

Original Research Article

FMNL1 promotes growth and metastasis of breast cancer by inhibiting BRCA1 via upregulation of HMGA1

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Abstract

Purpose: To investigate the role and mechanism of formin-like protein 1 (FMNL1) in breast cancer progression.

Methods: Expression of FMNL1 in breast cancer cells was evaluated using quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and western blotting. Colony formation and CCK8 assays were performed to assess cell proliferation. Cell migration and invasion were determined using wound-healing and Transwell assays, respectively.

Results: Data from UALCAN prediction (<http://ualcan.path.uab.edu/analysis.html>) showed that FMNL1 was significantly upregulated in primary breast cancer tissue compared to normal tissue ($p < 0.01$). Enhanced FMNL1 mRNA and protein expression was also identified in breast cancer cells. shRNA-mediated FMNL1 knockdown decreased viability of breast cancer cells and reduced cell proliferation, migration, and invasion. Expression of protein high mobility group AT-hook 1 (HMGA1) was reduced, whereas breast cancer gene 1 (BRCA1) expression was enhanced, in breast cancer cells transfected with shRNA-FMNL1. Overexpression of HMGA1 attenuated FMNL1-knockdown-induced decreased HMGA1 expression and increased BRCA1 expression in breast cancer cells. BRCA1 knockdown counteracted the suppressive effects of FMNL1 silencing on breast cancer cell proliferation, migration, and invasion.

Conclusion: FMNL1 promotes breast cancer cell growth and metastasis by inhibiting BRCA1 via upregulation of HMGA1, providing a potential therapeutic target for breast cancer.

Keywords: Formin-like protein 1, High mobility group AT-hook 1, Breast cancer gene 1, Breast cancer, Cell growth, Metastasis

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INTRODUCTION

Breast cancer is one of the most common malignancies among women worldwide [1]. Early diagnosis and improvement of therapeutic

strategies has reduced the death rate among breast cancer patients [1]. However, metastasis is still the major cause of death in breast cancer patients, accounting for more than 90 % of cancer-related mortality [1]. Therefore, strategies

to suppress breast cancer metastasis are important for the treatment of breast cancer.

Formin-like protein 1 (FMNL1) is expressed in hematopoietic and lymphoid tissues, binds to actin filaments, and participates in cellular processes, such as phagocytosis, cell adhesion, and cell migration [2]. FMNL1 has been reported to be overexpressed in human hematological malignancies, including leukemic and Non-Hodgkin's lymphoma cell lines [3], and contributes to leukemic cell proliferation and migration [4]. Downregulation of FMNL1 suppressed bone metastasis in non-small cell lung cancer through reduction of transforming growth factor beta 1 (TGF β 1) [5]. FMNL1 was overexpressed in basal type breast cancer [6], and the invasiveness of breast carcinoma cells was suppressed through downregulation of FMNL1 [7]. However, the mechanism involved in FMNL1-mediated breast cancer progression has not yet been reported.

High mobility group AT-hook 1 (HMGA1) interacts with the transcription machinery to regulate transcription of target genes through alteration of chromatin structure and participates in benign and malignant neoplasias [8]. Overexpression of HMGA1 promoted breast cancer invasion and metastasis [9]. Silencing of HMGA1 slowed breast cancer growth and metastasis [10]. FMNL1 was hypothesized to mediate HMGA1 during the progression of breast cancer.

The involvement of FMNL1 in breast cancer cell growth and metastasis was investigated in this study, and targeting the downstream pathway involved in the metastatic pattern of FMNL1-mediated breast cancer might provide a novel strategy for treatment.

EXPERIMENTAL

Cell culture

Breast cancer cells (MDA-MB-231, MCF-7, MDA-MB-361) and human mammary epithelial cells (HMECs) were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were cultured in Dulbecco's modified Eagle medium (DMEM, Hyclone, Logan, UT, USA) with 10 % fetal bovine serum (FBS, Hyclone) in a 37 °C incubator.

