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ORIGINAL RESEARCH

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High expression of PAFAH1B3 promotes the growth as well as motility and inhibits apoptosis of breast cancer cells

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Abstract

Background: Breast cancer (BC) is the most common type of cancer in women. It is urgent to identify new therapeutic targets and their mechanisms. Platelet activating factor acetylhydrolase 1B3 (PAFAH1B3) is a multimeric enzyme which is a vital metabolic enzyme that mediates lipid metabolism and affects several tumors. This study was performed to elucidate the function of PAFAH1B3 in BC progression and investigate its underlying mechanisms. **Methods**: Gene Expression Profiling Interactive Analysis (GEPIA) database and immunoblot showed the expression of PAFAH1B3 in breast cancer tissues. cell counting kit-8 (CCK-8), colony formation, and transwell assays showed the effects on breast cancer cell growth and migration. Flow cytometry (FCM) and immunoblot assays exhibited the effects on the apoptosis of breast cancer cells. Mechanically, immunoblot was further conducted to confirm the mechanism. **Results**: Our findings reveal that PAFAH1B3 is highly expressed in BC and that the depletion of PAFAH1B3 inhibits BC cell growth and migration while promoting apoptosis. Mechanistically, PAFAH1B3 depletion disrupts the Phosphatidylinositol 3-kinase (PI3K)/Protein Kinase B (AKT) pathway, thereby suppressing BC progression. **Conclusions**: We found that PAFAH1B3 enhances BC cell growth as well as motility via the PI3K/AKT axis and could be a target for BC.

Keywords

Apoptosis; Breast cancer; Migration; PAFAH1B3; PI3K/AKT axis

1. Introduction

Breast cancer (BC) is the most common tumor type diagnosed in women, with a global incidence of approximately 51 cases per 1 million people annually. Its incidence has been increasing, with the total number of BC patients worldwide reaching 21 million and accounting for nearly 2.6% of the female population [1]. In addition, despite advancements in cancer treatments, its mortality remains on the rise in China [2]. The etiology and pathogenesis of BC are associated with factors such as estrogen exposure, genetic predispositions, chronic stress, radiation and environmental factors [2]. While early diagnosis and adjuvant treatments can enhance survival and prognosis, mortality rates remain significant. Triple-negative BC (TNBC), comprising 15–20% of BC cases, is particularly challenging due to the lack of effective therapeutic targets [3]. Therefore, identifying novel targets and understanding their mechanisms is essential to improve treatment outcomes.

PAFAH1B is a heterotrimeric enzyme with 100 kDa [4]. PAFAH1B3, a component of this complex, is essential for brain development [5] and plays a significant role in signal transduction, cellular stress responses and protein transport [4]. It has been implicated in cancer progression [6], functioning as a ribosomal subunit in cell membranes and influencing cell metabolism and lipid metabolism. PAFAH1B3 is widely expressed in various human tumors, with evidence suggesting its role in cancer development across approximately 20 cancer types [7]. Additionally, PAFAH1B3 is a target gene of Kruppel-like factor 9 (KLF9), which promotes pancreatic cancer growth and metastasis [7]. Despite our current understanding on PAFAH1B3, its specific role and mechanisms in BC remain unclear and warrant further investigation.

We assess the role of PAFAH1B3 in BC progression and investigate its underlying mechanisms. Our findings indicate that PAFAH1B3 promotes BC cell growth as well as motility while inhibiting apoptosis via PI3K/AKT pathway, suggesting that PAFAH1B3 may serve as a target for BC.

2. Materials and methods

2.1 Cell culture

Human MCF-10A and BC cell lines MDA-MB-231 and MCF-7 were obtained from American Type Culture Collection (ATCC). Cells were cultured in DMEM (Dulbecco's Modified Eagle Medium, Beyotime, C11995500BT, Grand Island, NY, USA) supplemented with 10% FBS (Fetal Bovine Serum,

Gibco, 10099141, Grand Island, NY, USA) at 37 °C in a 5% CO_2 atmosphere.

