Technology in Cancer Research & Treatment
Volume 19: 1-7

DOI: 10.1177/1533033820917959
journals.sagepub.com/home/tct
SAGE

Inhibition of *PTEN* Gene Expression by Small Interfering RNA on *PI3K/Akt/FoxO3a* Signaling Pathway in Human Nasopharyngeal Carcinoma

Liang Zhong Yao, MM[1], Yan Li Zhu, MM[1], and Jun Jie Liu, BS[1]

Abstract

The objective of this article is to study the effect of inhibiting phosphatase and tensin homolog deleted chromatosome 10 gene on phosphoinositide 3-kinase/protein kinase B (*Akt*)/Forkhead homeobox O3a signaling pathway in human nasopharyngeal carcinoma HK-1 cells. Nasopharyngeal carcinoma HK-1 cell lines were divided into *PTEN* gene interference group (siPTEN), nonspecific small interfering RNA group (siNC), empty vector group (Vector), and no transfection control group (Normal). The mRNA and protein expression levels of *PTEN, PI3K*, *p-Akt*, and *FoxO3a* were detected by real-time fluorescence quantitative polymerase chain reaction and Western blot. Immunofluorescence was used to detect the subcellular localization of *PTEN, PI3K, p-Akt*, and *FoxO3a* in HK-1 cells. The proliferation of HK-1 cells was detected by MTT assay, and the apoptosis of HK-1 cells was detected by flow cytometry. Compared with the siNC group, the expression levels of *PTEN, FoxO3a* messenger RNA, and protein in the siPTEN group were significantly decreased ($P < .05$), while the expression levels of *PI3K*, *p-Akt* messenger RNA, and protein were significantly increased ($P < .05$). The growth rate of HK-1 cells in the siPTEN group was significantly higher than the siNC group ($P < .05$), while the apoptosis rate was significantly lower than that of the siNC group ($P < .05$). Small interfering RNA can inhibit the expression of *PTEN* in HK-1 cells, and *PTEN* can participate in the development of NPC by affecting *PI3K/Akt/FoxO3a* signaling pathway.

Keywords

PTEN, phosphoinositide 3-kinase, protein kinase B, small interfering RNA, nasopharyngeal cancer

Abbreviations

cDNA, complementary DNA; DMSO, dimethyl sulfoxide; *FoxO3a*, Forkhead homeobox O3a; mRNA, messenger RNA; NPC, nasopharyngeal carcinoma; PBS, phosphate-buffered saline; PI, propidium iodide; *PI3K*, phosphoinositide 3-kinase; *PTEN*, phosphatase and tensin homolog deleted chromatosome 10; qRT-PCR, real-time fluorescence quantitative polymerase chain reaction; RNAi, RNA interference; V-FITC, Annexin V-fluorescein isothiocyanate

Received: November 19, 2019; Revised: March 1, 2020; Accepted: March 13, 2020.

Nasopharyngeal carcinoma (NPC) is a malignant tumor characterized by region and race. It occurs mostly in Southeast Asia and southern China. Nasopharyngeal carcinoma generally originates from nasopharyngeal epithelial squamous cells.[1] There is no specific clinical manifestation in the early stage, so it is not easy to attract the attention of patients. Most patients have been in the middle or late stage when they visit the clinic. At this time, NPC has a high degree of invasiveness and metastasis, which makes treatment more difficult and seriously affects the quality of life of patients. Nowadays, radiotherapy and

[1] Department of Otorhinolaryngology, The First Affiliated Hospital of Guangdong Pharmaceutical University, Guangzhou, Guangdong, China

Corresponding Author:
Liang Zhong Yao, Department of Otorhinolaryngology, The First Affiliated Hospital of Guangdong Pharmaceutical University, No. 19, Nonglin Xia Road, Guangzhou, Guangdong 510080, China.
Email: yaoliangz88@126.com

