, Shanghai, China) containing 100× Protease inhibitor (Beyotime Biotechnology, Shanghai, China) and 50× phosphatase inhibitor (Beyotime Biotechnology, Shanghai, China) for 30 minutes on ice. After centrifugation at 14,000 at 4℃ for 20 minutes, supernatants containing total cellular proteins were collected. The concentration of protein was determined using a BCA assay kit (Tiangen Biochemical Technology, Beijing, China). An equal amount of each protein sample was separated by electrophoresis in 10% sodium dodecyl sulfate-polyacrylamide gels and followed by transferring to a polyvinylidene fluoride membrane on ice. Proteins were transferred onto PVDF membranes (Millipore, MA, USA), and membranes were blocked by 5% fat-free milk powder in TBS with 0.1% Tween-20 (Sigma-Aldrich; Merck, Germany) at room temperature for 1 hour. After blockage, membranes were incubated with specific primary antibodies at the dilution ratio of 1:1000 at 4°C overnight. Antibodies were purchased from Cell Signaling Technology Inc. (Boston, U.S.A.). After incubation with a determined HRP-conjugated secondary antibody, protein expression was detected by using the enhanced chemiluminescence technique (ECL, Millipore, MA, USA). β-actin is used as an internal control. All antibodies used for this research are listed in Table S1.

2.5. Real-time PCR assays

Total RNA was extracted from the cells using TRIzol reagent (Tiangen Biochemical Technology, Beijing, China) following the manufacturer's protocol. RNA purity and concentration were determined by measuring the absorbance ratio at 260/280 nm using a NanoDrop spectrophotometer (ND-100, Thermo Fisher Scientific, Waltham, MA). The integrity of the isolated RNA was further assessed by agarose gel electrophoresis. For gene expression analysis, qRT-PCR was performed using the One-Step SYBR PrimeScript RT-PCR Kit (Takara Bio, Inc., Japan) in accordance with the kit's instructions. Reactions were run on an ABI 7500 sequence detection system (Applied Biosystems), ensuring accurate quantification of mRNA levels. The primers used for amplification were custom-designed and synthesized by JinWeizhi Biological Technology (Guangzhou, China). To ensure the accuracy of our quantitative PCR (qPCR) results, we assessed primer efficiency by constructing a standard curve from a series of tenfold dilutions of the target DNA. All primers exhibited efficiencies between 90% and 110%. Additionally, all the primers were verified by agarose gel electrophoresis, which showed a single band at the expected size for each target gene. All reactions were carried out in triplicate to ensure reproducibility and the relative gene expression levels were normalized to an internal control gene.

2.6. Statistical analysis

All experimental data were analyzed using SPSS 22.0 software for Windows and GraphPad Prism 6 for Windows. The results were presented as the mean ± standard deviation (SD). Statistical analysis involved one-way analysis of variance (ANOVA) to evaluate differences across multiple groups, followed by Tukey's test where appropriate. For comparisons between two specific groups, an independent sample T-test was employed. A p-value of less than 0.05 ($P<0.05$) was considered indicative of statistical significance, highlighting meaningful differences between the groups. In all cases, data were thoroughly assessed to ensure the robustness and validity of the statistical outcomes.

3. Results

3.1. mRNA levels expression of MRPS23

The *MRPS23* gene was both knocked down and overexpressed in MDA-MB-231 BC cells to investigate its role in cell proliferation and migration. Following transfection, the efficiency of the gene knockdown and overexpression was evaluated using qRT-PCR and statistical analysis of relative fluorescence intensity. The results confirmed the successful overexpression and suppression of MRPS23, as seen in the control, MRPS23 overexpressed, sh-Ctrl, and sh-MRPS23 groups. Figures 1 and 2 clearly illustrate the efficiency of the transfection by displaying the relative mRNA expression levels of MRPS23. In addition, MRPS23's expression correlates with other genes involved in cell proliferation and migration pathways.