2.2 siRNA transfection

siRNAs targeting PAFAH1B3 were purchased from RiboBio and transfections were performed using Lipofectamine 3000 (Invitrogen, L3000015, Grand Island, NY, USA).

2.3 Immunoblot analysis

Proteins were separated by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and transferred to poly (vinylidene fluoride) (PVDF) membranes (Millipore, IPVH00010). The membranes were blocked with 5% non-fat milk and incubated overnight at 4 °C with primary antibodies: PAFAH1B3 (Abcam, ab15673, Cambridge, UK), GAPDH (Glyceraldehyde-3-Phosphate Dehydrogenase, Abcam, ab8245, Cambridge, UK), Bax (BCL-2-associated X protein, Abcam, ab32503, Cambridge, UK), BCL-2 (B-cell lymphoma-2, Abcam, ab32124, Cambridge, UK), Cleaved Caspase-3 (Cell Signaling Technology, 9664, Cambridge, UK), PI3K (Abcam, ab86714, Cambridge, UK), p-PI3K (Abcam, ab182651, Cambridge, UK), AKT (Abcam, ab38449, Cambridge, UK), and p-AKT (Abcam, ab38449, Cambridge, UK). Following incubation with the primary antibodies, the membranes were incubated with Horseradish Peroxidase (HRP) secondary antibodies (Abcam, ab6721, Cambridge, UK) for 1 hour. Then, the protein bands were visualized using an Enhanced Chemiluminescence (ECL) detection kit (Beyotime, P0018, Beijing, China).

2.4 Cell proliferation assay

Cell proliferation was evaluated using the cell counting kit-8 (CCK-8) assay (Beyotime, C0037, Beijing, China).

2.5 Colony formation assay

The cells were plated in 6-well plates and cultured for 14 days. The obtained colonies were then fixed with 4% paraformaldehyde (PFA) and stained with 0.1% crystal violet (Beyotime, C0121, Beijing, China). Colony formation was assessed using a microscope (Zeiss Axio Observer, Oberkochen, BW, Germany).

2.6 Transwell migration and invasion assays

Cell migration and invasion were assessed using Transwell chambers (Corning, 3422, Corning, NY, USA) with or without Matrigel (Corning, 356234, Corning, NY, USA). 5×10^4 cells were seeded in Matrigel-coated Transwell inserts.

2.7 Flow cytometry for apoptosis

Apoptosis was measured using the Annexin V-Fluorescein Isothiocyanate (FITC)/Propidium Iodide (PI) Apoptosis Detection Kit (Beyotime, C1062L, Beijing, China).

2.8 Statistical analysis

Data are expressed as mean ± standard deviation (SD). Statistical analysis was performed by GraphPad Prism (8.0, Graphpad Company, San Diego, CA, USA). A p-value of < 0.05 was considered significant.

3. Results

3.1 PAFAH1B3 is highly expressed in BC

To investigate the role of PAFAH1B3 in BC progression, we first assessed its expression in BC tissues. Analysis of the GEPIA database revealed elevated trascripts per million (TPM) values of PAFAH1B3 in BC tissues (Fig. 1A). Notably, PAFAH1B3 expression was higher in the BC cell lines MDA-MB-231 as well as MCF-7 compared to the normal MCF-10A cells, as confirmed by immunoblot analysis (Fig. 1B). These results indicate that PAFAH1B3 is highly expressed in BC.

3.2 PAFAH1B3 depletion inhibits the growth and migration of BC cells

Next, we examined the effects of PAFAH1B3 depletion on BC cell growth and migration by transfecting breast cancer cells with PAFAH1B3-specific siRNAs. Immunoblotting confirmed that the siRNA transfection effectively reduced PAFAH1B3 expression in these cells, demonstrating successful silencing (Fig. 2A). PAFAH1B3 depletion led to a significant reduction in cell growth, as evidenced by a lower optical density (OD)450 value (Fig. 2B). Similarly, PAFAH1B3 knockdown significantly decreased the number of colonies (Fig. 2C).

