Submitted: 11 November, 2024 Accepted: 10 January, 2025 Published: 15 March, 2025 DOI:10.22514/ejgo.2025.042

ORIGINAL RESEARCH

European Journal of Gynaecological Oncology

Knockdown of NEIL3 inhibits the growth of breast cancer cells by mediating the PI3K/Akt/mTOR axis

Wanqiong Zheng[1,*], Ye Tian[2], Yiming Zhang[1], Binnan Li[1]

[1]Department of General surgery, Wenzhou Hospital of Integrated Traditional Chinese and Western Medicine, 325000 Wenzhou, Zhejiang, China
[2]Department of Oncology, Affiliated Hospital of Shaanxi University of Traditional Chinese Medicine, 712000 Xi'an, Shaanxi, China

***Correspondence**
zwq5171717@163.com
(Wanqiong Zheng)

Abstract

Background: To explore the role of Nei endonuclease VIII-like 3 (NEIL3) in breast cancer (BC) cell proliferation and motility and its underlying mechanism. **Methods**: BC cell lines were analyzed. NEIL3 expression levels in BC tissues was assessed using the UALCAN and Kaplan-Meier Plotter databases. NEIL3 knockdown was performed using siRNAs, and its effects on cell growth, motility, Epithelial-Mesenchymal Transition (EMT) markers, and the Phosphoinositide 3-Kinase/Protein Kinase B/Mammalian Target of Rapamycin (PI3K/Akt/mTOR) axis were evaluated using Immunoblotting, cell counting kit-8 (CCK-8) assays, colony formation, Transwell as well as wound healing assays. **Results**: NEIL3 was highly expressed in BC tissues and cell lines. NEIL3 knockdown inhibited cell growth, motility and reduced EMT marker expression. It also decreased phosphorylation levels of proteins, indicating inhibition of the PI3K/Akt/mTOR axis. **Conclusions**: NEIL3 mediates BC cell proliferation as well as motility by activating the PI3K/Akt/mTOR axis. Targeting NEIL3 may offer a new approach to combat BC.

Keywords

NEIL3; BC; PI3K/Akt/mTOR axis; Growth; Motility; EMT

1. Introduction

Breast cancer (BC) is a common type of cancer among women [1]. Each year, approximately 1.3 million women are diagnosed with BC, resulting in an estimated 400,000 deaths [2]. Since 2008, the incidence of BC has significantly increased, with the mortality rates rising by up to 14% [2]. Currently, primary treatment options for BC include targeted therapy and so on [3]. Despite these advancements, challenges such as treatment resistance, significant toxicity and relapse after initial response persist, underscoring the requirement for novel therapeutic targets as well as strategies [4]. A deeper understanding of BC progression will aid in the combating BC [5].

NEIL3 is located at chromosome 4q34.3 and encodes a member of DNA glycosylase family [6]. NEIL3 affects the progression of various tumors [7, 8]. It promotes the occurrence of Hepatocellular Carcinoma (HCC) by mediating PI3K/Akt/mTOR axis [9]. NEIL3 is upregulated in Non-Small Cell Lung Cancer (NSCLC) and is involved in the initiation and lung cancer, significantly enhancing the growth as well as motility of NSCLC [10]. In Clear Cell Renal Cell Carcinoma (ccRCC), NEIL3 expression is elevated, contributing to increased cell proliferation, DNA replication, and cell cycle [11, 12]. However, the role of NEIL3 in BC remain unknown.

The PI3K/Akt/mTOR axis is aberrantly expressed in various tumors, contributing to the progression of BC, gastric cancer, nasopharyngeal carcinoma and pancreatic cancer [13, 14]. This pathway is associated with cell growth, translation, as well as metabolism. Multiple studies have demonstrated that targeting this pathway with drugs or drug combinations can effectively inhibit tumor development and progression [13]. Therefore, research into therapeutic agents or molecular targets within the PI3K/Akt/mTOR axis is significant importance for improving BC prognosis.

Here, we investigated NEIL3's role in BC progression. We found its knockdown suppressed growth as well as motility of BC cells via PI3K/Akt/mTOR axis.

2. Materials and methods

2.1 Cell culture

MDA-MB-231 as well as MCF-7 BC cell lines were obtained from American Type Culture Collection (ATCC) in Dulbecco's Modified Eagle Medium (DMEM, 11965092, Gibco, Waltham, MA, USA) with 10% Fetal Bovine Serum (FBS, Gibco, 26140-079, Waltham, MA, USA) at 37 °C in 5% CO_2.

2.2 siRNA transfection

Cells were seeded in 6-well plates and allowed to adhere overnight. Specifically, 50 pmol of siRNA was diluted in 250 μL of Opti-MEM (31985070, Thermo, Waltham, MA, USA) and mixed with 5 μL of Lipofectamine 2000 (11668019, Invitrogen, Waltham, MA, USA). Cells were harvested 48 hours post-transfection for further analysis. No additional star-

vation steps were required. Cells were transfected with NEIL3-specific siRNAs (si-NEIL3#1 and si-NEIL3#2, si-NC, C1, bought from Riobio, Guangzhou, China). The sequences of siRNAs: si-NEIL3#1: 5′-GGAUUCUACCUACAGUUCA-3′; si-NEIL3#2: 5′-GGACUUCUAUCAGGUUCCA-3′; si-NC: 5′-UUCUCCGAACGUGUCACGU-3′.

