

## Original Research Article

# FAM172A inhibits breast cancer cell growth and metastasis by upregulating Notch 3

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### Abstract

**Purpose:** To investigate the role of family with sequence similarity 172 member A (FAM172A) in breast cancer.

**Methods:** FAM172A expression was evaluated by western blot and quantitative reverse transcription polymerase chain reaction (qRT-PCR). Colony formation and CCK-8 assays were used to assess cell proliferation, while cell migration and invasion were evaluated by Transwell assay.

**Results:** FAM172A expression was reduced in breast cancer cells and tissues when compared to controls ( $p < 0.001$ ). Overexpression of FAM172A repressed proliferation of breast cancer cells ( $p < 0.001$ ) and suppressed cell migration and invasion ( $p < 0.001$ ). Overexpression of FAM172A increased Notch 3 expression in breast cancer cells. Knockdown of Notch 3 attenuated the FAM172A overexpression-induced decrease in breast cancer cell proliferation. Silencing of Notch 3 reversed the FAM172A ( $p < 0.001$ ).

**Conclusion:** FAM172A represses breast cancer cell growth and metastasis via upregulation of Notch 3; thus, FAM172A and Notch 3 are potential targets for breast cancer therapy.

**Keywords:** FAM172A, Notch 3, Breast cancer, Cell growth, Metastasis

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## INTRODUCTION

Breast cancer is one of the most common malignancies in women worldwide [1]. Metastasis is the main cause of death among breast cancer patients [2]. Therefore, appropriate treatment involves suppression of breast cancer metastasis [3]. Although research has increasingly focused on the carcinogenic and metastatic mechanisms of breast cancer, the exact mechanisms remain unclear [2]. Therefore, further understanding of the mechanisms involved in breast cancer

metastasis are urgently needed to treat breast cancer.

Family with sequence similarity 172 member A (FAM172A) is expressed widely in human aortic tissues, macrophages, vascular endothelial cells, and smooth muscle cells [4]. It was shown that deletion of FAM172A promoted endoplasmic reticulum stress and enhanced hepatic steatosis [4]. FAM172A was also found to be involved in cancer pathogenesis, for example, FAM172A was upregulated in colorectal carcinoma tissues

and predicted poor prognosis [5]. As an oncogene, FAM172A promoted papillary thyroid carcinoma cell proliferation and migration [6]. However, FAM172A repressed the epithelial to mesenchymal transition of pancreatic cancer cells [7]. The oncogenic and/or tumor suppressive roles of FAM172A in breast cancer were investigated in this study.

A previous study showed that FAM172A interacted with NF- $\kappa$ B and Notch 1–4 and slowed proliferation of hepatocellular carcinoma cells [8]. Notch signaling has been shown to be involved in breast cancer progression [9] and is considered a therapeutic target of breast cancer [10]. Therefore, FAM172A may play a role in the development of breast cancer by regulating Notch signaling.

In this study, FAM172A expression and the effects of FAM172A expression on breast cancer cell proliferation and metastasis were investigated.

## METHODS

### Tissues

Forty-five paired breast cancer and adjacent noncancer tissues were collected from patients in Nanjing First Hospital, Nanjing Medical University via surgical resection. Patients who gave written informed consent and were diagnosed with breast cancer via imaging modalities and pathology examination were enrolled in this study. This study was approved by the Ethics Committee of Nanjing First Hospital, Nanjing Medical University (approval no. 2017-026) and was in accordance with the 1964 Helsinki Declaration and its later amendments for ethical research involving human subjects [11].

### Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNAs were isolated from tumor cells and tissues and then reverse-transcribed into cDNAs. qRT-PCR analysis of FAM172A was performed using SYBR Green Master Mix (Roche, Mannheim, Germany) with GAPDH as the endogenous control. The primer sequences are shown in Table 1.