Cell transfection

MCF-7 cells were plated in a 96-well plate and transfected with shRNA targeting FMNL1 (shFMNL1) or negative control shRNA (shNC)

using Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA). MCF-7 cells were cotransfected with shFMNL1 and pcDNA3.1-HMGA1 (RiboBio, Guangzhou, China) or pcDNA vector with Lipofectamine 2000 (Thermo Fisher Scientific). MCF-7 cells were also cotransfected with shFMNL1 and shBRCA1 using Lipofectamine 2000.

Cell viability

Two days post-transfection, MCF-7 cells that had been cultured in 96-well plates for 24, 48, or 72 hours were incubated with 10 μ L CCK8 solution (Dojindo, Kumamoto, Japan) for 2 hours. Absorbance at 490 nm was measured using an ELISA reader (BioTek, Winooski, VT, USA).

Cell proliferation

Two days post-transfection, MCF-7 cells were plated in a 6-well plate. The DMEM was replaced every 3 days, and 14 days later formaldehyde-fixed cells were stained with 0.4 % crystal violet (Sigma-Aldrich, St. Louis, MO, USA). The cells were counted under a light microscope (Olympus, Tokyo, Japan).

Wound-healing assay

Two days post-transfection, MCF-7 cells were plated in a 6-well plate, and a scratch wound was made in the monolayer with a pipette. Twenty-four hours later, cellular debris was removed, and wound width was photographed under a microscope (Olympus).

Transwell assay

The upper chamber of a transwell chamber (BD Biosciences, San Jose, CA, USA) was coated with Matrigel (Corning, Mountain View, CA, USA), and 100 μ L MCF-7 cell suspension in serum-free DMEM was added to the chamber. The lower chamber was filled with DMEM with 15 % FBS (400 μ L). Forty-eight hours later, formaldehyde-fixed cells in the lower chamber were stained with 0.1 % crystal violet (Sigma-Aldrich) and counted under a microscope (Olympus).

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

RNA was isolated from the breast cancer cells using Trizol (Thermo Fisher Scientific). Complementary DNA (cDNA) was synthesized using the Reverse Transcription System (Thermo Fisher Scientific), and expression of FMNL1 mRNA was determined using Power SYBR

Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) with β -actin as the endogenous control. The primer sequences used are shown in Table 1.

Table 1: Primer sequences used in PCR

ID	Sequence (5'-3')
β -actin F	TATTGGCAACGAGCGGTTCC
β -actin R	GGCATAGAGGTCTTTACGGATGTC
FMNL1 F	GAAGCTGAAGAGCTATGTGG
FMNL1 R	CCTGCGTGGACTCCTGAACT

F = forward, R = reverse. FMNL1 = formin-like protein 1

Western blotting

Cells were lysed in RIPA Lysis and Extraction Buffer (Thermo Fisher Scientific), and the protein concentration was determined using the bicinchoninic acid protein kit (Thermo Fisher Scientific). SDS-PAGE was used to separate the samples, and the samples were then electro-transferred onto PVDF membranes (Millipore, Bedford, MA, USA). The membranes were blocked in 5 % bovine serum albumin and probed overnight with primary antibodies: anti-FMNL1 (1:2000, Cell Signaling Technology, Danvers, MA, USA), anti-HMGA1 and anti-BRCA1 (1:2500, Cell Signaling Technology), and anti- β -actin (1:4000, Cell Signaling Technology). Following incubation with the corresponding horseradish peroxidase-labeled secondary antibody (1:5000; Cell Signaling Technology), the density of immunoreactive bands on the membranes was detected using enhanced chemiluminescence (KeyGen, Nanjin, China).

Statistical analysis

Data are expressed as mean \pm standard error of the mean (SEM), and comparison between groups was determined by one-way analysis of variance (ANOVA) or Student's *t*-test. The $p < 0.05$ was considered statistically significant.

RESULTS

Enhanced FMNL1 in breast cancer

Ualcan analysis (<http://ualcan.path.uab.edu/analysis.html>) showed that FMNL1 was upregulated in breast cancer tissue ($n = 1097$) compared to normal tissue ($n = 114$) based on The Cancer Genome Atlas (TCGA) samples (Figure 1 A). Moreover, higher expression levels of FMNL1 mRNA (Figure 1 B) and protein (Figure 1 C) were validated in breast cancer cells (MDA-MB-231, MCF-7, MDA-MB-361) compared with HMECs.