2.3 Immunoblot

Samples were separated by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and transferred to Polyvinylidene Fluoride (PVDF). Membranes were blocked with 5% non-fat milk and incubated with antibodies overnight at 4 °C. The antibodies were used: NEIL3 (Abcam, Cambridge, UK, ab154906, 1:1000), E-cadherin (CST, Danvers, MA, USA, 14472, 1:1000), N-cadherin (CST, Danvers, MA, USA, 13116, 1:1000), Vimentin (CST, Danvers, MA, USA, 5741, 1:1000), p-PI3K (CST, Danvers, MA, USA, 4228, 1:1000), PI3K (CST, Danvers, MA, USA, 4249, 1:1000), p-Akt (CST, Danvers, MA, USA, 4060, 1:1000), Akt (CST, Danvers, MA, USA, 4685, 1:1000), p-mTOR (CST, Danvers, MA, USA, 5536, 1:1000), mTOR (CST, Danvers, MA, USA, 2983, 1:1000), and β-actin (Abcam, Cambridge, UK, ab8226, 1:1000). Membranes were then incubated with Horseradish Peroxidase (HRP) secondary antibodies (CST, Danvers, MA, USA, 7074, 1:5000). Bands were visualized using the Enhanced Chemiluminescence (ECL, Thermo, Waltham, MA, USA, 32106).

2.4 Quantitative polymerase chain reaction (qPCR) assay

RNA was reverse-transcribed by Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase (M1701, Promega, Madison, WI, USA). Quantitative PCR was performed by the use of a SYBR Taq kit (RR420A, Takara, Kusatsu, Japan).

2.5 Cell growth assay

The Cell Counting Kit-8 (CCK-8, Beyotime, Beijing, China, Catalog Number: C0038) assay was used to evaluate cell viability. 10 μL of CCK-8 solution was added. Absorbance was measured at a wavelength of 450 nm.

2.6 Colony formation assay

Cells were cultured for 14 days. Colonies were fixed with 4% Paraformaldehyde (PFA) (P1110, Solarbio, Beijing, China) for 20 minutes and stained with 0.1% crystal violet solution (C3886, Sigma, St. Louis, MO, USA) for 15 min.

2.7 Wound healing assay

A scratch was made using a sterile 10 μL pipette tip, ensuring uniform scratch widths. Images of the wound area were captured at 0 h and 24 h using a microscope (Inverted Microscope, Olympus, Tokyo, Japan).

2.8 Transwell assay

The Transwell invasion assay was performed using 24-well Transwell inserts (Corning) with an 8.0 μm pore size. The upper surface of the membrane was coated with Matrigel (354234, BD, San Jose, CA, USA). Transfected cells were suspended in serum-free DMEM. Cells were fixed with 4% PFA (Solarbio) and stained with crystal violet (Sigma).

2.9 Statistical analysis

We performed statistical analyses using GraphPad 9.0 (GraphPad Software, LLC, San Diego, CA, USA). Data are presented as mean $\pm$ Standard Deviation (SD). One-way Analysis of Variance (One-way ANOVA) followed by Tukey's *post hoc* test was used for multiple group comparisons. A p-value < 0.05 was considered statistically significant.

3. Results

3.1 High NEIL3 expression correlates with poor prognosis in BC

To investigate the role of NEIL3 in BC, its expression levels were analyzed using The Cancer Genome Atlas (TCGA) data. NEIL3 expression was significantly elevated in primary BC tissues (n = 1097 *vs.* n = 114, Fig. 1A). Patients with high NEIL3 expression had poorer overall survival (Hazard Ratio (HR) = 1.24, 95% Confidence Interval (CI): 1.02–1.49, Fig. 1B). These results suggest that NEIL3 overexpression is in BC.

3.2 NEIL3 ablation inhibited the growth of BC cells

To evaluate the effects of NEIL3 on BC cell growth, NEIL3 siRNAs were transfected into BC cell models. Both qPCR and Immunoblot assays confirmed that NEIL3 expression was significantly higher in BC cell lines including MDA-MB-231 cells and MCF7 cells, compared to MCF10A cells (Fig. 2A,B). Fig. 2C confirmed that NEIL3 protein levels are significantly reduced in BC cells following transfection with its. NEIL3 knockdown significantly inhibited the growth rates of both cell lines, as evidenced by decreased OD450 value (Fig. 2D). Furthermore, NEIL3 knockdown significantly reduced the number of colonies (Fig. 2E). These results collectively indicate that NEIL3 ablation suppressed the BC cell growth.