Additionally, transwell assays indicated that PAFAH1B3 depletion impaired cell migration and invasion, as reflected by a reduced number of migrating and invading cells (Fig. 2D). These findings demonstrate that PAFAH1B3 depletion inhibits both the growth and migration of BC cells.

3.3 PAFAH1B3 depletion stimulates the apoptosis of BC cells

We further investigated the impact of PAFAH1B3 on BC cell apoptosis. Flow cytometry (FCM) analysis showed that PAFAH1B3 depletion increased the percentage of apoptotic cells (Fig. 3A). Consistent with this, immunoblotting revealed increased levels of Bax and cleaved caspase-3, and decreased levels of BCL-2, indicating enhanced apoptosis (Fig. 3B). Thus, based on these findings, it could be deduced that PAFAH1B3 depletion can promote apoptosis in BC cells.

3.4 PAFAH1B3 depletion inhibits the PI3K/AKT axis in BC cells

Lastly, to elucidate the mechanism through which PAFAH1B3 depletion affects BC progression, we investigated the PI3K/AKT signaling pathway. We observed that PAFAH1B3 depletion led to reduced phosphorylation levels of both PI3K and AKT, suggesting suppression of the PI3K/AKT pathway (Fig. 4). These results indicate that PAFAH1B3 depletion inhibits the PI3K/AKT signaling pathway in BC cells.