In MDA-MB-231 cells, *MRPS23* mRNA expression was significantly higher compared to both normal breast

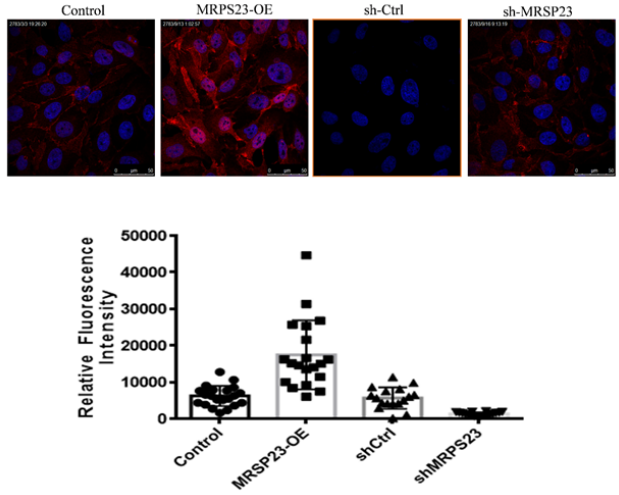

Fig. 1. Expression Analysis of MRPS23 in BC Cells (MDA-MB-231) Relative mRNA expression levels of *MRPS23* in normal, MRPS23-overexpressing, and MRPS23-knockdown cells. Statistical analysis of relative fluorescence intensity is presented for the control, MRPS23-overexpressing, sh-Ctrl, and sh-MRPS23 groups.

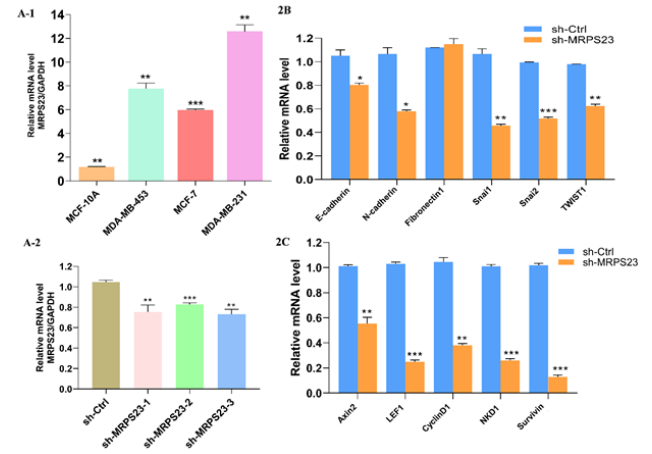

Fig. 2. MRPS23 expression and its effects on downstream targets in BC cell lines. (A1) Quantification of *MRPS23* mRNA expression levels in various BC cell lines using qRT-PCR. **(A2)** Evaluation and quantification of MRPS23 knockdown efficiency at the mRNA level in BC cells by qRT-PCR. **(B)** qRT-PCR analysis of migration-related gene expression in MRPS23 knockdown cells. **(C)** qRT-PCR analysis of proliferation-related gene expression in MRPS23 knockdown cells.

cells and other BC cell lines, as shown in Figure 2A. These findings underscore the elevated MRPS23 expression in aggressive BC phenotypes. Upon MRPS23 knockdown, a notable decrease in the expression levels of cadherin, SNAI1, and TWIST1 was observed, suggesting a direct link between MRPS23 depletion and the suppression of proteins associated with cancer cell migration ($P > 0.05$) (Figure 2B). This result indicates that MRPS23 may play a critical role in promoting cell motility and invasion. Moreover, MRPS23 knockdown also resulted in a significant reduction in the expression of cell proliferation-related genes, including *Cyclin D1*, *Axin 2*, *LEF1*, *NKD1*, and *Survivin*, as depicted in Figure 2C. These genes are known regulators of cell cycle progression and survival, indicating that MRPS23 is likely involved in controlling not only migration but also the growth and survival of BC cells. Our data suggest that MRPS23 overexpression is closely associated with the upregulation of key genes involved in cell migration and proliferation in BC cells. The knockdown of MRPS23, on the other hand, diminishes the expression of these genes, pointing to its potential as a therapeutic target for inhibiting BC cell invasion and growth.