3.3 NEIL3 knockdown suppressed the motility of BC cells

We then investigated the effects of NEIL3 on BC cell motility using wound healing and transwell assays. Notably, NEIL3 knockdown suppressed the migration of BC cells, with the decreased migration rates (Fig. 3A). Interestingly, the results of transwell assays confirmed the knockdown of NEIL3 suppressed the invasive capacity of these BC cells, as evidenced by a decreased invasive cell numbers (Fig. 3B). These findings demonstrate that NEIL3 knockdown effectively blocked the motility of BC cells.

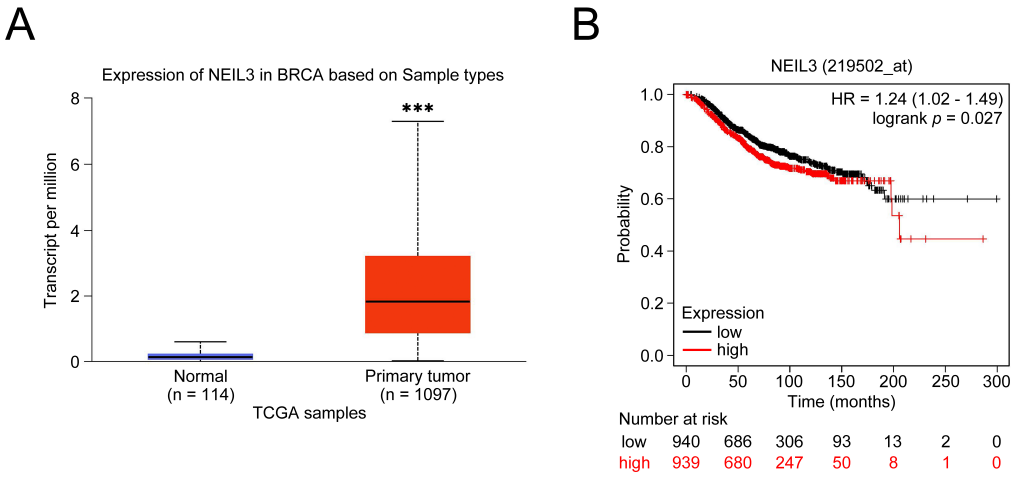

FIGURE 1. Expression of NEIL3 in breast cancer and its impact on patient survival. (A) Box plot showing the expression levels of NEIL3 in normal breast tissues (n = 114) and primary breast cancer tissues (n = 1097) based on TCGA samples. (***$p < 0.001$). (B) Kaplan-Meier survival analysis of breast cancer patients stratified by NEIL3 expression levels. Patients with high NEIL3 expression (red line) exhibit poorer overall survival compared to those with low NEIL3 expression (black line). NEIL3: Nei Endonuclease VIII-like 3; *BRCA*: Breast Cancer; TCGA: The Cancer Genome Atlas; HR: Hazard Ratio.

