Original Article

The effects of the tumor suppressor gene *PTEN* on the proliferation and apoptosis of breast cancer cells via AKT phosphorylation

Junhua Zhang[1#], Ying Zhang[1#], Xiaomeng Lin[1], Xiaoxu Han[1], Kenneth L. Meredith[2], Zhong Li[1]

[1]Department of Breast Surgery, Affiliated Hospital of Hebei University, Baoding, China; [2]Florida State University College of Medicine, Tallahassee, FL, USA

Contributions: (I) Conception and design: J Zhang; (II) Administrative support: Z Li; (III) Provision of study materials or patients: Y Zhang; (IV) Collection and assembly of data: X Lin; (V) Data analysis and interpretation: X Han; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Zhong Li, BM. Department of Breast Surgery, Affiliated Hospital of Hebei University, No. 212 Yuhua East Road, Lianchi District, Baoding 071030, China. Email: lizhong12456789@sina.com.

Background: The proliferation and apoptosis of cancer cells play important roles in breast carcinomas. However, to date, there have been few reports on the correlation between the expression of PTEN and AKT phosphorylation in breast cancer. This present study investigated the effects of the phosphatase and tensin homology deleted from chromosome 10 (*PTEN*) gene on the proliferation and apoptosis of breast cancer cells through protein kinase B (AKT) phosphorylation.

Methods: Human breast cancer MDA-MB-231 cells were transfected with the pcDNA3.0 control vector or the pcDNA3.0-*PTEN* vector for 48 hours. The Cell Counting Kit 8 (CCK-8) was used to detect cell survival rates, double staining was performed to detect apoptosis, and Western blot (WB) analysis was conducted to detect protein expression. The effects of PTEN expression on the cell cycle and apoptosis of human breast cancer cell line MDA-MB-231, and on the levels of phosphorylated AKT protein were further analyzed. Moreover, the relationship between the *PTEN* gene and clinical features were also analyzed.

Results: The cell survival rate of cells transfected with pcDNA3.0-*PTEN* was significantly lower than that of cells transfected with the control pcDNA3.0 vector (55.65%±12.18% *vs.* 97.32%±12.45%, P=0.004). Compared with the pcDNA3.0 group, the apoptosis rate of the pcDNA3.0-*PTEN* group was significantly increased (20.65±2.18 *vs.* 2.32±0.45, P=0.001). The expression of PTEN protein in pcDNA3.0-*PTEN* group was higher than that in the pcDNA3.0 group, and the expression of the AKT and mTOR proteins was significantly lower than that in pcDNA3.0 group (P<0.05). The expression of PTEN in the lymph node metastasis positive group was significantly higher than that in the lymph node metastasis negative group (P<0.05). The expression of the AKT protein in breast cancer was higher than that in normal breast tissue, and the difference was statistically significant (P<0.01).

Conclusions: Overexpression of the *PTEN* gene can promote AKT phosphorylation, increase the apoptotic index of breast cancer cells, and reduce the proliferative activity of breast cancer cells. This provided a new direction for the next treatment of breast cancer, but further clinical research is needed.

Keywords: *PTEN* gene; breast cancer; cell proliferation; phosphorylation; mechanism research

Submitted May 15, 2023. Accepted for publication Jun 28, 2023. Published online Jul 28, 2023.
doi: 10.21037/tcr-23-826
View this article at: https://dx.doi.org/10.21037/tcr-23-826

© Translational Cancer Research. All rights reserved.

Introduction

Phosphatase and tensin homology deleted from chromosome 10 (*PTEN*) is a typical tumor suppressor gene (1). It can inhibit the proliferation, invasion, and metastasis of tumor cells by downregulating tumor-related signal transduction pathway (2). Inactivation of the *PTEN* gene is related to malignant transformation, fine cell signal transduction, proliferation, invasion and metastasis of tumors. While the precise mechanisms are yet to be fully elucidated, studies have shown that *PTEN* is a negative regulator of the activation process of the phosphoinositide 3 kinase (PI3K)/protein kinase B (AKT) signaling pathway, via the dephosphorylation of phosphatidylinositol-3, 4, 5-triphosphate (PIP3) to phosphatidylinositol-4, 5-bisphosphate (PIP2) (3-5).

The oncogenic activity of PI3K inhibits the transformation of endothelial cells into mesenchymal cells, thereby inhibiting the proliferation of tumor cells. Target of rapamycin (TOR) is a non-canonical serine or threonine protein kinase (6). Studies (7-9) have shown that mTOR plays a central role in cell proliferation and apoptosis, as well as the occurrence and development of tumors. The signal transduction system of cells bridges the inside and outside of cells, as well as between individual cells. Any abnormalities will affect cell growth, differentiation, metabolism, and other functions. A study has found that most proteins involved in signal transduction are products of oncogenes or tumor suppressor genes, and their abnormal expression is closely related to the occurrence and development of tumors (10). The AKT pathway is an important signaling pathway that regulates cell proliferation. The AKT protein is regulated by the tumor suppressor gene *PTEN*.