3.2. MRPS23-silencing reduces BC cell proliferation and viability

We next investigated the impact of MRPS23 knockdown on the proliferation and viability of BC cells using CCK-8 and EdU assays. As shown in Figure 3A, the CCK-8 assay revealed a significant reduction in cell viability in MRPS23-deficient cells compared to wild-type controls. This result was further corroborated by the EdU assay, which also demonstrated a marked decrease in the proliferative capacity of MRPS23 knockdown cells (Figure 3B).

These findings align with our earlier results, where MRPS23 knockdown led to a reduction in the expression levels of key cell proliferative genes. The observed decline in gene expression following MRPS23 silencing directly correlates with the impaired proliferation seen in both the CCK-8 and EdU assays. Specifically, the knockdown of MRPS23 not only suppressed cell growth but also downregulated critical proliferation-associated pathways, reinforcing the idea that MRPS23 regulates cancer cell proliferation at both molecular and cellular levels. Together, these results suggest that MRPS23 plays a pivotal role in promoting BC cell proliferation by modulating the expression of proliferative genes. Its knockdown significantly impairs this process, highlighting MRPS23 as a key driver of BC progression. Therefore, targeting MRPS23 could serve as a potential therapeutic strategy for limiting tumor growth in aggressive BC subtypes.

3.3. Effect of MRSP23 Transfection on Migration of BC cells

We investigated the impact of MRPS23 on the aggressive behavior of BC cells, specifically focusing on their migration and invasion capabilities. To assess the role of MRPS23 overexpression in cell migration, scratch assays were performed. The results demonstrated that, compared to the control group, overexpression of MRPS23 significantly enhanced the migration rate of BC cells after 48 hours (Figure 3C). In contrast, the knockdown of MRPS23 resulted in a marked reduction in cell migration, indicating the crucial role of MRPS23 in promoting this behavior. Taken together, these results suggest that MRPS23 overexpression plays a significant role in enhancing the migration capacity of BC cells. These observations underscore the potential of MRPS23 as a critical regulator of tumor progression and a possible target for therapeutic intervention.

3.4. Effect of MRSP23 transfection on the invasion of BC cells

A transwell assay was performed to evaluate the impact of MRPS23 knockdown and overexpression on the invasive abilities of BC cells. MDA-MB-231 cells were transfected with MRPS23 overexpression and knockdown constructs, and their invasive behavior was assessed after 24 hours. In the MRPS23 overexpression group, a significant increase in cell invasion was observed compared to the control group, indicating that elevated MRPS23 levels enhance the invasive potential of BC cells. Conversely, the knockdown of MRPS23 resulted in a marked reduction in cell invasion compared to the sh-Ctrl group, highlighting the inhibitory effects of MRPS23 silencing on BC cell

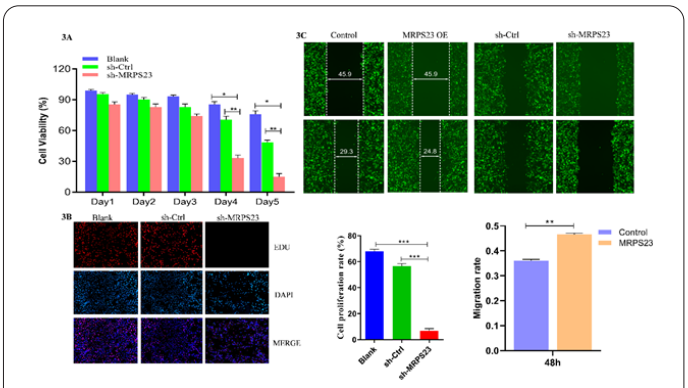

Fig. 3. Impact of MRPS23 knockdown on BC cell viability, proliferation, and migration. (A) Cell viability was assessed using the CCK-8 assay. **(B)** Cell proliferation was evaluated through the EdU assay and visualized via fluorescence microscopy. **(C)** Comparative analysis of MRPS23 overexpression and knockdown effects on BC cells after 48 hours using scratch assay, relative to the control group.

invasiveness (Figure 4A, B, C). These results underscore the crucial role of MRPS23 in regulating the invasion of MDA-MB-231 cells. The data suggest that MRPS23 acts as a pro-invasive factor in BC, promoting more aggressive cellular behavior and potentially contributing to cancer progression.