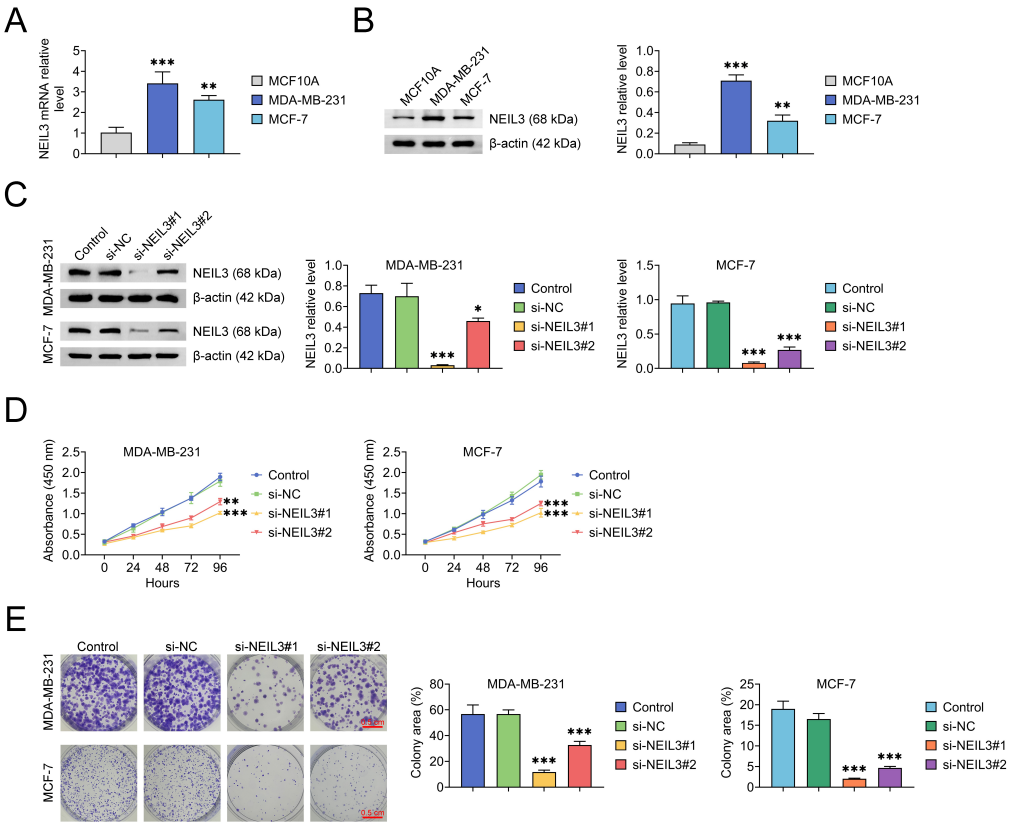

FIGURE 2. Effects of NEIL3 knockdown on breast cancer cell growth. (A) qPCR analysis of NEIL3 mRNA levels in MDA-MB-231 and MCF-7 breast cancer cells or MCF10A normal breast cells. (B) Immunoblot analysis of NEIL3 protein levels in MDA-MB-231and MCF-7 breast cancer cells or MCF10A normal breast cells. (C) Immunoblot analysis of NEIL3 protein levels in MDA-MB-231 and MCF-7 breast cancer cells following transfection with control siRNA (si-NC) or NEIL3-specific siRNAs (si-NEIL3#1 and si-NEIL3#2). Quantification of NEIL3 protein levels is shown on the right. (D) CCK-8 assays showing the proliferation rates of MDA-MB-231 and MCF-7 cells after NEIL3 knockdown. Absorbance at 450 nm was measured at the indicated time points. (E) Colony formation assays of MDA-MB-231 and MCF-7 cells after NEIL3 knockdown. Representative images of colonies are shown. Quantification of colony areas is presented below. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to si-NC. NEIL3: Nei Endonuclease VIII-like 3; si-NC: si-Negative Control.