These results suggest a possible correlation between FMNL1 and breast cancer progression.

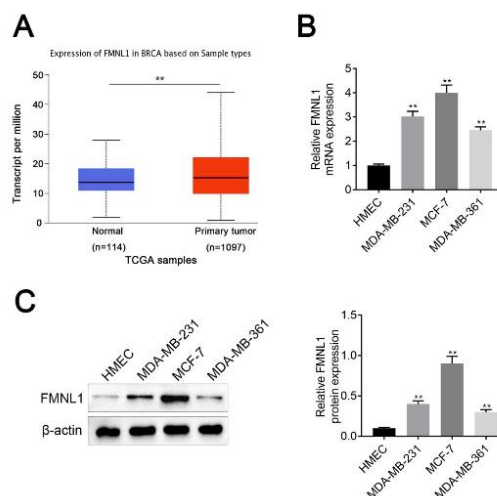


Figure 1: Enhanced FMNL1 in breast cancer. (A) FMNL1 was upregulated in breast cancer tissue ($n = 1097$) compared to normal tissue ($n = 114$) based on TCGA samples and UALCAN analysis (<http://ualcan.path.uab.edu/analysis.html>). (B) FMNL1 mRNA expression was increased in breast cancer cells (MDA-MB-231, MCF-7, MDA-MB-361) compared to human mammary epithelial cells (HMECs). (C) Expression of FMNL1 protein was increased in breast cancer cells (MDA-MB-231, MCF-7, MDA-MB-361) compared to HMECs. **MDA-MB-231, MCF-7, MDA-MB-361 vs. HMECs, $p < 0.01$. FMNL1, formin-like protein 1; TCGA, The Cancer Genome Atlas

FMNL1 contributed to breast cancer cell proliferation

MCF-7 cells were transfected with shRNA targeting FMNL1 (shFMNL1) to investigate the role of FMNL1 in breast cancer progression. Expression of FMNL1 protein was lower in MCF-7 cells transfected with shFMNL1 than in cells transfected with shNC (Figure 2 A). Silencing of FMNL1 reduced viability of MCF-7 cells (Figure 2 B) and suppressed cell proliferation (Figure 2 C), demonstrating that knockdown of FMNL1 exerted an anti-proliferative effect on breast cancer cells.

FMNL1 contributed to breast cancer cell metastasis

In addition to the anti-proliferative effect on breast cancer cells, silencing of FMNL1 inhibited MCF-7 cell migration (Figure 3 A) and invasion (Figure 3 B), indicating that knockdown of FMNL1 played an anti-invasive role in breast cancer cells.

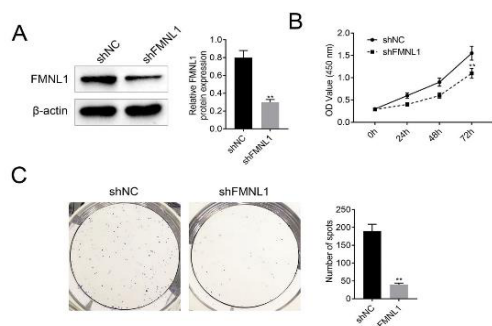


Figure 2: FMNL1 contributed to breast cancer cell proliferation. (A) Expression of FMNL1 protein was lower in MCF-1 cells transfected with shFMNL1 than in those transfected with shNC. (B) Silencing of FMNL1 reduced MCF-7 cell viability. (C) Silencing of FMNL1 suppressed the proliferation of MCF-7 cells. ** shFMNL1 vs. shNC, $p < 0.01$