chemotherapy are still the standard therapy of NPC. But the related data showed that the 5-year survival rate for NPC patients is only 60%.[2] Therefore, it has become most important to understand the growth mechanism of NPC cells and search for new molecular targeted therapies. Phosphoinositide 3-Kinase (*PI3K*) can activate protein kinase B (*Akt*), form *PI3K/Akt* signaling pathway by phosphorylated Akt, and regulate the accumulation of Forkhead homeobox O3a (*FoxO3a*) from nucleus to cytoplasm, leading to its inactivation, thus promoting the growth and migration of cancer cells.[3] In NPC, the activation of *PI3K/Akt/ FoxO3a* signaling pathway is usually closely related to the rapid growth of HK-1 cells. Moreover, previous studies found that phosphatase and tensin homolog deleted chromatosome 10 (*PTEN*) protein expression in tumor cells was significantly lower than that in normal tissue cells in NPC, suggesting that *PTEN* may be involved in the development of NPC disease. However, it is unclear whether the abnormal expression of *PTEN* gene will participate in the disease progression of NPC by affecting the *PI3K/Akt/FoxO3a* signaling pathway. *PTEN* is a phosphorylase-mediated oncogene, which is prone to mutation or deletion. It has been proved that it can inhibit tumor cell proliferation and promote apoptosis in many tumor diseases.[4] Therefore, this study intends to synthesize small interfering RNA *in vitro* by using RNA interference (RNAi), to study the effect of *PTEN* gene expression on NPC *PI3K/Akt/FoxO3a* signaling pathway.

Materials and Methods

Materials and Reagents

Nasopharyngeal carcinoma HK-1 cell line was purchased from ATCC (Washington D.C., USA) and preserved by our studying team; fetal bovine serum, RPMI-1640, and Opti-MEM medium were purchased from Gibco (Beijing, China); dimethyl sulfoxide (DMSO) was purchased from Sigma (Shanghai, China); phosphate-buffered saline (PBS) powder was purchased from Wuhan PhD Bioengineering Co., Ltd (Wuhan, China); Lipofectamine 2000 transfection reagent was purchased from Invitrogen (Wuhan, China); Radio-Immunoprecipitation Assay (RIPA) lysate, phenylmethylsulfonyl fluoride, bicinchoninic acid (BCA) protein concentration assay kit, SDS-polyacrylamide gelelectrophoresis (SDS-PAGE) gel rapid preparation kit (P0012 AC), *PTEN*, *PI3K*, *p-Akt*, *FoxO3a* first antibody and internal reference ACTB antibody, and goat antirabbit fluorescent second antibody were purchased from Beyotime Biotechnology.

Methods

Cell culture. HK-1 cells were incubated in RPMI 1640 medium containing 10% inactivated fetal bovine serum, incubated at a 37°C, 5% CO_2 constant-temperature incubator, and the medium was changed every 2 days. When the cell adherence grew logarithmically to cover 80% to 90% of the basement, 0.25% trypsin containing EDTA was used for digestion passage, and the cells with better growth condition were taken for subsequent experiments.

Cell transfection. Transfection was divided into 4 groups, including *PTEN* gene interference group (siPTEN), nonspecific small interfering RNA group (siNC), empty vector group (Vector), and nontransfected control group (Normal). The transfection reagent was Lipofectamine 2000, and the siRNA sequence of the siPTEN group was synthesized by Shanghai Jima Pharmaceutical Technology Co, Ltd. Its forward sequence was 5′-ACCAGGACCAGAGGAAACCT-3′, and the reverse sequence was 5′-TTTGTCAGGGTGAGCACAAG-3′. The HK-1 cells of the siNC group were treated with a nonspecific siRNA-Lipofectamine 2000, in which the nonspecific small interfering RNA (siRNA) base pair sequence was the forward sequence 5′-AUUGGCUACUACCGAAGAG-3′, and the reverse sequence 5′-CUCUUCGGUAGUAGCCAAU-3′. One day before transfection, 0.4 to 1.0×10^5 cells/well were inoculated into a 24-well plate culture containing an appropriate amount of complete medium and were cultured at 37°C 5% CO_2 to 70% to 80% confluence; 1 hour before transfection, the 24-well plate culture was replaced with fresh, nonresistant complete medium. An amount of 0.8 μg siRNA plasmid was added into 50-μL Opti-MEM medium, and 4.8-μL Lip2000 reagent was diluted with 50-μL serum-free Opti-MEM, mixed and placed at room temperature for 5 minutes. The diluted siRNA and Lipofectamine 2000 reagents were mixed and placed at room temperature for 30 minutes to form siRNA/Lipofectamine 2000 complex. The 100-μL siRNA/Lipofectamine 2000 complex was added to the wells of the culture plate containing cells and medium, and the cell culture plate was shaken gently to and fro to make the siRNA/Lip2000 mixture cover the cells evenly. After the cells were incubated in CO_2 incubator at 37°C for 48 hours, the cells expressing green fluorescent protein were observed by fluorescence microscopy, and the transfection efficiency was observed.