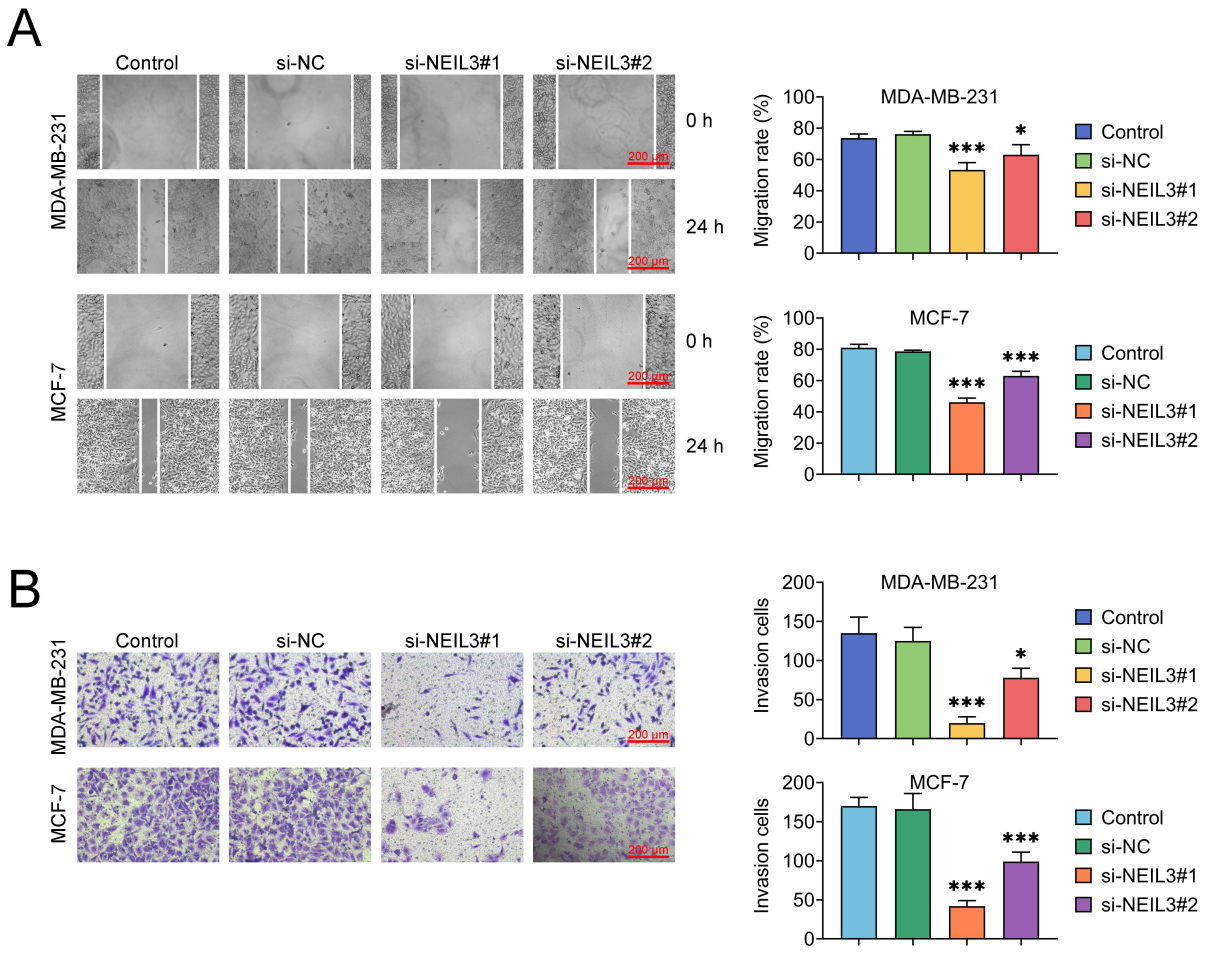

FIGURE 3. Effects of NEIL3 knockdown on breast cancer cell motility. (A) Wound healing assays showing the migration rates of MDA-MB-231 and MCF-7 cells following transfection with control siRNA (si-NC) or NEIL3-specific siRNAs (si-NEIL3#1 and si-NEIL3#2). Representative images at 0 hours and 24 h are shown. Quantification of migration rates is presented on the right. (B) Transwell invasion assays of MDA-MB-231 and MCF-7 cells after NEIL3 knockdown. Representative images of invaded cells are shown. Quantification of invasion cells is presented on the right. $*p < 0.05$, $***p < 0.001$ compared to si-NC. NEIL3: Nei Endonuclease VIII-like 3; si-NC: si-Negative Control.

3.4 Knockdown of NEIL3 affected the expression of EMT markers in BC cells

We then investigated the effects of NEIL3 knockdown on EMT markers in BC cells. We noticed NEIL3 knockdown significantly increased E-cadherin and decreased N-cadherin expression in BC cells, confirmed by immunoblot analysis (Fig. 4A). Furthermore, NEIL3 ablation also significantly reduced the expression of Vimentin in both cell lines (Fig. 4B). These results indicate that NEIL3 knockdown inhibited the EMT process in BC cells.

3.5 NEIL3 ablation suppressed the PI3K/Akt/mTOR axis in BC cells

Subsequently, we examined the possible underlying mechanism by which NEIL3 knockdown suppresses BC progression. The effects of NEIL3 knockdown on the PI3K/Akt/mTOR axis were detected. NEIL3 knockdown significantly reduced the levels of phosphorylated PI3K and phosphorylated Akt (Fig. 5A). Furthermore, NEIL3 knockdown also led to a significantly decrease in the levels of phosphorylated mTOR in both cell lines (Fig. 5B).

4. Discussion

Breast cancer is a leading cause of women mortality, despite advancements in therapeutic strategies [15]. Challenges such as resistance to therapy, significant toxicity and high recurrence rates underscore the need for novel therapeutic targets [16]. We identified NEIL3 as a key regulator of BC progression, demonstrating its role in promoting cell growth, motility and EMT through the PI3K/Akt/mTOR axis.

Cell growth, migration and EMT are critical processes in cancer progression [17, 18]. Proliferation allows cancer cells to grow uncontrollably, while motility and EMT facilitate invasion into surrounding tissues and metastasis to distant organs [17]. EMT, in particular, is associated with increased metastatic potential and poor prognosis in BC patients [17]. Here, NEIL3 knockdown significantly inhibited cell proliferation, migration, and EMT, suggesting that NEIL3 is crucial for maintaining the aggressive characteristics of BC cells.