**Table 1:** Primers used in PCR study

Gene	Forward (5'-3')	Reverse (5'-3')
GAPDH	ACCACAGTCCATGCCATCAC	TCCACCACCCTGTTGCTGTA
FAM172A	GGTACCATGTCTATTTCTTGAGCTC	AAGCTTCAGCTCTTCGTGCTTGATG

### Cell culture and transfection

MDA-MB-231, MCF-7, and T47D breast cancer cells and human mammary epithelial cells (HMECs) were obtained from the Chinese Academy of Sciences (Shanghai, China) and were cultured in RPMI 1640 medium containing streptomycin-penicillin and 10% fetal bovine serum (Lonza, Basel, Switzerland).

pcDNA-FAM172A and shRNA targeting *NOTCH3* (sh*NOTCH3*) were constructed by RiboBio (Guangzhou, China). MDA-MB-231 and MCF-7 cells were transfected with pcDNA-FAM172A or co-transfected with pcDNA-FAM172A and sh*NOTCH3* using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

### Cell proliferation assay

MDA-MB-231 and MCF-7 cells were seeded into 96-well plates, transfected, and incubated for 24, 48, 72, or 96 h. Then, CCK8 solution (Dojindo, Tokyo, Japan) was added, and the plates were incubated for 2 h. Absorbance at 450 nm was measured using a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA).

MDA-MB-231 and MCF-7 cells were seeded into six-well plates, transfected, and incubated for 10 d. Then, the colonies were fixed and stained with crystal violet. Cells were observed using a light microscope (Olympus, Tokyo, Japan).

### Transwell assay

MDA-MB-231 and MCF-7 cells were suspended in serum-free RPMI 1640 medium and placed in the upper chamber of a Matrigel-coated transwell (Corning, Tewksbury, MA, USA). Medium containing 15% fetal bovine serum was placed in the lower chamber. After 24 h, the invasive cells in the lower chamber were stained with crystal violet and photographed under the microscope. Cell migration was also investigated using a transwell without Matrigel coating.

### Western blot assay

Tissues and cells were lysed in RIPA buffer (Beyotime, Beijing, China) and then centrifugated at 12,000  $\times$  g for 1 h to collect supernatants.

Proteins in the supernatants were separated by SDS-PAGE. Samples were electrotransferred onto PVDF membranes and the membranes were blocked in 5% BSA. The membranes were then incubated with primary antibody: anti-FAM172A (1:1500; Abcam, Cambridge, UK), anti-Notch 3 (1:2000; Abcam), or anti- $\beta$ -actin (1:3000; Abcam). Following incubation with horseradish peroxidase-labelled secondary antibody (1:4000; Abcam) and tetramethylbenzidine, immunoreactivity was visualized using enhanced chemiluminescence (KeyGen, Nanjin, China).

### Statistical analysis

All data were expressed as mean  $\pm$  SEM ( $n = 3$ ) and analyzed by Student's t-test or one-way analysis of variance (ANOVA) using SPSS 11.5.  $P$  values  $< 0.05$  indicated statistically significant differences.

## RESULTS

### FAM172A expression was reduced in breast cancer

Analysis of breast cancer tissues showed that FAM172A mRNA expression was downregulated in breast cancer tissue when compared to normal tissue (Figure 1A). FAM172A protein expression was also downregulated in breast cancer tissues when compared to normal tissues (Figure 1B). Moreover, FAM172A expression was lower in MDA-MB-231, MCF-7, and T47D breast cancer cells than in HMECs (Figures 1C and D), suggesting that FAM172A may play a role in breast cancer progression.

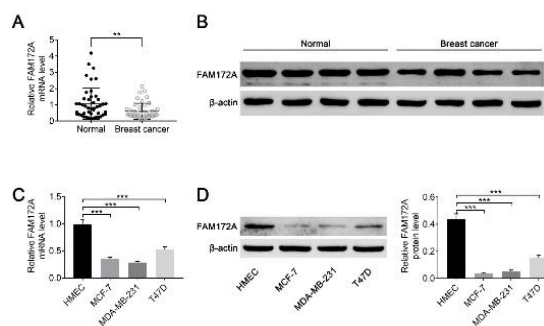
### FAM172A repressed breast cancer cell proliferation

MCF-7 and MDA-MB-231 breast cancer cells were transfected with pcDNA-FAM172A to upregulate FAM172A expression (Figure 2A). MCF-7 and MDA-MB-231 cell viabilities decreased upon transfection with pcDNA-FAM172A (Figure 2B). Moreover, FAM172A overexpression reduced the numbers of MCF-7 and MDA-MB-231 colonies formed (Figure 2C), demonstrating the anti-proliferative effect of FAM172A on breast cancer cells.