Real-time fluorescence quantitative polymerase chain reaction. Real-time fluorescence quantitative polymerase chain reaction (qRT-PCR) was used for RNA quantitative analysis. First, total RNA was extracted by TRIzol reagent, and the OD value was determined by nucleic acid protein analyzer. When the ratio of OD260/OD280 was between 1.8 and 2.0, the prepared RNA was pure. Then complementary DNA (cDNA) was synthesized by single-stranded RNA reverse transcription according to the instructions of the cDNA kit. With ACTB as the internal reference, the quantitative PCR conditions followed: predenaturation at 95°C for 20 seconds, (95°C 10 seconds → 60°C 20 seconds → 72°C) × 40, and the relative expression level of the target gene was calculated by $2^{-\Delta\Delta CT}$. The primer sequences used in qRT-PCR were as follows: *PI3K* forward primer: 5′-AACTCTGGGGATGACCTGGA-3′, reverse primer: 5′-AGGCGGTCACAACACTCCTA-3′; *Akt* forward primer: 5′-AGGGTTGGCTGCACCGCG-3′, reverse primer: 5′-GTTGTTGAAGAGACACCG-3′; *FoxO3a* forward primer: 5′-AAACCCTCTCGGACTCTCTCTC-3′, reverse primer: 5′-

TAAAATCCAACCCATCAgCATC-3′; *ACTB* forward primer: 5′-CAGCGACACCCACTCCTC-3′, reverse primer: 5′-TGAGGTCCACCACCCTGT-3′.

Western blot detection. Protein samples were lysed using RIPA proteolytic buffer to determine protein concentration using the BCA Protein Quantitation Kit according to the instructions. Then, the protein in the sample was separated by SDS-PAGE gel electrophoresis, 5 μL of protein loaded in each lane, and the pre-stained marker loaded on both sides, and the excess wells loaded with 1× SDS-PAGE loading buffer. The wet system was used to transfer to PVDF film after the electrophoresis finished. After transfer completed, according to the prestained marker prediction target band interval, cut the PVDF membrane into the small grid, and the target protein was incubated corresponding to the primary antibody dilution *PTEN* (AP686, 1:1000) and *PI3K* (AF1549, 1:1000) at 60 to 70 rpm shaker at 4°C, *p-Akt* (AF1546, 1:1500), *FoxO3a* (AF609, 1:1000) overnight (12-16 hours). The goat antirabbit secondary antibody with HRP locus (A0208, 1:2000) was selected according to the source of the primary antibody and incubated for 1 hour at room temperature on a 60 to 70 rpm shaker and washed with tris buffered saline-Tween (TBS-T) buffer at 100 rpm for 10 minutes, 3 times; finally, the developer was added and developed by chemiluminescence imaging system.

Immunofluorescence detection. The well-crawled slides were immersed in PBS in the culture plate, fixed with 4% paraformaldehyde for 15 minutes; 0.5% cell penetrant (Triton X-100) was at room temperature for 20 minutes; normal goat serum were added dropwise on the slides, which was sealed at room temperature for 30 minutes. Each slide was added enough amount of diluted primary antibody *PTEN* (AP686, 1:100), *PI3K* (AF1549, 1:200), *p-Akt* (AF1546, 1:200), and *FoxO3a* (AF609, 1:200) and put into a wet box overnight at 4°C. Then, the diluted fluorescent secondary antibody (A0208, 1:200) was added and incubated at 20°C to 37°C for 1 hour in a wet box. 4′,6-diamidino-2-phenylindole (DAPI) was added to incubate for 5 minutes in the dark, and the samples were stained. Finally, seal the film with a sealing liquid containing an antifluorescence quencher to observe the collected image under the fluorescence microscope.

MTT detection. The HK-1 cells after transfection were treated with trypsin digestion and then seeded in 96-well plates. The marginal wells were filled with sterile water or PBS, and the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide, Thiazolyl Blue Tetrazolium Bromide (MTT) dilution was added to 37°C, incubated in a 5% CO_2 incubator for 4 hours, and each group was set to 3 to 5 duplicate wells; then DMSO solution was added dropwise, and the OD value was measured at 490 nm for 3 days, and a growth curve was drawn.

Flow cytometry detection. Annexin V-fluorescein isothiocyanate (V-FITC) cell apoptosis detection kit (Keygen, No .KGA106) was used to detect the apoptosis of cells. EDTA-free trypsinase digestive cells were used to terminate digestion and centrifuged at 300*g* and 4°C for 5 minutes to collect cells; 0.5 mL cell suspension was added to resuspend, and the same amount of untreated live cells were mixed in addition to that. Cell suspension was added to 1.5 mL, divided into 3 tubes, one of which was a blank control tube and the 2 tubes were single-stained tubes; 5 μL Annexin V-FITC or 10 μL propidium iodide (PI) was added to the single-stained tubes, and the compensation of the fluorescent channels was adjusted with a single-stained tube. In the sample group, Annexin V-FITC (Ex = 488 nm; Em = 530 nm) was detected by FITC detection channel and PI was detected by PE detection channel.