NEIL3, a DNA glycosylase has been implicated in the

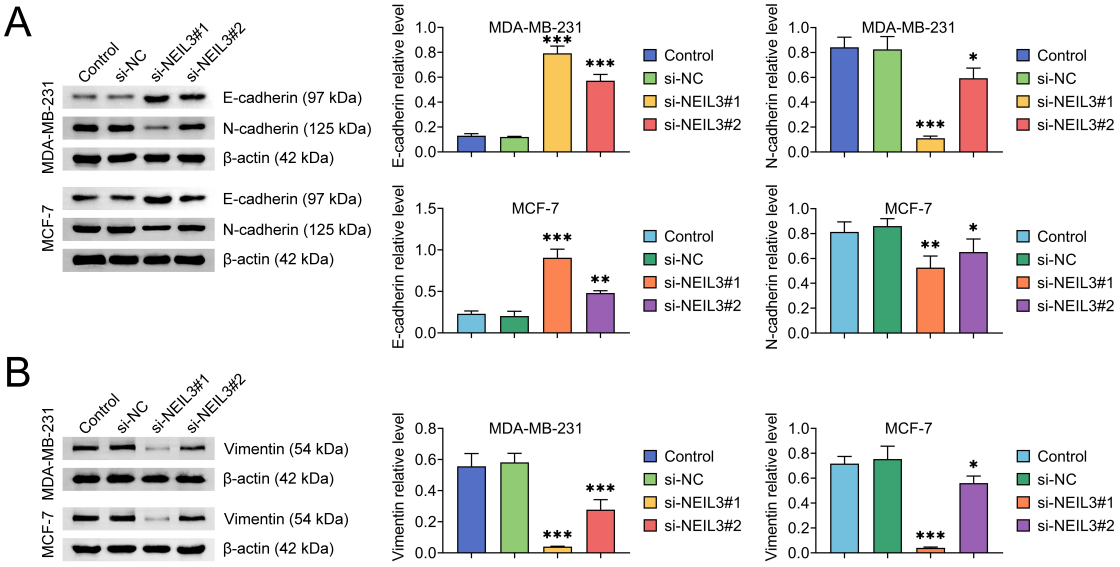

FIGURE 4. Effects of NEIL3 knockdown on epithelial-mesenchymal transition (EMT) markers in breast cancer cells. (A) Immunoblot analysis of E-cadherin and N-cadherin protein levels in MDA-MB-231 and MCF-7 cells following transfection with control siRNA (si-NC) or NEIL3-specific siRNAs (si-NEIL3#1 and si-NEIL3#2). Quantification of E-cadherin and N-cadherin protein levels is shown on the right. (B) Immunoblot analysis of Vimentin protein levels in MDA-MB-231 and MCF-7 cells after NEIL3 knockdown. Quantification of Vimentin protein levels is shown on the right. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ compared to si-NC. NEIL3: Nei Endonuclease VIII-like 3; si-NC: si-Negative Control.

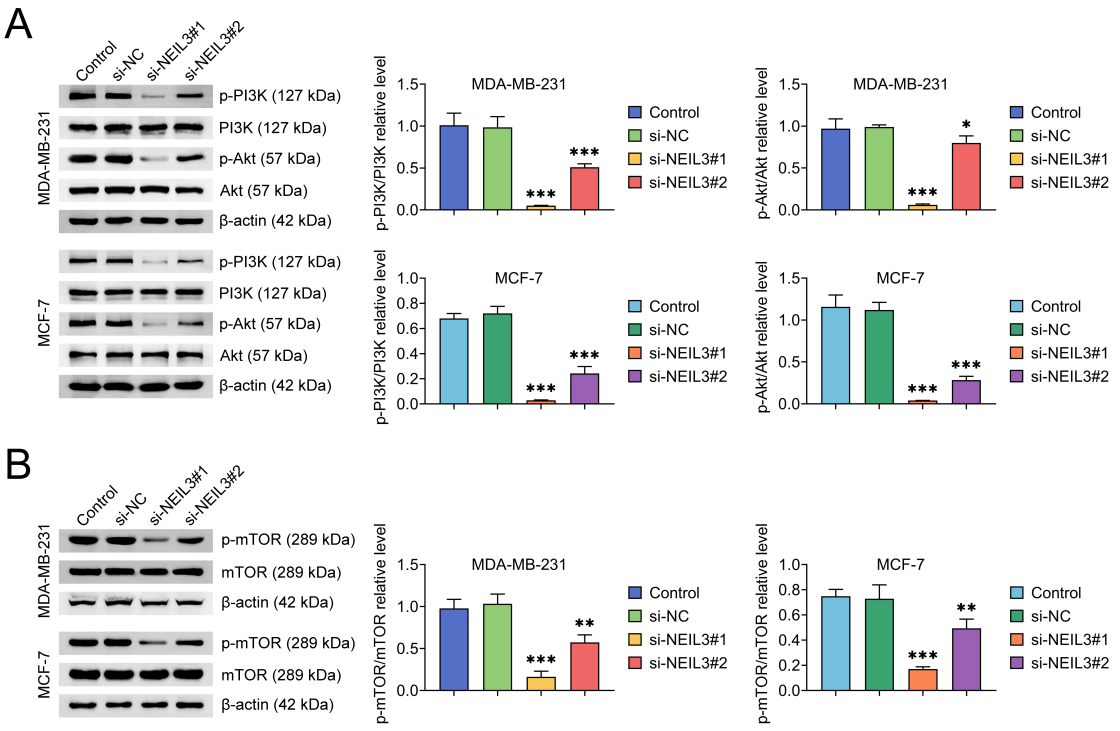

FIGURE 5. Effects of NEIL3 knockdown on PI3K/Akt/mTOR signaling pathway in breast cancer cells. (A) Immunoblot analysis of phosphorylated PI3K (p-PI3K), total PI3K, phosphorylated Akt (p-Akt), and total Akt protein levels in MDA-MB-231 and MCF-7 cells following transfection with control siRNA (si-NC) or NEIL3-specific siRNAs (si-NEIL3#1 and si-NEIL3#2). Quantification of p-PI3K/PI3K and p-Akt/Akt protein levels is shown on the right. (B) Immunoblot analysis of phosphorylated mTOR (p-mTOR) and total mTOR protein levels in MDA-MB-231 and MCF-7 cells after NEIL3 knockdown. Quantification of p-mTOR/mTOR protein levels is shown on the right. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ compared to si-NC. NEIL3: Nei Endonuclease VIII-like 3; si-NC: si-Negative Control; Akt: Protein Kinase B; PI3K: Phosphoinositide 3-Kinase; mTOR: Mammalian Target of Rapamycin.

progression of various cancers [6]. It plays a crucial role in regulating genomic stability by repairing oxidative DNA damage [12]. NEIL3 is involved in the progression of various cancers, including HCC, NSCLC and ccRCC [7, 9–11]. In these cancers, NEIL3 has been shown to promote cell growth as well as motility, suggesting its role as an oncogene [7, 11, 19]. In BC, we found that NEIL3 knockdown significantly inhibited cell growth, motility and EMT, while also suppressing PI3K, Akt and mTOR phosphorylation.