Breast cancer is the most common malignant tumor in women. Comprehensive treatment with surgery is the primary form of management, with postoperative chemotherapy as the auxiliary treatment. While disease control, recurrence, and survival time can be effectively improved, early diagnosis remains the key to improving disease-free survival and quality of life. Studies (11-13) have shown that the PTEN-PI3K/AKT pathway in breast cancer tissue is abnormally activated. *PTEN* is a tumor suppressor gene with phosphatase activity and the protein kinase AKT is a proto-oncogene. The abnormal expression of these genes is closely related to tumorigenesis (14).

Breast cancer is considered as a genetic disease, involving the activation of multiple genes and the inactivation of tumor suppressor genes. While many studies have investigated the etiology and inducing factors of breast cancer, gene mutations and deletions, and the abnormal expression of signaling proteins are believed to play important roles in the occurrence, development, and prognosis of breast cancer. The PI3K/AKT cell signaling pathway regulates cell proliferation, activates survival signals, and maintains cell activity, so as to effectively control normal blood vessel growth and tumor blood vessel formation. In normal tissues, the PTEN protein can inhibit the activity of the PI3K/AKT signal transduction pathway, while the downregulation or deletion of the PTEN protein in malignant tumors leads to increased AKT phosphorylation (15).

Breast cancer is a common malignant tumor in Chinese women, and its mechanism of action has attracted increasing attention. It has been found that *PTEN* is located at the 10q23 locus of the human chromosome, which is 200 bp long and contains 8 introns and 9 exons (16). The biological functions of *PTEN* include inducing cell cycle arrest and apoptosis, inhibiting local cell adhesion, migration and angiogenesis, and playing an important role in the occurrence and development of many tumors. The PI3K/AKT-mediated signaling pathway is one of the important signal transduction pathways in cells (17). AKT phosphorylation can promote the proliferation and metastasis of breast cancer cells. However, to date, there have been few reports on the correlation between the expression of PTEN and AKT phosphorylation in breast cancer.

Highlight box

Key findings

- PTEN gene overexpression can promote AKT phosphorylation, increase the apoptosis index of breast cancer cells, and reduce the proliferation of breast cancer cells.

What is known and what is new?

- The AKT pathway is an important signaling pathway that regulates cell proliferation. The AKT protein is regulated by the tumor suppressor gene PTEN.
- The effects of the *PTEN* gene on the proliferation and apoptosis of breast cancer cells through AKT phosphorylation.

What is the implication, and what should change now?

- Overexpression of the PTEN gene can promote AKT phosphorylation, increase the apoptotic index of breast cancer cells, and reduce the proliferative activity of breast cancer cells. This provided a new direction for the next treatment of breast cancer, but further clinical research is needed.

© Translational Cancer Research. All rights reserved.

This current study investigated the effects of *PTEN* gene expression on the proliferation and apoptosis of breast cancer cells through AKT phosphorylation in the AKT-mTOR pathway. We present this article in accordance with the MDAR reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-826/rc).

Methods

Research materials

The human breast cancer MDA-MB-231 cell line was purchased from Shanghai Cell Bank Chinese Academy of Sciences (including stage I breast cancer, stage II breast cancer, stage III breast cancer, stage IV breast cancer and breast cancer with lymph node metastasis). Moreover, the normal cell from normal breast tissues was also purchased. Sheep anti-mouse *PTEN*, AKT and β-actin reference antibodies were purchased from Santa Cruz. QuickShuttle transfection reagent was purchased from Sigma and the Annexin V FITC/PI Apoptosis Kit was purchased from Shanghai Shenggong. Breast cancer tissue and normal tissue were obtained from patients of the Affiliated Hospital of Hebei University. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Ethics Committee of the Affiliated Hospital of Hebei University (No. 202100584) and individual consent for this analysis was obtained from all patients.

Cell culture and transfection

MDA-MB-231 cells in logarithmic growth phase (about 80% growth density) were collected, and 1×10^6 cells and 5 mL Dulbecco's Modified Eagle Medium (DMEM) medium (containing 10% fetal bovine serum) were added to each culture flask (25 cm^2). After 24 hours, cells were randomly divided into two groups. One group was transfected with 2 µg pcDNA3.0 plasmid and the other group was transfected with 2 µg pcDNA3.0-*PTEN* plasmid. Cells were cultured at 37 ℃ and 5% CO_2 for 48 hours. Three repetitions were done per each experiment.

Detection of cell survival rate using the Cell Counting Kit-8 (CCK-8)

The transfected MDA MB 231 cells were seeded into 96 well plates at a density of 1×10^4 cells/mL and 5 mL CCK-8 solution were added. After 2 hours of continuous culture, the cell survival rate was measured with a microplate reader at 450 nm. Three repetitions were done per each experiment.

Detection of apoptosis by immunohistochemistry

Cells were transfected for 48 hours and washed twice with sterile phosphate buffered saline (PBS). Thereafter, cells were resuspended in buffer solution to achieve a cell concentration of 5×10^5 cells/mL. Annexin-FITC (10 µL) and 10 µL propidium iodide (PI) was added to 200 µL cell suspension and incubated in the dark at room temperature for 10 minutes. PBS (500 µL) was added, and the apoptosis index was analyzed via flow cytometry. Three repetitions were done per each experiment.