Statistical Processing Analysis

The statistical software SPSS 19.0 was used for data analysis. The measurement data were expressed as mean ± standard deviation. The comparison among groups was analyzed by variance and the comparison between groups by q-snk. The difference was statistically significant at $P < .05$.

Results

Detection of the Protein Level of PTEN, PI3K, p-Akt, and FoxO3a by Western Blot

From Figure 1, *PTEN*, *PI3K*, *p-Akt*, and *FoxO3a* proteins were expressed in all 4 groups of cells. Compared with the siNC group, the *PTEN* and *FoxO3a* protein levels in the siPTEN group were significantly decreased ($P < .05$), the *PI3K* protein levels significantly increased ($P < .05$), and the *Akt* phosphorylation level significantly upregulated ($P < .05$).

Detection of the Messenger RNA Expression Levels of PTEN, PO3K, p-Akt, and FoxO3a by qRT-PCR

The expression levels of *PTEN* and *FoxO3a* messenger RNA (mRNA) in the siPTEN group were significantly lower than those in the siNC group ($P < .05$), while expression levels of *PI3K* and *p-Akt* mRNA were significantly higher than those in the siNC group ($P < .05$). There were no significant differences in the expression of *PTEN*, *PI3K*, *p-Akt*, and *FoxO3a* mRNA among the Normal group, the Vector group, and the siNC group ($P > .05$), as shown in Figure 2.

MTT Assay for Proliferation of HK-1 Cells

Within 24 hours, the growth rate of HK-1 cells in Normal group, Vector group, siNC group, and siPTEN group was not significantly different ($P > .05$), but after 24 hours, the growth rate of HK-1 cells in the siPTEN group was significantly higher than that in the siNC group ($P < .05$), and the difference became more obvious over time, as shown in Figure 3.

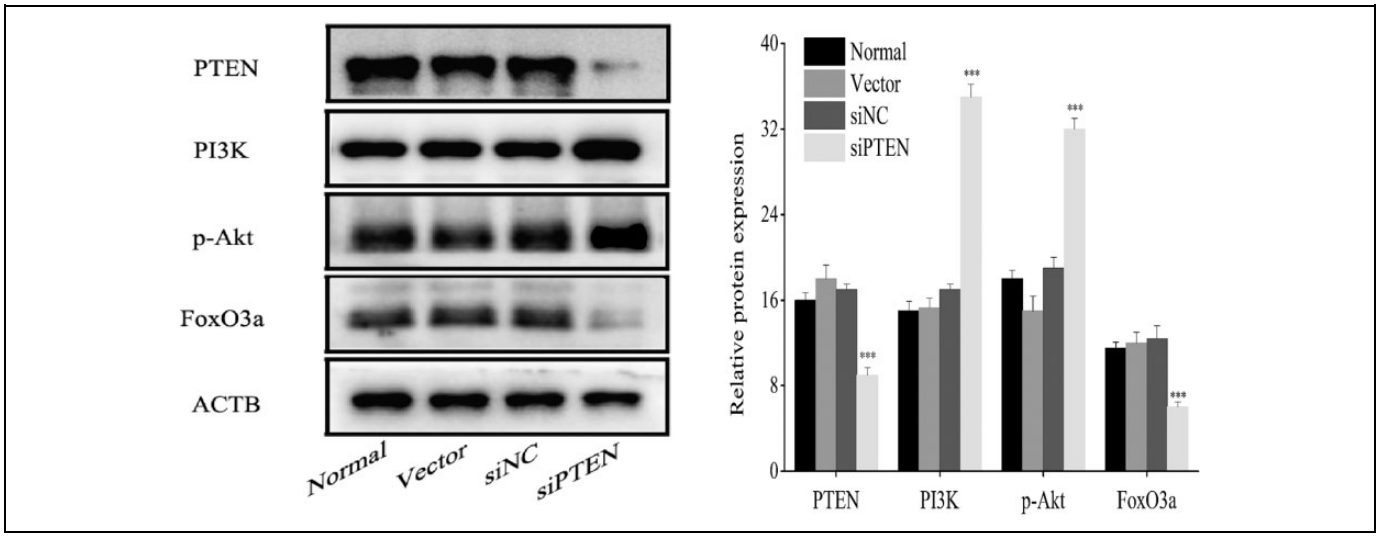

Figure 1. Western blot showed *PTEN* and *FoxO3a* protein levels decreased significantly, *PI3K* protein level increased significantly, *Akt* phosphorylation level increased significantly. ***$P < .001$. ACTB was used as a loading control.