3.5. MRPS23-inhibition promotes cell apoptosis

To further investigate the impact of MRPS23 on cell physiology, we assessed apoptosis using the TUNEL assay. Our results revealed a significantly higher rate of apoptosis in MRPS23-depleted cells compared to control cells (Figure 4D). This finding suggests that inhibition of MRPS23 induces apoptosis, indicating a role for MRPS23 in cell survival regulation. Moreover, the observed increase in apoptotic activity in MRPS23-depleted cells correlates with reduced cell growth in BC cells. These findings highlight MRPS23 as a potential regulator of cell survival mechanisms and provide insight into its role in BC progression.

3.6. Western Blot Analysis

To further corroborate the inhibitory effects of MRPS23 silencing on BC progression, protein expression levels were assessed through western blot analysis. Specifically, the expression of epithelial and mesenchymal (EMT) markers, including E-Cadherin, N-Cadherin, Fibronectin, and the transcription factor TWIST1, was evaluated in MRPS23 knockdown and overexpression models of BC cells and compared to control groups. Notably, MRPS23 was significantly overexpressed in BC cells compared to normal breast cells (Figure 5D), suggesting that MRPS23 overexpression may contribute to the malignant phenotype observed in BC. Figure 5A shows the results of western blot analysis assessing the expression levels of MRPS23 following gene knockdown in BC cells. The results demonstrated a significant reduction in the levels of E-Cadherin, N-Cadherin, Snail1, and TWIST1 proteins following MRPS23 knockdown in BC cells, suggesting that silencing MRPS23 attenuates the EMT, a process critical to cancer cell invasion and metastasis (Figure 5B). Notably, the decrease in these markers in the MRPS23 knockdown group compared to the control group underscores the potential of MRPS23 silencing as a therapeutic strategy against BC progression. Conversely, in MRPS23-overexpressing cells, elevated expression levels of N-cadherin, fibronectin, and TWIST1 were observed, which are associated with enhanced EMT and invasive behavior, further emphasizing MRPS23's role as a promoter of cancer cell aggressiveness (Figure 5C). Overall, these results indicate that MRPS23 plays a crucial role in modulating key proteins involved in EMT, and its silencing may offer a promising approach to suppress the invasion and progression of BC.

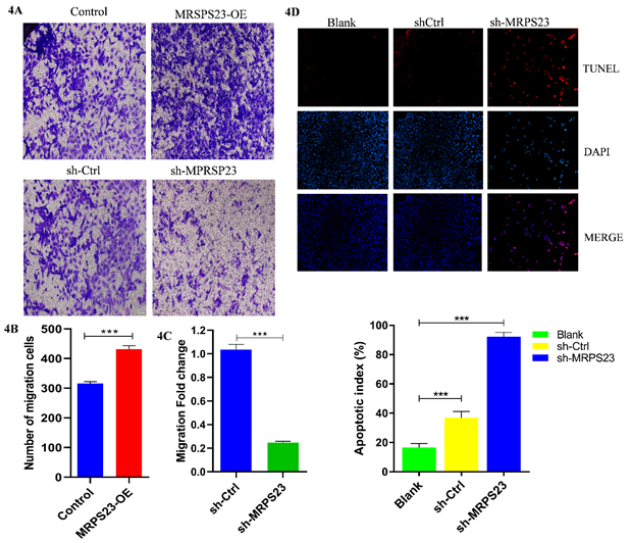

Fig. 4. Impact of MRPS23 overexpression and knockdown on BC cell invasion and apoptosis. (A) Representative images showing the effects of MRPS23 knockdown and overexpression on BC cell invasion. **(B, C)** Quantitative analysis of the relative changes in invasion following MRPS23 knockdown or overexpression. **(D)** Apoptosis rates of BC cells after MRPS23 knockdown, with images captured at 100× magnification.