The connection between NEIL3 and its impact on BC cell growth and motility was established using a combination of literature review and experimental validation. Initially, existing studies indicated NEIL3's role in tumor progression, particularly its involvement in cell proliferation and motility in cancers such as hepatocellular carcinoma and lung cancer, through mechanisms like the PI3K/Akt/mTOR axis. This provided a strong rationale to investigate its role in BC.

We observed that NEIL3 knockdown reduced both the growth and motility of BC cells. This suggests that NEIL3 plays a crucial role in promoting these malignant behaviors. By inhibiting NEIL3, we were able to suppress EMT markers and reduce the aggressive traits of BC cells. The significant reduction in cell motility as well as growth upon NEIL3 knockdown indicates that NEIL3 could be critical for tumor growth and metastasis.

The PI3K/Akt/mTOR axis mediates cell growth and survival [20–22]. Aberrations in this axis are common in various cancers, including BC, leading to enhanced tumor progression and resistance to therapies [23]. Activation of the PI3K/Akt/mTOR axis promotes cell cycle, inhibits apoptosis, and enhances cellular metabolism, contributing to the aggressive phenotype of cancer cells [8]. Therefore, targeting components of this axis has been a focus of cancer research and drug development.

Our findings indicate that NEIL3 exerts its effects on BC cells via PI3K/Akt/mTOR axis. This suggests that NEIL3 may contribute to BC by activating the PI3K/Akt/mTOR axis, and targeting NEIL3 could disrupt this oncogenic signaling. The downregulation of PI3K, Akt, and mTOR phosphorylation upon NEIL3 knockdown underscores the axis's involvement in NEIL3-mediated tumorigenesis [24].

Despite the promising results, our study has several limitations. The *in vitro* nature of our experiments necessitates further validation *in vivo* to confirm the therapeutic potential of targeting NEIL3 in BC. Animal models and clinical samples should be used to validate our findings and gain a deeper understanding of NEIL3's role in tumor growth. Additionally, understanding the precise molecular mechanisms by which NEIL3 regulates the PI3K/Akt/mTOR axis requires more detailed investigation. Future studies should explore the upstream regulators and downstream effectors of NEIL3 within the context of BC.

Our findings suggest that NEIL3 knockdown correlates with decreased EMT marker expression. However, the direct mechanistic link between NEIL3-mediated PI3K/Akt signaling and EMT regulation remains unclear. It is possible that NEIL3 influences EMT indirectly by modulating cellular processes such as proliferation or motility, which are downstream effects of PI3K/Akt pathway activation. Further studies are required to elucidate whether NEIL3 directly interacts with EMT-related transcription factors or signaling mediators to regulate this process.

5. Conclusions

NEIL3 was highly expressed in BC tissues and cell lines. In addition, NEIL3 knockdown inhibited cell growth, motility and reduced EMT marker expression. It also decreased phosphorylation levels of proteins, indicating inhibition of the PI3K/Akt/mTOR axis. There NEIL3 mediates BC cell proliferation as well as motility by activating the PI3K/Akt/mTOR axis, and targeting NEIL3 may offer a new approach to combat BC.

AVAILABILITY OF DATA AND MATERIALS

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

AUTHOR CONTRIBUTIONS

WQZ—performed material preparation and the experiments. YT and YMZ—performed data collection and analysis. BNL—written the first draft of the manuscript. All authors contributed to the study conception and design. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

ACKNOWLEDGMENT

Not applicable.

FUNDING

This research received no external funding.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Hu D, Zhuo W, Gong P, Ji F, Zhang X, Chen Y, *et al*. Biological differences between normal and cancer-associated fibroblasts in breast cancer. Heliyon. 2023; 9: e19803.

[2] Barzaman K, Karami J, Zarei Z, Hosseinzadeh A, Kazemi MH, Moradi-Kalbolandi S, *et al*. Breast cancer: biology, biomarkers, and treatments. International Immunopharmacology. 2020; 84: 106535.

[3] Kaboli PJ, Imani S, Jomhori M, Ling KH. Chemoresistance in breast cancer: PI3K/Akt pathway inhibitors *vs* the current chemotherapy. American Journal of Cancer Research. 2021; 11: 5155–5183.

[4] Garcia-Martinez L, Zhang Y, Nakata Y, Chan HL, Morey L. Epigenetic mechanisms in breast cancer therapy and resistance. Nature Communications. 2021; 12: 1786.