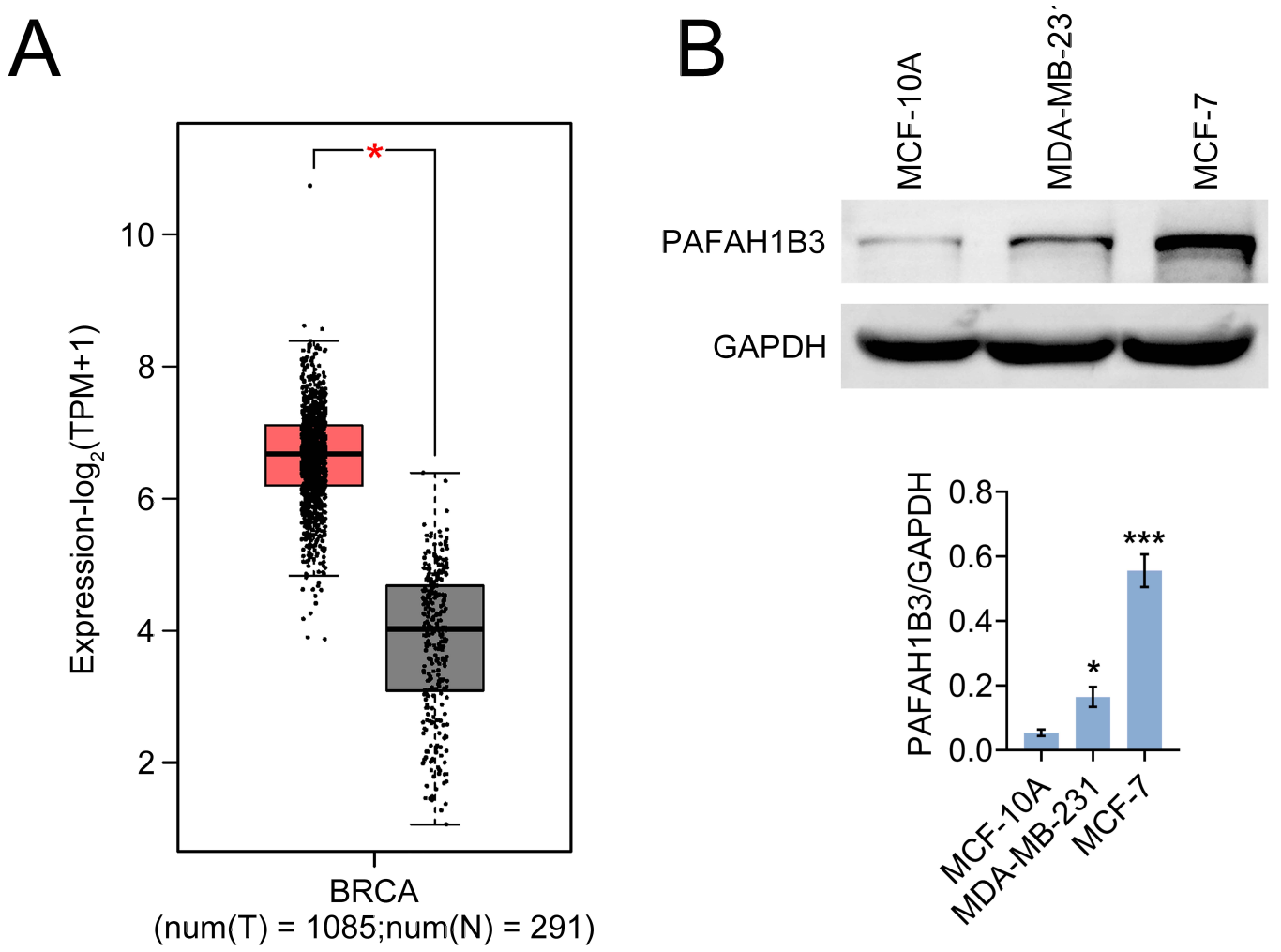

FIGURE 1. PAFAH1B3 is highly expressed in breast cancer. (A) Transcript levels of PAFAH1B3 in breast cancer tissues (n = 1085) and normal tissues (n = 291) as determined by the GEPIA database, expressed as transcripts per million (TPM). (B) Immunoblot analysis of PAFAH1B3 expression in MCF-10A, MDA-MB-231 and MCF-7 cells. *$p < 0.05$, ***$p < 0.001$ compared to MCF-10A. TPM, trascripts per million; BRCA, breast cancer; GAPDH, Glyceraldehyde-3-Phosphate Dehydrogenase; PAFAH1B3, Platelet activating factor acetylhydrolase 1B3.

4. Discussion

BC remains one of the leading causes of mortality among women despite significant advances in treatment [8]. The disease's complexity, including subtypes such as hormone receptor-positive, human epidermal growth factor receptor-2 (HER2)-enriched and TNBC, presents substantial challenges for effective therapeutic strategies [9, 10]. TNBC, in particular, is known for its poor prognosis due to the lack of effective therapies. In this context, our study has focused on the role of PAFAH1B3, a protein found to be highly expressed in BC, especially in the MCF-7 and MDA-MB-231 cell lines. Our findings underscore the importance of identifying new molecular targets like PAFAH1B3, which could enhance treatment options and address existing resistance mechanisms in BC therapy.

The progression of BC is regulated by various molecular pathways that control cell growth, motility, and apoptosis [2]. Herein, our study demonstrated that PAFAH1B3 plays an essential role in promoting the growth and motility of breast tumor cells while inhibiting their apoptosis. Silencing PAFAH1B3 led to a significant decrease in cell viability and invasive capabilities, as evidenced by Transwell assays. Additionally, PAFAH1B3 knockdown increased apoptotic activity, indicated by upregulation of pro-apoptotic markers such as Bax and cleaved caspase-3, and downregulation of the anti-apoptotic factor BCL-2. These findings highlight the critical role of PAFAH1B3 in regulating key aspects of BC cell behavior.