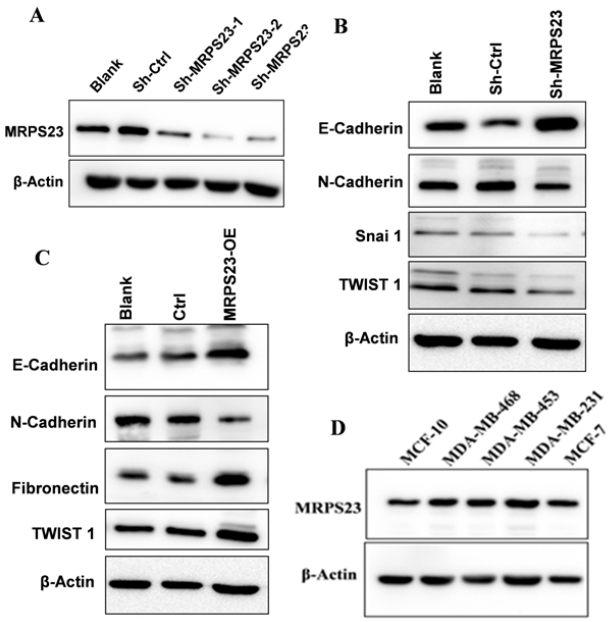

Fig. 5. Protein expression analysis by Western blot. (A) Evaluation of E-cadherin, N-cadherin, and MRPS23 protein levels in BC cells. **(B)** Validation of MRPS23 gene knockdown efficiency in BC cells. **(C)** Analysis of E-cadherin, N-cadherin, Fibronectin, and TWIST1 expression in MRPS23-depleted BC cells. **(D)** Comparative expression of MRPS23 protein in normal breast cells and three BC cell lines.

4. Discussion

In this study, we explored the impact of MRPS23 knockdown on BC progression by examining changes in both genomic and protein expression using qRT-PCR and western blot analysis. Our key finding highlights the significant association between MRPS23 knockdown and the regulation of proteins involved in cell division, as well as those mediating cancer cell migration and invasion. These proteins are crucial in determining the progression and metastatic potential of BC cells. Notably, our results suggest that the suppression of MRPS23 disrupts pathways essential for cancer cell survival, proliferation, and dissemination. As outlined in prior studies, MRPS23 is consistently upregulated across various cancers, where it plays an integral role in processes like cell differentiation, uncontrolled proliferation, and resistance to apoptosis [9,10,16,17]. By silencing MRPS23, we observed a reduction in cancer cell

motility and invasiveness, further supporting the hypothesis that this gene contributes to metastatic behavior. Based on these findings, we propose that MRPS23 represents a promising molecular target for BC therapy. Suppressing MRPS23 could provide a dual benefit: inhibiting tumor growth and limiting the metastatic spread of cancer cells. Consequently, MRPS23 knockdown may offer a novel approach in developing more effective therapeutic strategies and diagnostic tools for BC, particularly in aggressive and treatment-resistant forms of the disease.

To the best of our knowledge, this study is the first to explore the direct association of MRPS23 with BC progression and development. Through our qRT-PCR expression analysis, we observed that the downregulation of MRPS23 led to a significant decrease in the expression of key migratory markers such as cadherin's (E and N), SNAI1/2, and TWIST1 in BC cells. These proteins are well-established contributors to cancer cell migration, as evidenced by previous studies across multiple cancer types, where cadherin's, fibronectin, SNAI1/2, and TWIST1 have been implicated in facilitating EMT and promoting metastatic behavior [29-33]. Our findings suggest that MRPS23 influences the expression of these critical EMT markers, and its knockdown leads to a reduction in the metastatic potential of BC cells, reinforcing the importance of MRPS23 in BC cell migration and invasion. Furthermore, our data demonstrated that MRPS23 downregulation also significantly reduced the expression of key proliferative genes such as *Cyclin D1*, *Axin 2*, *LEF1*, *NKD1*, and *Survivin*. These genes are critical regulators of cell cycle progression and survival pathways, and their decreased expression upon MRPS23 suppression indicates a loss of proliferative capacity in BC cells. This supports our conclusion that MRPS23 plays a pivotal role in regulating both BC cell proliferation and migration, thus contributing to overall cancer progression [34-38]. By targeting MRPS23, we observed a dual effect—suppressing not only the migratory and invasive abilities of the BC cells but also their proliferative capacity, highlighting the potential of MRPS23 as a novel therapeutic target in BC. Interestingly, our findings present a stark contrast to recent large-scale genomic studies, such as the one conducted on a cohort of Norwegian BC patients, where MRPS23 amplification was observed in 8% of primary tumors and 9% of lymph node metastases [39]. While that study emphasized the potential oncogenic role of MRPS23 through its amplification in tumor tissues, our research provides novel insights into the suppressive effects of MRPS23 knockdown on BC progression. This suggests that targeting MRPS23 could offer a promising therapeutic strategy for mitigating BC metastasis and growth.