[5] Wang M, Wu Y, Li X, Dai M, Li S. IGJ suppresses breast cancer growth and metastasis by inhibiting EMT via the NF-κB signaling pathway. International Journal of Oncology. 2023; 63: 105.
[6] Dickinson K, Hammond L, Akpa M, Chu LL, Lalonde CT, Goumba A, *et al*. WT1 regulates expression of DNA repair gene Neil3 during nephrogenesis. American Journal of Physiology. Renal Physiology. 2023; 324: F245–F255.
[7] Lai HH, Hung LY, Yen CJ, Hung HC, Chen RY, Ku YC, *et al*. NEIL3 promotes hepatoma epithelial-mesenchymal transition by activating the BRAF/MEK/ERK/TWIST signaling pathway. The Journal of Pathology. 2022; 258: 339–352.
[8] Wang W, Yin Q, Guo S, Wang J. *NEIL3* contributes toward the carcinogenesis of liver cancer and regulates PI3K/Akt/mTOR signaling. Experimental and Therapeutic Medicine. 2021; 22: 1053.
[9] Huang H, Hua Q. NEIL3 mediates lung cancer progression and modulates PI3K/AKT/mTOR signaling: a potential therapeutic target. International Journal of Genomics. 2022; 2022: 8348499.
[10] Zhao C, Liu J, Zhou H, Qian X, Sun H, Chen X, *et al*. NEIL3 may act as a potential prognostic biomarker for lung adenocarcinoma. Cancer Cell International. 2021; 21: 228.
[11] Sun X, Liu P. Prognostic biomarker NEIL3 and its association with immune infiltration in renal clear cell carcinoma. Frontiers in Oncology. 2023; 13: 1073941.
[12] Zhang M, Jiang Y, Wang J, Yue Y, Liu W, Wang L, *et al*. NEIL3 promotes cell proliferation of ccRCC via the cyclin D1-Rb-E2F1 feedback loop regulation. DNA Repair. 2024; 133: 103604.
[13] Alzahrani AS. PI3K/Akt/mTOR inhibitors in cancer: at the bench and bedside. Seminars in Cancer Biology. 2019; 59: 125–132.
[14] Varshney P, Saini N. PI3K/AKT/mTOR activation and autophagy inhibition plays a key role in increased cholesterol during IL-17A mediated inflammatory response in psoriasis. Biochimica et Biophysica Acta. Molecular Basis of Disease. 2018; 1864: 1795–1803.
[15] Yang Z, Zhang C, Liu X, Che N, Feng Y, Xuan Y. SETD5 regulates glycolysis in breast cancer stem-like cells and fuels tumor growth. The American Journal of Pathology. 2022; 192: 712–721.
[16] Derakhshan F, Reis-Filho JS. Pathogenesis of triple-negative breast cancer. Annual Review of Pathology. 2022; 17: 181–204.
[17] Li Z, Liu B, Li C, Sun S, Zhang H, Sun S, *et al*. NRBP2 functions as a tumor suppressor and inhibits epithelial-to-mesenchymal transition in breast cancer. Frontiers in Oncology. 2021; 11: 634026.
[18] Patel JR, Banjara B, Ohemeng A, Davidson AM, Boue SM, Burow ME, *et al*. Novel therapeutic combination targets the growth of letrozole-resistant breast cancer through decreased cyclin B1. Nutrients. 2023; 15: 1632.
[19] Yang H, Liu D, Wang YP. Physalin A exerts neuroprotective effects: inhibition of OGD/R-induced cellular pyroptosis and inflammatory responses in nerve cells. Signa Vitae. 2023; 19: 168–174.
[20] Porta C, Paglino C, Mosca A. Targeting PI3K/Akt/mTOR signaling in cancer. Frontiers in Oncology. 2014; 4: 64.
[21] Gao Z, Wu J, Wu X, Zheng J, Ou Y. SRPX2 boosts pancreatic cancer chemoresistance by activating PI3K/AKT axis. Open Medicine. 2020; 15: 1072–1082.
[22] Slomovitz BM, Coleman RL. The PI3K/AKT/mTOR axis as a therapeutic target in endometrial cancer. Clinical Cancer Research. 2012; 18: 5856–5864.
[23] Lou C, Xu X, Chen Y, Zhao H. Alisol A suppresses proliferation, migration, and invasion in human breast cancer MDA-MB-231 Cells. Molecules. 2019; 24: 3651.
[24] Roncolato F, Lindemann K, Willson ML, Martyn J, Mileshkin L. PI3K/AKT/mTOR inhibitors for advanced or recurrent endometrial cancer. Cochrane Database of Systematic Reviews. 2019; 10: CD012160.

How to cite this article: Wanqiong Zheng, Ye Tian, Yiming Zhang, Binnan Li. Knockdown of NEIL3 inhibits the growth of breast cancer cells by mediating the PI3K/Akt/mTOR axis. European Journal of Gynaecological Oncology. 2025; 46(3): 105-111. doi: 10.22514/ejgo.2025.042.