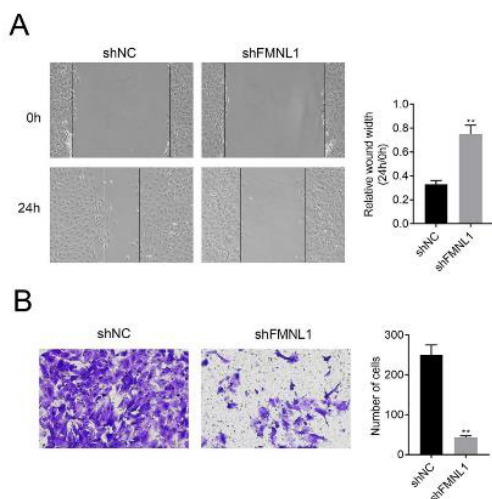


Figure 3: FMNL1 contributed to breast cancer cell metastasis. (A) Silencing of FMNL1 suppressed migration of MCF-7 cells. (B) Silencing of FMNL1 suppressed the invasion of MCF-7 cells. ** shFMNL1 vs. shNC, $p < 0.01$

FMNL1 inhibited BRCA1 through downregulation of HMGA1

Western blotting was used to assess the underlying mechanism involved in FMNL1-mediated breast cancer progression. Expression of HMGA1 protein was reduced, whereas BRCA1 expression was enhanced, in MCF-7 cells transfected with shFMNL1 (Figure 4). However, overexpression of HMGA1 downregulated expression of BRCA1 protein and upregulated HMGA1 protein in MCF-7 cells transfected with shFMNL1 (Figure 4), revealing that silencing of FMNL1 increased BRCA1 expression through downregulation of HMGA1 in breast cancer cells.

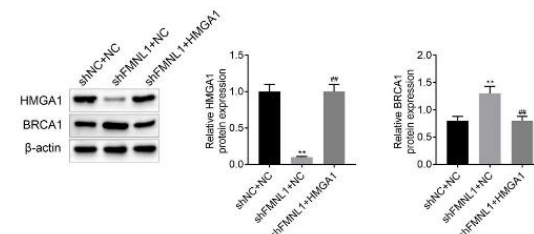


Figure 4: FMNL1 inhibited BRCA1 through downregulation of HMGA1. Silencing of FMNL1 reduced expression of HMGA1 protein and enhanced BRCA1 protein expression in MCF-7 cells. Overexpression of HMGA1 downregulated expression of BRCA1 protein and upregulated HMGA1 protein in MCF-7 cells transfected with shFMNL1. ** shFMNL1 + NC vs. shNC, $p < 0.01$. ## shFMNL1 + HMGA1 vs. shFMNL1 + NC, $p < 0.01$

Knockdown of BRCA1 attenuated FMNL1-induced inhibition of breast cancer progression

The suppressive effect of FMNL1 silencing on MCF-7 cell proliferation was reversed by silencing of BRCA1 (Figure 5 A). Moreover, knockdown of FMNL1 counteracted the suppressive effects of FMNL1 silencing on migration (Figure 5 B) and invasion (Figure 5 C) of MCF-7 cells, indicating that FMNL1 contributed to breast cancer cell metastasis through regulation of BRCA1.

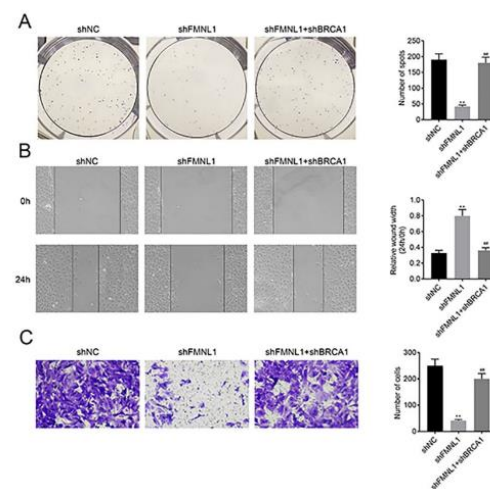


Figure 5: Knockdown of BRCA1 attenuated FMNL1-induced inhibition of breast cancer progression. (A) Knockdown of FMNL1 counteracted the suppressive effect of FMNL1 silencing on MCF-7 cell proliferation. (B) Knockdown of FMNL1 counteracted the suppressive effect of FMNL1 silencing on MCF-7 cell migration. (C) Knockdown of FMNL1 counteracted the suppressive effect of FMNL1 silencing on MCF-7 cell proliferation; **shFMNL1 vs. shNC, $p < 0.01$. ##shFMNL1 + shBRCA1 vs. shFMNL1, $p < 0.01$