Migration and invasion are critical processes that play key roles in cancer metastasis and progression. During these processes, individual cancer cells or collective clusters, strands, or cords of cells detach from the primary tumor, invade the surrounding tissue stroma, and enter the bloodstream. Once in circulation, these cells are transported to distant organs, where secondary tumors may form [40]. In our study, we demonstrated that MRPS23 knockdown significantly reduces the migration rate of BC cells, as observed in a wound healing assay. This suggests a direct involvement of MRPS23 in promoting cancer cell motility, which is a key factor in tumor progression. Our findings align with previous studies showing that MRPS23 is associated with cellular functions such as proliferation, invasion, and migration [41]. Furthermore, our invasion assay showed a substantial reduction in the invasive capacity of BC cells upon MRPS23 knockdown. This decreased invasiveness supports the hypothesis that MRPS23 plays a pivotal role in driving the metastatic behavior of BC cells. These observations reinforce the potential biological significance of MRPS23 in BC. For the first time, we have revealed that the downregulation of MRPS23 leads to a marked decrease in both the proliferation and metastasis of BC cells. This reduction in both migration and invasion underscores the gene's importance in regulating cancer progression. Given the central role of MRPS23 in these processes, our findings suggest that targeting MRPS23 could offer a novel therapeutic strategy for mitigating BC progression and metastasis.

Cancer encompasses a diverse group of diseases characterized by uncontrolled cell growth and the potential to invade or spread to other parts of the body. Recent research has highlighted the critical roles of various molecular players, including proteins such as RNF-121, in cancer progression [42].

Overall, our research adds to the growing body of evidence supporting the critical role of MRPS23 in cancer biology. By demonstrating its influence on key hallmarks of cancer, such as cell proliferation, migration, and invasion, we provide a strong rationale for exploring MRPS23 as a potential therapeutic target. Future studies should focus on identifying the precise molecular mechanisms by which MRPS23 influences these pathways. Specifically, *in-vivo* experiments using xenograft models of BC with MRPS23 knockdown will be essential to assess its impact on tumor growth and metastasis. Additionally, pharmacological inhibition of MRPS23 in these models could further evaluate its therapeutic potential. Investigating the downstream molecular pathways regulated by MRPS23 through RNA sequencing or proteomic analysis would also enhance our understanding of its role in cancer progression. These efforts will offer more robust evidence of MRPS23's therapeutic relevance in BC.

5. Conclusion

Our study identified a strong association between MRPS23 knockdown and reduced metastatic potential in BC cells. MRPS23 downregulation significantly decreased cell migration, invasion, and proliferation, highlighting its critical role in tumor progression. These findings suggest that MRPS23 is a key regulator of metastasis and growth in BC. Given its involvement in these processes, MRPS23 represents a promising therapeutic target. Targeting MRPS23 could offer a novel approach to limit tumor growth and metastasis, providing a potential new direction for BC treatment. Future research should focus on exploring the molecular mechanisms by which MRPS23 contributes to cancer progression to validate its therapeutic potential.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors consent to publication.

Availability of data and materials
All data generated or analyzed during this study are included in this published article and are available to the corresponding author by request.

Competing interests
All authors declare that they have no conflicts of interest.

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Author's contributions
FAK conducted the experimental work, drafted the initial manuscript, and took the lead in refining, editing, and revising the final version, as well as designing the tables and figures. DF, FSA, and US contributed to the visualization and revisions. JD and XYJ provided supervision for the study and critically reviewed and revised the manuscript. All listed authors have made significant, direct, and intellectual contributions to the work and have approved the final manuscript for publication.

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