PAFAH1B3 is a heterotrimeric enzyme involved in lipid metabolism and intracellular signaling [5]. It also participates in various processes, including signal transduction, cellular stress responses, and protein transport [11]. Additionally, PAFAH1B3 has been linked to multiple diseases, with research indicating its involvement in neurodevelopment and tumorigenesis [7]. Our study reveals that PAFAH1B3 is significantly upregulated in BC, suggesting its pivotal role in promoting BC progression.

The impact of PAFAH1B3 on tumor progression extends to various malignancies, where it has been demonstrated to influence cancer growth and metastasis through distinct mechanisms [12–14]. Previous studies have reported PAFAH1B3's

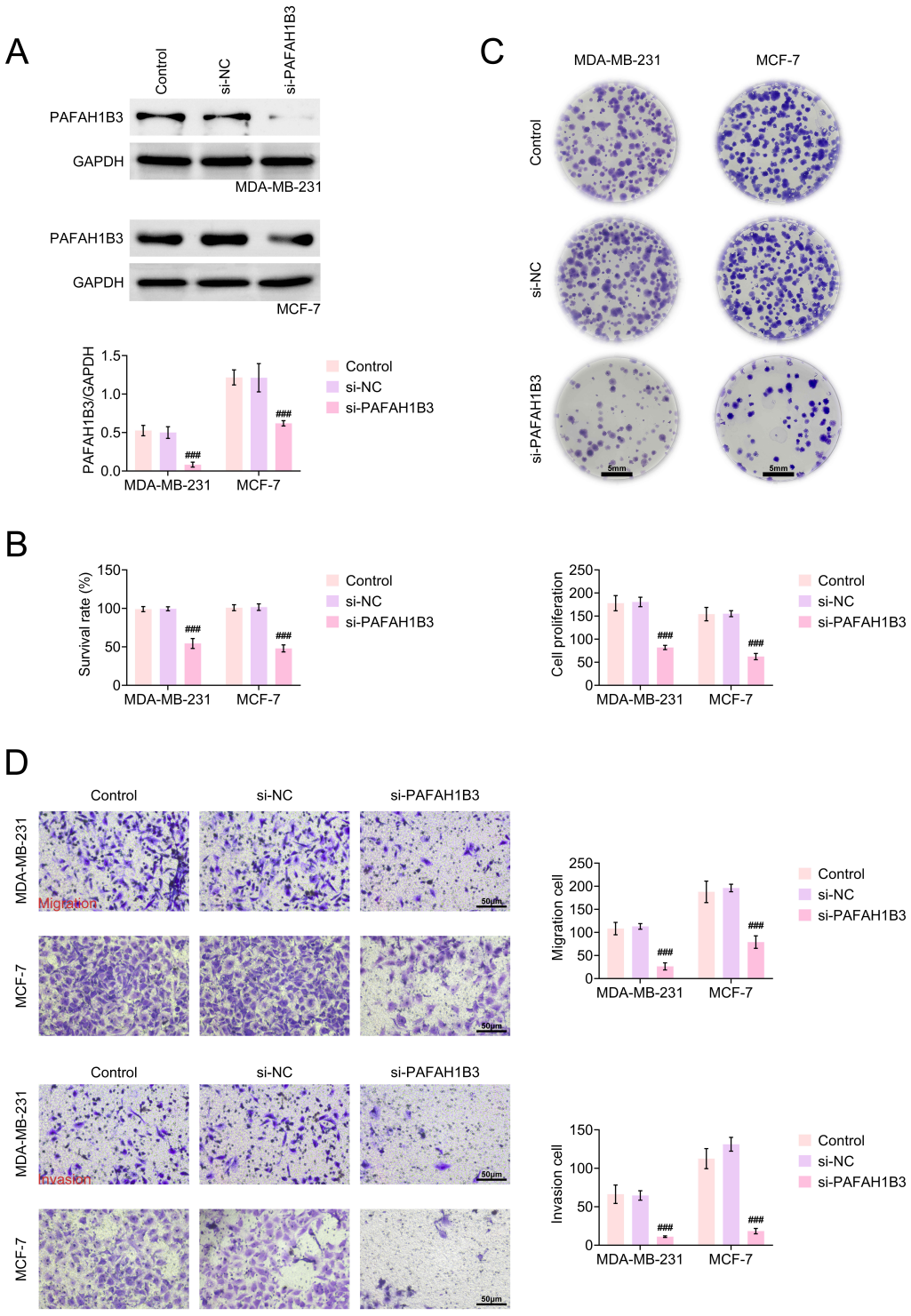

FIGURE 2. PAFAH1B3 depletion inhibits the growth and migration of breast cancer cells. (A) Immunoblot analysis of PAFAH1B3 expression in MDA-MB-231 (top) and MCF-7 (bottom) cells following transfection with si-NC or si-PAFAH1B3. (B) Cell growth assessed by CCK-8 assay in MDA-MB-231 (left) and MCF-7 (right) cells after 48 hours of transfection with si-NC or si-PAFAH1B3. OD450 values are shown. (C) Colony formation assay results showing colony counts of MDA-MB-231 (left) and MCF-7 (right) cells after 14 days of transfection with si-NC or si-PAFAH1B3. (D) Transwell assays showing migration (top) and invasion (bottom) of MDA-MB-231 (left) and MCF-7 (right) cells following 48 hours of transfection with si-NC or si-PAFAH1B3. The number of migrated and invaded cells per field is indicated. ###$p < 0.001$, si-PAFAH1B3 *vs.* si-NC. NC, negative control; PAFAH1B3, Platelet activating factor acetylhydrolase 1B3; GAPDH, Glyceraldehyde-3-Phosphate Dehydrogenase.