DISCUSSION

Formins regulate cytoskeletal remodeling through binding to actin filaments, thus modulating the migration and invasion of adherent cancer cells [11]. Inhibition of formin homology 2 domain containing 1 interfered with breast cancer cell proliferation and invasion [12]. Because a previous study showed that FMNL1 was overexpressed in basal type breast cancer [6], the role of FMNL1 in breast cancer progression was investigated in this study.

Firstly, FMNL1 was overexpressed in breast cancer cells. Arjonen *et al* showed that FMNL1 was upregulated in breast cancer with poor prognosis [13]. The relation between FMNL1 expression and clinicopathological parameters of patients with breast cancer should be investigated to explore the diagnostic and prognostic roles in breast cancer. Consistent with the findings of a previous study that downregulation of FMNL1 suppressed the invasiveness of breast carcinoma cells [7], knockdown of FMNL1 in this study reduced viability of breast cancer cells and repressed cell proliferation, migration, and invasion.

Results of this study demonstrated that expression of HMGA1 protein was reduced by knockdown of FMNL1. Ectopic expression of HMGA1 attenuated the FMNL1 silencing-induced decrease of HMGA1. Suppression of FMNL1 blocked the TGF β 1 pathway to reduce bone metastasis in non-small cell lung cancer [5]. TGF β 1 has been shown to induce specificity protein 1 and enhance the promoter activity of HMGA1 to contribute to breast cancer cell growth and metastasis [14]. Hence, silencing of FMNL1 in this study might suppress the promoter activity of HMGA1 through reduction of TGF β 1 to decrease HMGA1 expression and suppress breast cancer progression. A previous study has shown that HMGA1 binds to the promoter of *BRCA1*, and negatively regulates *BRCA1* expression in sporadic breast carcinoma [15]. Expression of *BRCA1* protein was enhanced by knockdown of FMNL1 in breast cancer cells, and ectopic expression of HMGA1 attenuated the FMNL1 silencing-induced increase in *BRCA1*. *BRCA1* has a pleiotropic biological function and may play a role in transcriptional regulation, chromatin remodeling, DNA damage repair, cell cycle regulation, and checkpoint control [16]. Reduced *BRCA1* expression is often observed in sporadic breast cancer, and decreased *BRCA1* expression is positively correlated with the invasion of human breast cancer [17]. High expression levels of *BRCA1* inhibited the proliferation of breast cancer cells [18].

Knockdown of *BRCA1* in this study attenuated FMNL1-induced inhibition of breast cancer progression. Therefore, silencing of FMNL1 might suppress breast cancer cell growth and metastasis through downregulation of HMGA1 and upregulation of *BRCA1*.

CONCLUSION

This study provides evidence that FMNL1 contributes to breast cancer cell growth and metastasis. Furthermore, knockdown of FMNL1 *in vitro* decreases HMGA1 and increases *BRCA1* to suppress breast cancer cell growth and metastasis. However, a clinically relevant animal model of breast cancer should be used to confirm the suppressive role of FMNL1 silencing on breast cancer growth.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Qian Zhang and Hua Yang designed the study and supervised the data collection. Caiyi Tang and Qian Wang analyzed and interpreted the data. Lili Ren, Chao Jia, and Hui Zou prepared the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