Detection of cellular protein expression by Western blot (WB)

Transfected cells were lysed in 400 µL RIPA buffer, at room temperature for 10 minutes. The samples were centrifuged at 12,000 r/min for 5 minutes. The protein concentration of the supernatant was measured, and WB analysis was performed. Samples were incubated with the primary antibody (1:1,000) at 4 ℃ overnight, followed by incubation with the secondary antibody (1:5,000) at 37 ℃ for 30 minutes. The target bands were detected using the odyssey fluorescence detector, and GAPDH was used as the internal reference.

Flow cytometry analysis

At 24–48 hours after transfection, cells were digested with trypsin/EDTA and washed twice with 500 µL precooled PBS. Cells were resuspended with 70% ethanol/PBS and fixed at 4 ℃ overnight. Prior to flow cytometry, the cells were incubated with 500 µL PBS solution containing 0.05 g/L RNase at 37 ℃ for 30 minutes, followed by 1 mL PI/PBS solution (final concentration of PI was 50–100 mg/L) for 30 minutes. Three repetitions were done per each experiment.

Diagnosis and analysis of pathological features

For histochemical analysis, brown or tan particles inside the cell with a clear background in the cytoplasm were considered positively stained cells. A total of 10 high-power

© Translational Cancer Research. All rights reserved.

Table 1 A comparison of the cell survival rate between cells overexpressing *PTEN* and control cells

	Group		t	P
	pcDNA3.0-*PTEN* group (n=3)	pcDNA3.0 group (n=3)		
Cell viability (%)	55.65±12.18	97.32±12.45	7.752	0.004

Data are shown as mean ± standard deviation.

Table 2 A comparison of the apoptosis rate between cells transfected with *PTEN* and control cells

	Group		t	P
	pcDNA3.0-*PTEN* group (n=3)	pcDNA3.0 group (n=3)		
Apoptosis rate (%)	20.65±2.18	2.32±0.45	27.752	0.001

Data are shown as mean ± standard deviation.

fields of view were observed in each section, and 100 tumor cells were counted in each high-power field. The number and intensity of positively stained cells was comprehensively determined. When 0–5% of tumor cells were positive, the sample was awarded 0 points; if 6–20% of cells were positive, the sample was awarded 1 point; if 21–50% of cells were positive, the sample was awarded 2 points; and if more than 50% of cells were positive, the sample was awarded 3 points. A score of 0 to 1 was considered negative, and a score of 2–3 was considered positive.

The effect of AKT inhibitor on human breast cancer MDA-MB-231 cells

To examine which AKT affects PTEN phosphorylation, human breast cancer MDA-MB-231 cells were treated with an AKT inhibitor. We detected the effect of *AT7519*, *AZD5438*, *PP242*, *LY294002* and *U0126* on the PTEN-SP phosphorylation. Furthermore, PTEN phosphorylation was detected in AKT-small interfering (si)RNA-treated cells.

Statistical analysis

All statistical analyses were conducted using SPSS 23.00 software. All measurement data are expressed as mean ± standard deviation. The Student's *t*-test was used for comparisons and all statistical tests were two-sided test. The Pearson correlation test was used for correlation analysis. A P value <0.05 was considered statistically significant (two-tailed).

Results

The effect of PTEN overexpression on cell viability

Breast cancer cells transfected with the pcDNA3.0-*PTEN* vector showed significantly lower cell viability compared with cells transfected with the pcDNA3.0 control (P=0.004; *Table 1*).

The effect of PTEN overexpression on cell apoptosis

The apoptotic rate of cells in the pcDNA3.0-*PTEN* group was significantly increased compared to that in the pcDNA3.0 group (P=0.001; *Table 2*; *Figure 1*).

Expression of PTEN and AKT in breast cancer tissues

The surface reach of PTEN in adenocarcinoma was lower than that in normal breast tissue, and the difference was statistically significant (P<0.01). The expression of PTEN decreased with the increase of tumor stage and histological grade. Furthermore, the expression of PTEN in the lymph node metastasis positive group was significantly higher than that in the lymph node metastasis negative group (P<0.05). The expression of the AKT protein in breast cancer was higher than that in normal breast tissue, and the difference was statistically significant (P<0.01). The expression of AKT increased gradually with the increase of tumor stage and histological grade. Furthermore, AKT expression was significantly higher in patients with lymph node metastasis was compared to patients without lymph node metastasis

© Translational Cancer Research. All rights reserved.