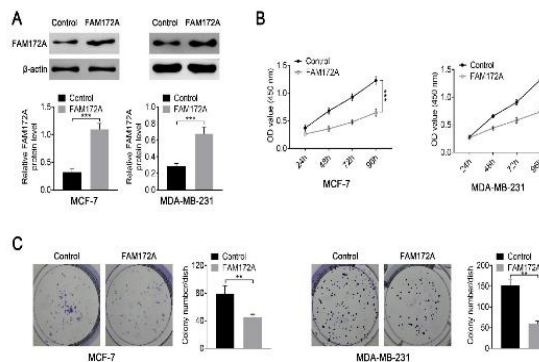
### FAM172A repressed breast cancer cell metastasis

MCF-7 and MDA-MB-231 cell migration was suppressed upon transfection of pcDNA-FAM172A (Figure 3); thus, overexpression of FAM172A repressed breast cancer cell invasion,

indicating the anti-invasive effect of FAM172A on breast cancer.



**Figure 1:** FAM172A expression was reduced in breast cancer. (A) The FAM172A mRNA level was lower in breast cancer tissue than in normal tissue. (B) FAM172A protein expression in breast cancer tissues compared to normal tissues. (C) FAM172A mRNA levels in MDA-MB-231, MCF-7, and T47D breast cancer cells and HMECs. (D) FAM172A protein expression in MDA-MB-231, MCF-7, and T47D breast cancer cells and HMECs.  $**P < 0.01$ ,  $***p < 0.001$  vs. normal tissues or HMECs



**Figure 2:** FAM172A overexpression repressed breast cancer cell proliferation. (A) FAM172A protein expression increased in MDA-MB-231 and MCF-7 cells upon transfection with pcDNA-FAM172A. (B) FAM172A overexpression reduced MDA-MB-231 and MCF-7 cell viabilities. (C) FAM172A overexpression reduced the numbers of MDA-MB-231 and MCF-7 colonies formed.  $**P < 0.01$ ,  $***p < 0.001$  vs. control

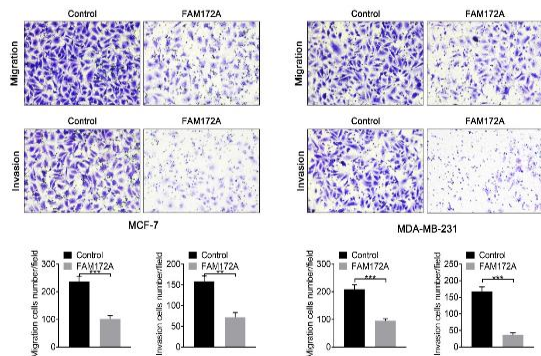
### FAM172A repressed breast cancer cell proliferation and metastasis through upregulation of Notch 3

Notch 3 protein expression was upregulated in MCF-7 and MDA-MB-231 cells overexpressing FAM172A (Figure 4A). In MCF-7 and MDA-MB-231 cells co-transfected with the FAM172A overexpression plasmid and shNOTCH3, the increase in Notch 3 expression upon transfection with the FAM172A overexpression plasmid was reversed (Figure 4B). Silencing of NOTCH3 also reversed the reduction in MCF-7 and MDA-MB-231 cell proliferation that resulted from FAM172A

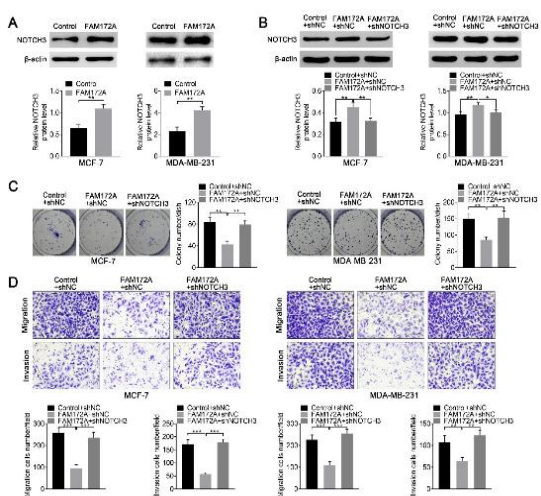
overexpression (Figure 4 C). Knockdown of Notch 3 attenuated the FAM172A overexpression-induced decrease in cell migration and invasion (Figure 4 D). These results showed that FAM172A repressed breast cancer cell proliferation and metastasis through upregulation of Notch 3.