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REFERENCES

- Liang Y, Zhang H, Song X, Yang Q. Metastatic heterogeneity of breast cancer: Molecular mechanism and potential therapeutic targets. *Semin Cancer Biol* 2020; 60: 14-27.
- Katoh M, Katoh M. Identification and characterization of human FMNL1, FMNL2 and FMNL3 genes in silico. *Int J Oncol* 2003; 22(5): 1161-1168.
- Favaro PMB, Traina F, Vassallo J, Brousset P, Delsol G, Costa FF, Saad STO. High expression of FMNL1 protein in T non-Hodgkin's lymphomas. *LEukemia Res* 2006; 30(6): 735-738.
- Favaro P, Traina F, Machado-Neto JA, Lazarini M, Lopes MR, Pereira JKN, Costa FF, Infante E, Ridley AJ, Saad STO. FMNL1 promotes proliferation and migration of leukemia cells. *J Leukocyte Biol* 2013; 94(3): 503-512.
- Yang X-Y, Liao J-J, Xue W-R. FMNL1 downregulation suppresses bone metastasis through reducing TGF- β 1 expression in non-small cell lung cancer (NSCLC). *Biomed Pharmacother* 2019; 117: 109126.
- Gardberg M, Heuser VD, Ijijn K, Kampf C, Uhlen M, Carpén O. Characterization of Leukocyte Formin FMNL1 Expression in Human Tissues. *J Histochem Cytochem* 2014; 62(6): 460-470.
- Mogg J, Heuser V, Peippo M, Gardberg M, Carpen O: Formin-like protein 1: a new drug target in breast cancer. *J Pathol* 2017, 241: S8-S8.
- Fusco A, Fedele M. Roles of HMGA proteins in cancer. *Nat Rev Cancer* 2007; 7(12): 899-910.
- Méndez O, Peg V, Salvans C, Pujals M, Fernández Y, Abasolo I, Pérez J, Matres A, Valeri M, Gregori J et al. Extracellular HMGA1 Promotes Tumor Invasion and Metastasis in Triple-Negative Breast Cancer. *Clin Cancer Res* 2018; 24(24): 6367.
- Di Cello F, Shin J, Harbom K, Brayton C. Knockdown of HMGA1 inhibits human breast cancer cell growth and metastasis in immunodeficient mice. *Biochem Bioph Res Co* 2013; 434(1): 70-74.
- DeWard AD, Eisenmann KM, Matheson SF, Alberts AS. The role of formins in human disease. *BBA-Proteins Proteom* 2010; 1803(2): 226-233.
- Heuser VD, Mansuri N, Mogg J, Kurki S, Repo H, Kronqvist P, Carpén O, Gardberg M. Formin Proteins FHOD1 and INF2 in Triple-Negative Breast Cancer: Association With Basal Markers and Functional Activities. *J Breast Cancer* 2018; 12: 1178223418792247-1178223418792247.
- Arjonen A, Kaukonen R, Ivaska J. Filopodia and adhesion in cancer cell motility. *Cell Adhes Migr* 2011; 5(5): 421-430.
- Zu X, Zhong J, Tan J, Tan L, Yang D, Zhang Q, Ding W, Liu W, Wen G, Liu J et al. TGF- β 1 induces HMGA1 expression in human breast cancer cells: implications of the involvement of HMGA1 in TGF- β signaling. *Int J Mol Med* 2015; 35(3): 693-701.
- Baldassarre G, Battista S, Belletti B, Thakur S, Pentimalli F, Trapasso F, Fedele M, Pierantoni G, Croce CM, Fusco A. Negative regulation of BRCA1 gene expression by HMGA1 proteins accounts for the reduced BRCA1 protein levels in sporadic breast carcinoma. *Mol Cell Biol* 2003; 23(7): 2225-2238.
- Mullan PB, Quinn JE, Harkin DP. The role of BRCA1 in transcriptional regulation and cell cycle control. *Oncogene* 2006; 25(43): 5854-5863.
- Wilson CA, Ramos L, Villaseñor MR, Anders KH, Press MF, Clarke K, Karlan B, Chen J-J, Scully R, Livingston D et al. Localization of human BRCA1 and its loss in high-grade, non-inherited breast carcinomas. *Nat Genet* 1999; 21(2): 236-240.
- Zhang W, Luo J, Yang F, Wang Y, Yin Y, Strom A, Gustafsson JÅ, Guan X. BRCA1 inhibits AR-mediated proliferation of breast cancer cells through the activation of SIRT1. *Sci Rep* 2016; 6(1): 22034.