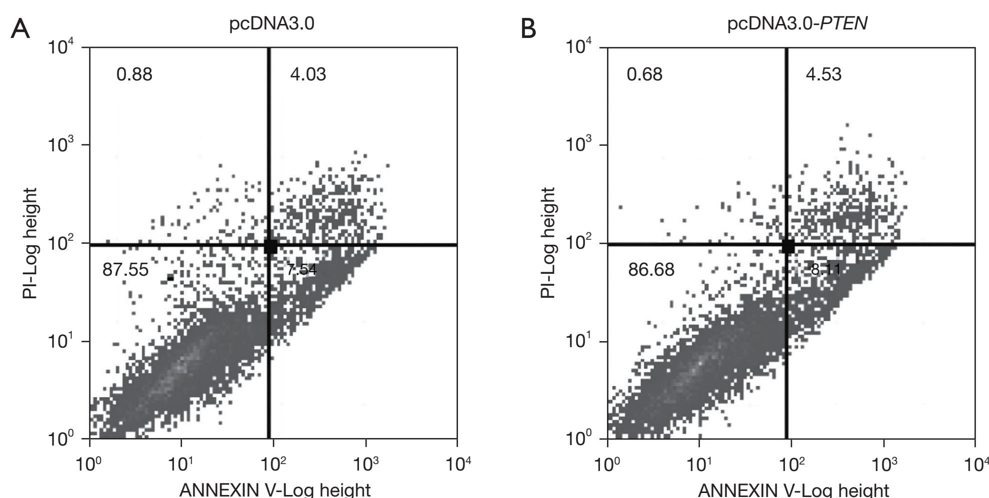

Figure 1 A comparison of the rate of apoptosis in cells transfected with *PTEN* and control cells. (A) pcDNA3.0 group; (B) pcDNA3.0-*PTEN* group. PI, propidium iodide; *PTEN*, phosphatase and tensin homology deleted from chromosome 10.

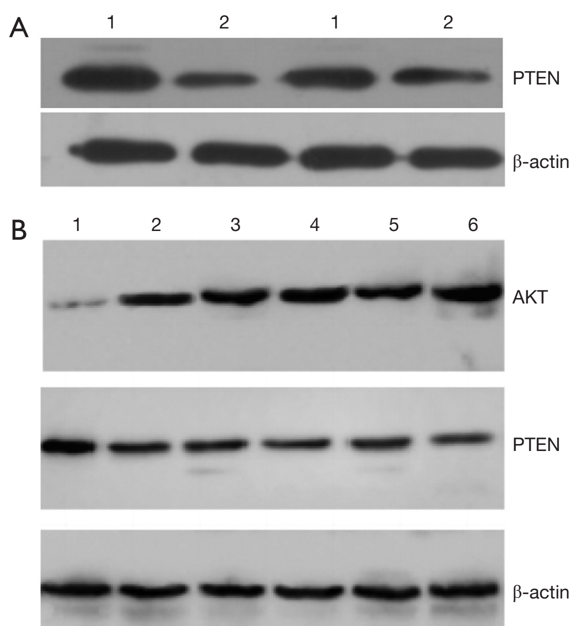

Figure 2 The expression of PTEN and AKT protein in breast cancer tissues. 1: normal breast tissues; 2: stage I breast cancer; 3: stage II breast cancer; 4: stage III breast cancer; 5: stage IV breast cancer; 6: breast cancer with lymph node metastasis. (A) The expression of PTEN in breast cancer tissues; (B) the expression of AKT and PTEN in breast cancer tissues. PTEN, phosphatase and tensin homology deleted from chromosome 10; AKT, protein kinase B.

(P<0.05; *Figure 2*).

Immunohistochemical analysis of PTEN protein expression in cancerous and benign breast tissues

The expression of the PTEN protein in breast invasive ductal carcinoma tissues was lower than that in breast benign hyperplasia tissues. In breast invasive ductal carcinoma tissues, the expression of the PTEN protein was correlated with WHO grade (WHO grade was mainly used to judge the malignant degree of breast cancer) and lymph node metastasis status (P<0.05), as well as the molecular subtypes of breast cancer (P<0.001). Furthermore, and the expression rate of the PTEN protein in Luminal A and Luminal B types was higher than that in Her-2 overexpression types and basal-like subtypes (*Figure 3*).

Expression of AKT and phosphorylated (p)-AKT protein in the transfected breast cancer cells transfected with pcDNA3.0 or pcDNA3.0-PTEN

WB analysis showed that the relative expression of p-AKT in the PTEN overexpression (PTEN+) group (0.87±0.09) was significantly lower than that in the negative control (PTEN−) group (1.76±0.15). The relative expression of AKT in the PTEN positive group (0.68±0.05) was lower than that in the

© Translational Cancer Research. All rights reserved.