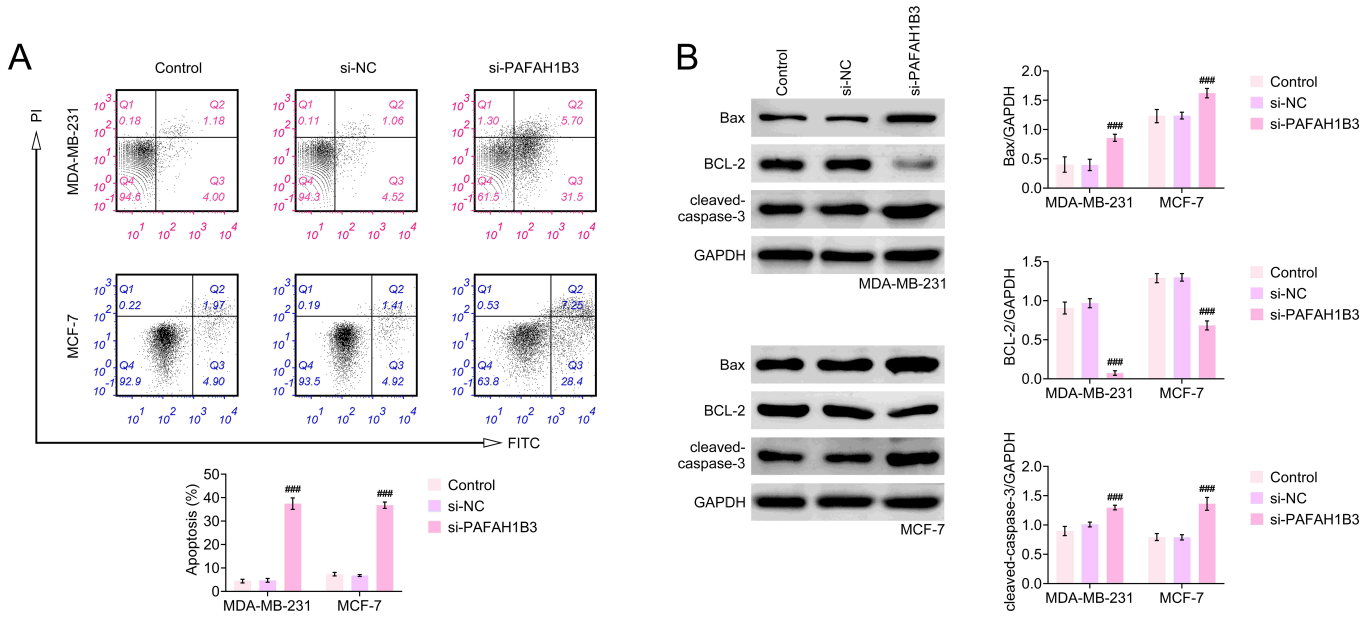

FIGURE 3. PAFAH1B3 depletion promotes breast cancer cell apoptosis. (A) Flow cytometry analysis of apoptosis in MDA-MB-231 (top) and MCF-7 (bottom) cells after 48 hours of transfection with si-NC or si-PAFAH1B3. The percentage of apoptotic cells is shown. (B) Immunoblot analysis of apoptosis-related proteins Bax, BCL-2 and cleaved caspase-3 in MDA-MB-231 (top) and MCF-7 (bottom) cells following 48 hours of transfection with si-NC or si-PAFAH1B3. ###$p < 0.001$, si-PAFAH1B3 *vs.* si-NC. NC, negative control; PAFAH1B3, Platelet activating factor acetylhydrolase 1B3; FITC, Fluorescein Isothiocyanate; PI, Propidium