## DISCUSSION

Family with sequence similarity (FAM) proteins function as stress responsive proteins and are implicated in nervous system and brain development [12]. FAM proteins also regulate tumor cell proliferation and metastasis and are poor prognosis predictors [13]. FAM proteins have been shown to be essential for breast cancer progression [14]. Because FAM172A plays distinct roles in tumors, the role of FAM172A in breast cancer was investigated in this study. In our study, FAM172A expression was reduced in breast cancer cells and tissues. In a previous study, high FAM172A expression associated with lymph node involvement and the tumor-node-metastasis stage of patients with colorectal carcinoma, indicating that high FAM172A expression predicted poor prognosis [5]. The associations between FAM172A expression and the clinicopathological parameters of breast cancer patients should be investigated to determine the diagnostic and prognostic potential of FAM172A in breast cancer.



**Figure 3:** FAM172A repressed metastasis of breast cancer cells. Overexpression of FAM172A suppressed MDA-MB-231, and MCF-7 cell migration and invasion. \*\* $P < 0.01$ , \*\*\* $p < 0.001$  vs. control



**Figure 4:** FAM172A repressed breast cancer cell proliferation and metastasis through upregulation of Notch 3. (A) Notch 3 protein expression in MDA-MB-231 and MCF-7 cells upon overexpression of FAM172A. (B) Co-transfection of MDA-MB-231 and MCF-7 cells with shNOTCH3 and the FAM172A overexpression plasmid reversed the increase in Notch 3 resulting from FAM172A overexpression. (C) Co-transfection with shNOTCH3 and the FAM172A overexpression plasmid reversed the decreases in MDA-MB-231 and MCF-7 cell proliferation resulting from FAM172A overexpression. (D) Co-transfection with shNOTCH3 and the FAM172A overexpression plasmid reversed the decrease in MDA-MB-231 and MCF-7 cell migration and resulting from FAM172A overexpression. \* $P < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  vs. control or FAM172A + shNC

A previous study showed that FAM172A functions as either a tumor suppressor or an oncogene in distinct tumors. This study showed that overexpression of FAM172A reduced breast cancer cell viability and suppressed breast cancer cell proliferation, migration, and invasion. FAM172A was shown to promote cell apoptosis and suppress the epithelial to mesenchymal transition in pancreatic cancer [7]. The role of FAM172A on cell apoptosis and the epithelial to mesenchymal transition in breast cancer should be investigated in future research.

It was demonstrated by co-immunoprecipitation assay that FAM172A interacts with NF- $\kappa$ B and Notch 1–4 and has been shown that FAM172A induces cell cycle arrest, thereby slowing proliferation of hepatocellular carcinoma cells [8]. FAM172A upregulated Notch 3 protein expression and induced cell cycle arrest of HepG2 cells [15]. In this study, Notch 3 protein expression was upregulated in breast cancer cells as a result of FAM172A overexpression. Notch 3 signaling was shown to be involved in breast cancer metastasis [16] and chemoresistance [17], and Notch 3 suppressed breast cancer metastasis and tumorigenesis [18]. In this study, silencing of NOTCH3 attenuated the FAM172A overexpression-induced decrease in breast cancer cell proliferation. In addition, the FAM172A overexpression-induced decrease in breast cancer cell migration and invasion was reversed by knockdown of Notch 3. Thus, our study indicates that the suppressive effects of

FAM172A on breast cancer cell proliferation and metastasis are regulated by Notch 3 signaling.

## CONCLUSION

This study has identified FAM172A as a novel tumor suppressor in breast cancer. FAM172A expression is downregulated in breast cancer tissues and cells and FAM172A overexpression represses breast cancer cell proliferation and metastasis through upregulation of Notch 3. Thus, FAM172A/Notch 3 signaling pathway may be a novel mechanism in the carcinogenesis and metastasis of breast cancer. However, the *in vivo* role of FAM172A in breast cancer progression needs to be evaluated using an animal model.

## DECLARATIONS

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None provided.

### Funding

None provided.

### Ethical approval

None provided.

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Conflict of Interest

No conflict of interest associated with this work.

### 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. Chongyin Tang designed the study and supervised the data collection. Guozhu Wang analyzed and interpreted the data. Chongyin Tang and Guozhu Wang prepared the manuscript for publication and reviewed the draft of the manuscript. All authors read and approved the manuscript.

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