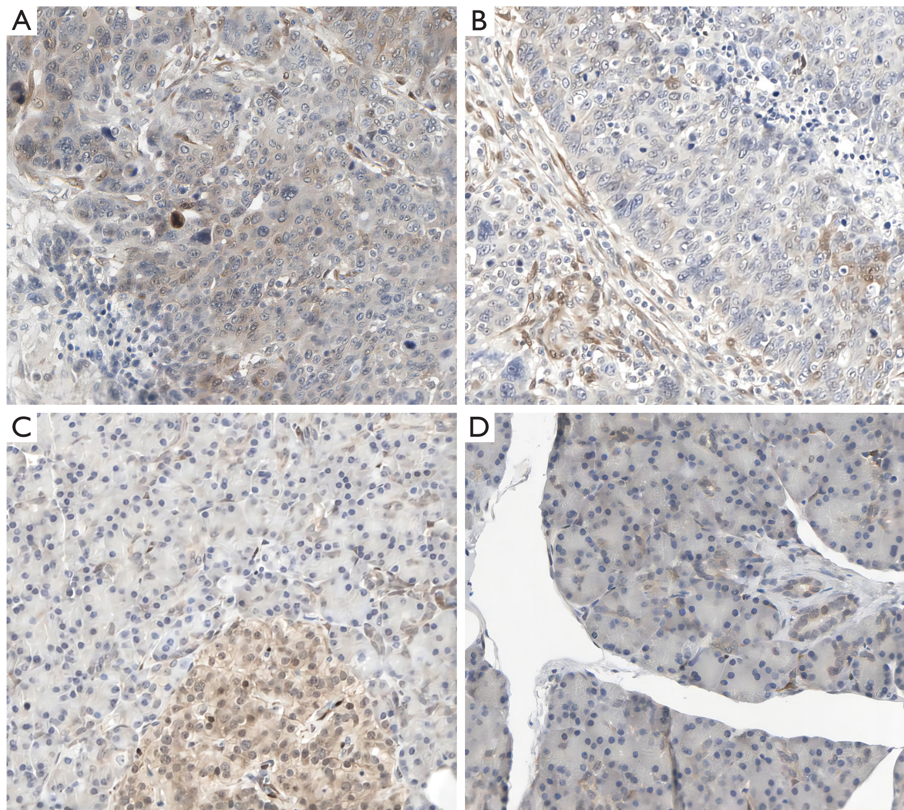

Figure 3 Immunohistochemical analysis of PTEN protein expression in breast cancer tissues (A,B) and normal breast tissues (C,D). Staining methods: HE tissue staining; magnification: 50×. PTEN, phosphatase and tensin homology deleted from chromosome 10; HE, hematoxylin-eosin staining.

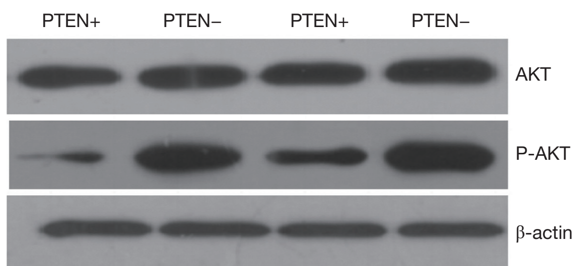

Figure 4 Expression of AKT and p-AKT protein in breast cancer tissues of PTEN positive and negative groups. PTEN, phosphatase and tensin homology deleted from chromosome 10; AKT, protein kinase B.

negative group (0.72±0.06), however, there was no significant difference between the two groups (*Figure 4*).

PTEN is phosphorylated in the S/G2 phase

The PTEN protein contains four cyclin-bound structures (RXL), suggesting that S/G2-specific phosphorylation of *PTEN* may be regulated by cyclin/AKTs. Meanwhile, the PTEN protein has three putative SP/TP motifs, including S113P, S202P, and S559P. Phosphorylation of SP motifs (p-SP) was detected using anti-SP antibodies. The immunoprecipitated PTEN protein and anti-PSP antibody

© Translational Cancer Research. All rights reserved.

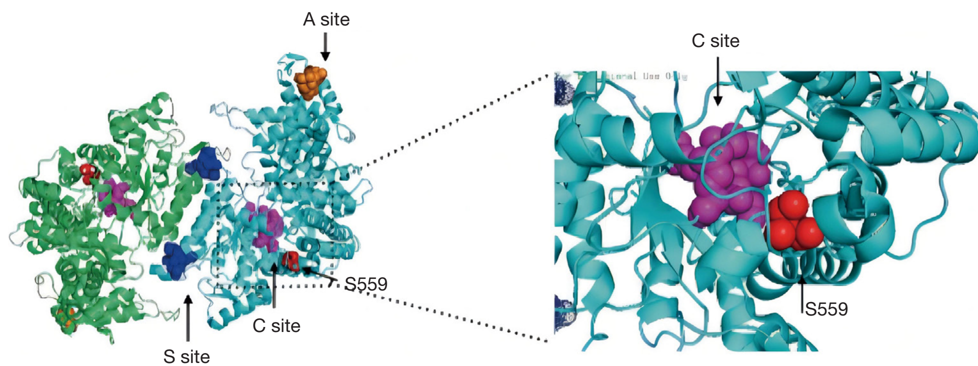

Figure 5 Structure of the PTEN protein. S559 is adjacent to the catalytic site (site C), but not adjacent to the active site (site A) nor the substance-specific site (site S). PTEN, phosphatase and tensin homology deleted from chromosome 10.

showed strong immunoactivity, which indicated that *PTEN* was a substrate of AKT kinase. To examine which AKT affects *PTEN* phosphorylation, human breast cancer MDA-MB-231 cells were treated with an AKT inhibitor. The results demonstrated that *AT7519* and *AZD5438* eliminated *PTEN*-SP phosphorylation, but PP242, LY294002, and U0126 had no effect on *PTEN* phosphorylation. Furthermore, *PTEN* phosphorylation was detected in AKT-small interfering (si)RNA-treated cells, suggesting that AKT may induce *PTEN* phosphorylation (*Figure 5*).