Iodide; Bax, BCL-2-associated X; BCL-2, B-cell lymphoma-2; GAPDH, Glyceraldehyde-3-Phosphate Dehydrogenase.

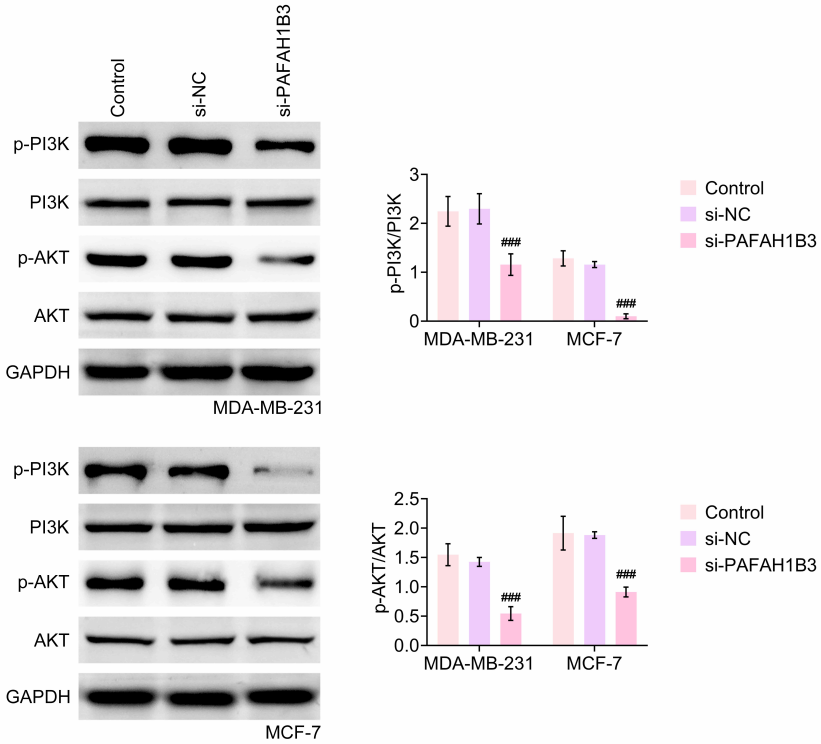

FIGURE 4. PAFAH1B3 depletion inhibits the PI3K/AKT pathway in breast cancer cells. Immunoblot analysis of PI3K and AKT expression and phosphorylation levels in MDA-MB-231 (top) and MCF-7 (bottom) cells following 48 hours of transfection with si-NC or si-PAFAH1B3. ###$p < 0.001$, si-PAFAH1B3 *vs.* si-NC. NC, negative control; PAFAH1B3, Platelet activating factor acetylhydrolase 1B3; PI3K, Phosphatidylinositol 3-kinase; AKT, Protein Kinase B; GAPDH, Glyceraldehyde-3-Phosphate Dehydrogenase.

