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

Chongyin Tang, Guozhu Wang*
Department of Surgery of Thyroid and Breast, Nanjing First Hospital, Nanjing Medical University, Nanjing, Jiangsu 210006, China

*For correspondence: Email: wgz0401_dr@126.com; Tel: +86-025-85453096

Sent for review: 2 March 2022  Revised accepted: 12 June 2022

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

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-κB 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    | ACCACAGTCCATGCCATCAG | TCCACCACCTGTTTGCTGTA |
| FAM172A  | GGTACCAGTCTATTTCCTTGAGCCTC | AAGCTTCAGCTTTCGCTTGTGATG |

**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 (shNOTCH3) 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 shNOTCH3 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 centrifuged at 12,000 × 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-β-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 ± 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.

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-κB 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

Acknowledgements
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.

Open Access
This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/road), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

REFERENCES

1. Dong Y, Liu Y, Zhao L, Qu C. Mediator complex subunit 19 regulates the proliferation, migration and invasion of human breast cancer cells. Trop J Pharm Res 2020; 19(11): 2273-2278.
2. Solanki M, Visscher D. Pathology of breast cancer in the last half century. Human Pathology 2020; 95: 137-148.
3. Zhao W, Prijic S, Urban BC, Tisza MJ, Zuo Y, Li L, Tan Z, Chen X, Mani SA, Chang JT. Candidate antimetastasis drugs suppress the metastatic capacity of breast cancer cells by reducing membrane fluidity. Cancer Res 2016; 76(7): 2037.
4. Xiao F, Gao M, Yang J, He L, Wei H. FAM172A Deletion may enhance hepatic steatosis by promoting ER stress. Digest Dis Sci 2021; 66(9): 3054-3061.
5. Liu W, Wang S, Qian K, Zhang J, Zhang Z, Liu H. Expression of family with sequence similarity 172 member A and nucleotide-binding protein 1 is associated with the poor prognosis of colorectal carcinoma. Oncol Lett 2017; 14(3): 3587-3593.
6. Li MF, Zhang R, Guo MG, Li LX, Lu HK, Lu JX, Jia WP. FAM172A protein promotes the proliferation of human papillary thyroid carcinoma cells via the p38 mitogen-activated protein kinase pathway. Mol Med Rep 2016; 13(1): 353-358.
7. Chen Y, Liu P, Shen D, Liu H, Xu L, Wang J, Shen D, Sun H, Wu H. FAM172A inhibits EMT in pancreatic cancer via ERK-MAPK signaling. Biol Open 2020; 9(2).
8. Shen W, Feng Z, Wang P, Zhang J. FAM172A controls endoplasmic reticulum (ER) stress related to NF-κB signaling pathway in hepatocellular carcinoma. RSC Adv 2017; 7(82): 51870-51878.
9. Kontomanolis EN, Kalagasidou S, Poullidou S, Anthoulaki X, Georgiou N, Papamanolis V, Fasoulakis ZN. The notch pathway in breast cancer progression. Sci World J 2018:2415489-2415489.
10. Han J, Hendzel MJ, Allalunis-Turner J. Notch signaling as a therapeutic target for breast cancer treatment? Breast Cancer Res 2011; 13(3): 210.
11. Association WM. World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. B World Health Organ 2001; 79(4): 373.
12. Nakajima H, Koizumi K. Family with sequence similarity 107: A family of stress responsive small proteins with diverse functions in cancer and the nervous system (Review). Biomed Rep 2014; 2(3): 321-325.
13. Chen C, Li HF, Hu YJ, Jiang MJ, Liu QS, Zhou J. Family with Sequence Similarity 83 Member H Promotes the viability and metastasis of cervical cancer cells and indicates a poor prognosis. Yonsei Med J 2019; 60(7): 611-618.

14. Snijders AM, Lee SY, Hang B, Hao W, Bissell MJ, Mao JH. FAM83 family oncogenes are broadly involved in human cancers: an integrative multi-omics approach. Mol Oncol 2017; 11(2): 167-179.

15. Feng Z, Li H, Liu S, Cheng J, Xiang G, Zhang J. FAM172A induces S phase arrest of HepG2 cells via Notch 3. Oncol Rep 2013; 29(3): 1154-1160.

16. Leontovich AA, Jalalirad M, Salisbury JL, Mills L, Haddox C, Schroeder M, Tuma A, Guicciardi ME, Zammataro L, Gambino MW et al. NOTCH3 expression is linked to breast cancer seeding and distant metastasis. Breast Cancer Res 2018; 20(1): 105.

17. Gu X, Lu C, He D, Lu Y, Jin J, Liu D, Ma X. Notch3 negatively regulates chemoresistance in breast cancers. Tumor Biol 2016; 37(12): 15825-15833.

18. Zhang YQ, Liang YK, Wu Y, Chen M, Chen WL, Li RH, Zeng YZ, Huang WH, Wu JD, Zeng D et al. Notch3 inhibits cell proliferation and tumorigenesis and predicts better prognosis in breast cancer through transactivating PTEN. Cell Death Dis 2021; 12(6): 502.