Discussion

The *PTEN* genome consists of 9 exons and 8 introns and is 200 kb in length. The PTEN protein is a tumor suppressor with bi-specific phosphatase activity, which is localized in the cytoplasm and distributed in a grid pattern (18). The normal expression of this protein can inhibit the growth, metastasis, and invasion of tumor cells. *PTEN* gene mutations and inactivation has been detected in liver cancer, ovarian cancer, breast cancer, endometrial cancer, and its downregulation can achieve tumor suppression. Recent studies (19-21) have shown that *PTEN* plays an important role in the occurrence of breast cancer and PTEN overexpression can inhibit the proliferation of breast cancer. Furthermore, *PTEN* deletion and downregulation can promote the proliferation of breast cancer. This study demonstrated that the cell survival rate of breast cancer cells transfected with pcDNA3.0-*PTEN* was significantly lower than that of cells transfected with the pcDNA3.0 control (P<0.05), suggesting that the overexpression of PTEN can inhibit the proliferation of breast cancer cells (22).

Cell apoptosis is an important indicator of the degree of cell damage. Basic studies have shown that *PTEN* can promote cell apoptosis by antagonizing the AKT pathway to promote the release of reactive oxygen species, inhibit nuclear factor (NF)-κB, and finally induce apoptosis. Other studies (23-25) have shown that *PTEN* can also induce apoptosis by activating caspase-3 and exert pro-apoptotic activity by antagonizing the PI3K pathway and affecting the Fas apoptosis pathway. This current study demonstrated that compared cells transfected with the pcDNA3.0 negative control, the apoptosis rate of cells transfected with pcDNA3.0-*PTEN* was significantly increased (P<0.05). Overexpression of PTEN can also downregulate PI3K levels and induce G1 arrest, thereby increasing cell apoptosis (26). The tumor suppressor function of *PTEN* is mainly involved in multiple signal transduction pathways such as PTEN/PI3K/AKT, PTEN/ERK, and PTEN/FAK/P130cas, which can inhibit tumor cell invasion and metastasis, arrest cell cycle, and inhibit tumor angiogenesis.

Recent studies (27-29) have shown that mTOR is related to tumorigenesis and development and can control the expression of the c-myc gene, thereby controlling the transcription and translation of cellular ribosomal proteins. mTOR is highly expressed in prostate cancer and breast cancer tissues, which may be closely related to tumor formation and active cell proliferation. We herein demonstrated that the protein expression of AKT and as was significantly lower in cells transfected with pcDNA3.0-*PTEN* compared to cells transfected with pcDNA3.0 alone (P<0.05). A previous study has also revealed that low expression of PTEN can enhance the activity of PI3K kinase, activate the PI3K/AKT/mTOR signaling pathway, and facilitate the proliferation of tumor cells (30).

The AKT pathway is an important signaling pathway

© Translational Cancer Research. All rights reserved.

that regulates cell proliferation. Activated AKT is vital for cell survival (31). As a serine or threonine protein kinase, it reduces apoptotic factors and increases the activity of anti-apoptotic proteins by phosphorylating multiple downstream targets. AKT can directly phosphorylate the pro-apoptotic molecule BCL2 associated agonist of cell death (BAD) to play an anti-apoptotic role. It can also inhibit apoptosis by promoting the expression of the caspase-8 analogue c-FLIP through NF-κB, and inhibit the apoptotic AKT signaling pathway by maintaining mitochondrial stability through activation of hexokinase, which also plays an important role in cancer cell migration (32). In mammalian cells, AKT rearranges the fine cytoskeleton and regulates cell motility through its downstream effectors such as Rho, Racl, and cdc42. The AKT signaling pathway can also upregulate matrix metalloproteinase 2 (MMP2) through a variety of ways to promote fine cell invasion (33). Furthermore, it can promote the expression and secretion of vascular endothelial growth factor and other angiogenic factors, and stimulate angiogenesis. This current report revealed that the expression of AKT in advanced breast cancer tissues was higher than that in early breast cancer tissues. It is speculated that the overexpression of AKT after carcinogenesis promotes cell proliferation, inhibits cell apoptosis, encourages cancer cells to grow and spread without restriction, and promotes angiogenesis by degrading extracellular mechanisms, thus promoting the development of breast cancer (34). The tumour-node-metastasis (TNM) stage and lymph node metastasis are important factors influencing the prognosis. Our results demonstrated a correlation between the expression of AKT and axillary lymph node metastasis of breast cancer. The expression of AKT in samples with axillary lymph node metastasis was significantly higher than that in samples without metastasis, suggesting that AKT is highly expressed during lymph node metastasis. This indicated that that AKT may be involved in the process of lymph node metastasis of breast cancer. The PTEN protein can dephosphorylate PIP3 and inhibit the PI3K/AKT pathway, thereby playing a biological role in inhibiting cell proliferation, promoting cell apoptosis, and the like (35). Finally, to examine which AKT affects *PTEN* phosphorylation, human breast cancer MDA-MB-231 cells were treated with an AKT inhibitor in the present study, showing that *AT7519* and *AZD5438* eliminated *PTEN*-SP phosphorylation. This provides a new direction for the next treatment of breast cancer, but further clinical research is needed.