role in pancreatic and colorectal cancers, where it facilitates tumor growth and metastasis [13, 15, 16]. Our presented findings align with these studies, showing that PAFAH1B3 enhances BC cell growth and motility while inhibiting apoptosis. Mechanistically, our research indicates that PAFAH1B3 exerts its effects by modulating the PI3K/AKT pathway, a critical regulator of cell survival, proliferation, and motility. This study provides valuable insights into the role of PAFAH1B3 in BC and contributes to a broader understanding of its function in cancer progression. PAFAH1B3 likely influences BC progression through the regulation of proliferation and motility via the PI3K/AKT axis.

The PI3K/AKT axis is essential for various cellular processes, including growth, metabolism and survival, and dysregulation of this pathway has been found to be a common feature in many cancers, including BC [17]. Our findings indicate that PAFAH1B3 positively regulates the PI3K/AKT pathway, thereby promoting BC cell growth and motility while inhibiting apoptosis. In addition, silencing PAFAH1B3 resulted in decreased phosphorylation of both PI3K and AKT, indicating a suppression of pathway activity [18–20], thereby suggesting that targeting PAFAH1B3 could disrupt PI3K/AKT axis and impede BC progression, presenting a potential therapeutic strategy.

PI3K activation occurs through two primary mechanisms. First, PI3K interacts with connexins that have phosphorylated tyrosine residues, leading to a conformational change in the PI3K dimer [18–20]. Second, PI3K can be activated by direct binding of Ras to the p110 subunit. Once activated, PI3K produces the second messenger, PIP3, on the plasma membrane. PIP3 then binds to signaling proteins AKT and PDK1, which contain a PH domain. This interaction prompts PDK1 to phosphorylate Ser308 on AKT, resulting in AKT activation [18–20]. However, the precise molecular mechanism through which PAFAH1B3 influences this pathway requires further investigation.

There are several limitations regarding this study that should be acknowledged. The *in vitro* nature of our experiments necessitates validation in animal models to confirm the therapeutic potential of targeting PAFAH1B3. Additionally, the specific mechanisms by which PAFAH1B3 modulates the PI3K/AKT pathway need to be elucidated further. Future research should address these aspects and evaluate the efficacy of PAFAH1B3 inhibitors in preclinical and clinical settings. Furthermore, understanding PAFAH1B3's interactions with other signaling pathways will be essential for developing comprehensive treatment strategies.

5. Conclusions

In conclusion, our study establishes PAFAH1B3 as a key regulator of BC progression. We demonstrate that PAFAH1B3 facilitates cell growth and motility while suppressing apoptosis through the PI3K/AKT. This research enhances our understanding of PAFAH1B3's role and mechanisms in BC, suggesting that targeting PAFAH1B3 may offer a promising strategy for developing novel treatments. These findings provide a foundation for advancing personalized therapeutic approaches to improve outcomes for BC patients.

AVAILABILITY OF DATA AND MATERIALS

The authors declare that all data supporting the findings of this study are available within the paper and any raw data can be obtained from the corresponding author upon request.

AUTHOR CONTRIBUTIONS

LC—designed the study and carried them out; prepared the manuscript for publication and reviewed the draft of the manuscript. LC, ZQY and WFX—supervised the data collection. LC and ZQY—analyzed the data; interpreted the data. All authors have read and approved the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This article does not contain any studies with human participants or animals performed by any of the authors.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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