Limitations

Due to the lack of data on the long-term prognosis of patients, we could not analyze the relationship between PTEN gene and prognosis. Moreover, it was more convincing to identify the pathway by AKT/mTOR signal pathway inhibitor. However, we failed to study it. In addition, we failed to study the effects of PTEN on the migration or invasion of breast cancer cells.

Conclusions

This investigation revealed that the expression of *PTEN* and AKT are negatively correlated in breast cancer, suggesting that during the development of breast cancer, the inactivation of *PTEN* gene inhibits the PI3K/AKT pathway, inhibits the apoptosis of breast cells, promotes unlimited proliferation, and drives the development of breast cancer. Therefore, abnormalities in the *PTEN* PI3K/AKT pathway play an important role in the occurrence and development of breast cancer, and this revelation may contribute to the development of novel research directions for further understanding and treating breast cancer.

Acknowledgments

Funding: The work was funded by the Baoding Science and Technology Project (No. 17ZF192).

Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-826/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-826/dss

Peer Review File: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-826/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-826/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all

© Translational Cancer Research. All rights reserved.

aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Ethics Committee of the Affiliated Hospital of Hebei University (No. 202100584) and individual consent for this analysis was obtained from all patients.

Open Access Statement: This is an Open Access article distributed in accordance with the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License (CC BY-NC-ND 4.0), which permits the non-commercial replication and distribution of the article with the strict proviso that no changes or edits are made and the original work is properly cited (including links to both the formal publication through the relevant DOI and the license). See: https://creativecommons.org/licenses/by-nc-nd/4.0/.

References

1. Chibaya L, Karim B, Zhang H, et al. Mdm2 phosphorylation by Akt regulates the p53 response to oxidative stress to promote cell proliferation and tumorigenesis. Proc Natl Acad Sci U S A 2021;118:e2003193118.
2. Yudushkin I. Control of Akt activity and substrate phosphorylation in cells. IUBMB Life 2020;72:1115-25.
3. Piazzi M, Bavelloni A, Gallo A, et al. AKT-Dependent Phosphorylation of ADAR1p110 and ADAR2 Represents a New and Important Link Between Cell Signaling and RNA Editing. DNA Cell Biol 2020;39:343-8.
4. Senoo H, Murata D, Wai M, et al. KARATE: PKA-induced KRAS4B-RHOA-mTORC2 supercomplex phosphorylates AKT in insulin signaling and glucose homeostasis. Mol Cell 2021;81:4622-4634.e8.
5. Jiang Q, Zhang X, Dai X, et al. S6K1-mediated phosphorylation of PDK1 impairs AKT kinase activity and oncogenic functions. Nat Commun 2022;13:1548.
6. Wu L, Zheng Y, Liu J, et al. Comprehensive evaluation of the efficacy and safety of LPV/r drugs in the treatment of SARS and MERS to provide potential treatment options for COVID-19. Aging (Albany NY) 2021;13:10833-52.
7. Qiu K, Ma C, Lu L, et al. DAPT suppresses proliferation and migration of hepatocellular carcinoma by regulating the extracellular matrix and inhibiting the Hes1/PTEN/AKT/mTOR signaling pathway. J Gastrointest Oncol 2021;12:1101-16.
8. Huang X, Tan J, Chen X, et al. Akt Phosphorylation Influences Persistent Chlamydial Infection and Chlamydia-Induced Golgi Fragmentation Without Involving Rab14. Front Cell Infect Microbiol 2021;11:675890.
9. Huang Y, Liu S, Shan M, et al. RNF12 is regulated by AKT phosphorylation and promotes TGF-β driven breast cancer metastasis. Cell Death Dis 2022;13:44.
10. Saneyasu T, Ueno M, Nagata K, et al. Central administration of insulin and refeeding lead to Akt and ERK phosphorylation in the chicken medulla. Neurosci Lett 2021;758:136008.
11. Mo Y, Wu Y, Li X, et al. Osthole delays hepatocarcinogenesis in mice by suppressing AKT/FASN axis and ERK phosphorylation. Eur J Pharmacol 2020;867:172788.
12. Vivero A, Ruz M, Rivera M, et al. Zinc Supplementation and Strength Exercise in Rats with Type 2 Diabetes: Akt and PTP1B Phosphorylation in Nonalcoholic Fatty Liver. Biol Trace Elem Res 2021;199:2215-24.
13. Wu L, Zhong Y, Wu D, et al. Immunomodulatory Factor TIM3 of Cytolytic Active Genes Affected the Survival and Prognosis of Lung Adenocarcinoma Patients by Multi-Omics Analysis. Biomedicines 2022;10:2248.
14. Ali MZ, Dholaniya PS. Oxidative phosphorylation mediated pathogenesis of Parkinson's disease and its implication via Akt signaling. Neurochem Int 2022;157:105344.
15. Azraq I, Craveiro RB, Niederau C, et al. Gene expression and phosphorylation of ERK and AKT are regulated depending on mechanical force and cell confluence in murine cementoblasts. Ann Anat 2021;234:151668.
16. Myöhänen TT, Mertens F, Norrbacka S, et al. Deletion or inhibition of prolyl oligopeptidase blocks lithium-induced phosphorylation of GSK3b and Akt by activation of protein phosphatase 2A. Basic Clin Pharmacol Toxicol 2021;129:287-96.
17. Wu CH, Tsai YC, Tsai TH, et al. Valproic Acid Reduces Vasospasm through Modulation of Akt Phosphorylation and Attenuates Neuronal Apoptosis in Subarachnoid Hemorrhage Rats. Int J Mol Sci 2021;22:5975.
18. Wu L, Zheng Y, Ruan X, et al. Long-chain noncoding ribonucleic acids affect the survival and prognosis of patients with esophageal adenocarcinoma through the autophagy pathway: construction of a prognostic model. Anticancer Drugs 2022;33:e590-603.
19. Hussain N, Chuang SJ, Johanns M, et al. Effects of PKB/Akt inhibitors on insulin-stimulated lipogenesis and phosphorylation state of lipogenic enzymes in white

adipose tissue. Biochem J 2020;477:1373-89.
20. Costa C, Wang Y, Ly A, et al. PTEN Loss Mediates Clinical Cross-Resistance to CDK4/6 and PI3Kα Inhibitors in Breast Cancer. Cancer Discov 2020;10:72-85.
21. Csolle MP, Ooms LM, Papa A, et al. PTEN and Other PtdIns(3,4,5)P(3) Lipid Phosphatases in Breast Cancer. Int J Mol Sci 2020;21:9189.
22. Prvanović M, Nedeljković M, Tanić N, et al. Role of PTEN, PI3K, and mTOR in Triple-Negative Breast Cancer. Life (Basel) 2021;11:1247.
23. Bassi C, Fortin J, Snow BE, et al. The PTEN and ATM axis controls the G1/S cell cycle checkpoint and tumorigenesis in HER2-positive breast cancer. Cell Death Differ 2021;28:3036-51.
24. Pappas K, Martin TC, Wolfe AL, et al. NOTCH and EZH2 collaborate to repress PTEN expression in breast cancer. Commun Biol 2021;4:312.
25. Li X, Wang M, Li S, et al. HIF-1-induced mitochondrial ribosome protein L52: a mechanism for breast cancer cellular adaptation and metastatic initiation in response to hypoxia. Theranostics 2021;11:7337-59.
26. Swellam M, Saad EA, Sabry S, et al. Alterations of PTEN and SMAD4 methylation in diagnosis of breast cancer: implications of methyl II PCR assay. J Genet Eng Biotechnol 2021;19:54.
27. Yue C, Bai Y, Piao Y, et al. DOK7 Inhibits Cell Proliferation, Migration, and Invasion of Breast Cancer via the PI3K/PTEN/AKT Pathway. J Oncol 2021;2021:4035257.
28. Izzo L, Messineo D, DI Cello P, et al. Correlation Between Onco-suppressors PTEN and NM23 and Clinical Outcome in Patients With T1 Breast Cancer. In Vivo 2021;35:169-74.
29. Wieland A, Strissel PL, Schorle H, et al. Brain and Breast Cancer Cells with PTEN Loss of Function Reveal Enhanced Durotaxis and RHOB Dependent Amoeboid Migration Utilizing 3D Scaffolds and Aligned Microfiber Tracts. Cancers (Basel) 2021;13:5144.
30. Chen J, Sun J, Wang Q, et al. Systemic Deficiency of PTEN Accelerates Breast Cancer Growth and Metastasis. Front Oncol 2022;12:825484.
31. Bao C, Liu T, Qian L, et al. Shikonin inhibits migration and invasion of triple-negative breast cancer cells by suppressing epithelial-mesenchymal transition via miR-17-5p/PTEN/Akt pathway. J Cancer 2021;12:76-88.
32. Wu L, Zhong Y, Yu X, et al. Selective poly adenylation predicts the efficacy of immunotherapy in patients with lung adenocarcinoma by multiple omics research. Anticancer Drugs 2022;33:943-59.
33. Wu J, Gao H, Ge W, et al. Over expression of PTEN induces apoptosis and prevents cell proliferation in breast cancer cells. Acta Biochim Pol 2020;67:515-9.
34. Brunner A, Suryo Rahmanto A, Johansson H, et al. PTEN and DNA-PK determine sensitivity and recovery in response to WEE1 inhibition in human breast cancer. Elife 2020;9:e57894.
35. Du MG, Peng ZQ, Gai WB, et al. The Absence of PTEN in Breast Cancer Is a Driver of MLN4924 Resistance. Front Cell Dev Biol 2021;9:667435.

(English Language Editor: J. Teoh)

Cite this article as: Zhang J, Zhang Y, Lin X, Han X, Meredith KL, Li Z. The effects of the tumor suppressor gene *PTEN* on the proliferation and apoptosis of breast cancer cells via AKT phosphorylation. Transl Cancer Res 2023;12(7):1863-1872. doi: 10.21037/tcr-23-826

© Translational Cancer Research. All rights reserved.