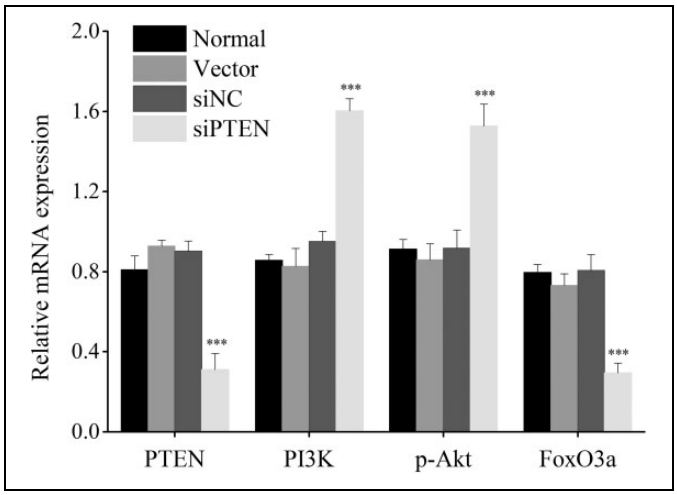

Figure 2. Real-time fluorescence quantitative polymerase chain reaction showed *PTEN* and *FoxO3a* messenger RNA (mRNA) levels decreased significantly; *PI3K* and *p-Akt* mRNA levels increased significantly. ***$P < .001$.

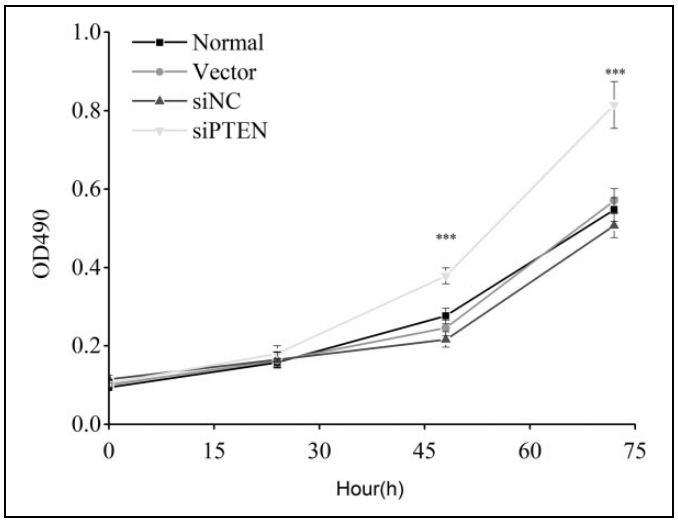

Figure 3. 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide, Thiazolyl Blue Tetrazolium Bromide (MTT) showed that after 24 hours, the growth rate of HK-1 cells in the siPTEN group was significantly higher than that in the siNC group, and the difference was more and more obvious with the passage of time. ***$P < .001$.

Immunofluorescence Detection of the Expression of PTEN, PI3K, p-Akt, *and* FoxO3a *in HK-1 Cells*

From Figure 4, *PTEN, PI3K* and *p-Akt* proteins were mainly located in the cytoplasm and expressed in a small amount in the nucleus. However, after *PTEN* gene was interfered, the expression of *PTEN*, *PI3K*, and *p-Akt* proteins in the cytoplasm decreased significantly, while the expression of *PTEN*, *PI3K*, and *p-Akt* proteins in the nucleus increased significantly, that is, from the cytoplasm to the nucleus (Figure 4A-C). *FoxO3a* protein was mainly located in the nucleus and expressed in a small amount in the cytoplasm. However, after *PTEN* gene was interfered, *FoxO3a* protein moved from the nucleus to the cytoplasm (Figure 4D).

Flow Cytometry Detection of Apoptosis in HK-1 Cells

Normal HK-1 cells were not stained with FITC and PI and were in the left lower quadrant of flow cytometry. HK-1 cells in the early stage of apoptosis were stained with FITC and were in the right lower quadrant. HK-1 cells in the late stage of apoptosis were simultaneously stained with FITC and PI and were in the right upper quadrant. Necrotic or mechanically damaged cells were in the left upper quadrant. In the siPTEN group, the proportion of HK-1 cells at the early stage of apoptosis was 6.1% and the total apoptotic rate of HK-1 cells at the early stage of apoptosis was 7.2%, which was significantly lower than that in the siNC group ($P < .05$; Figure 5A-H).

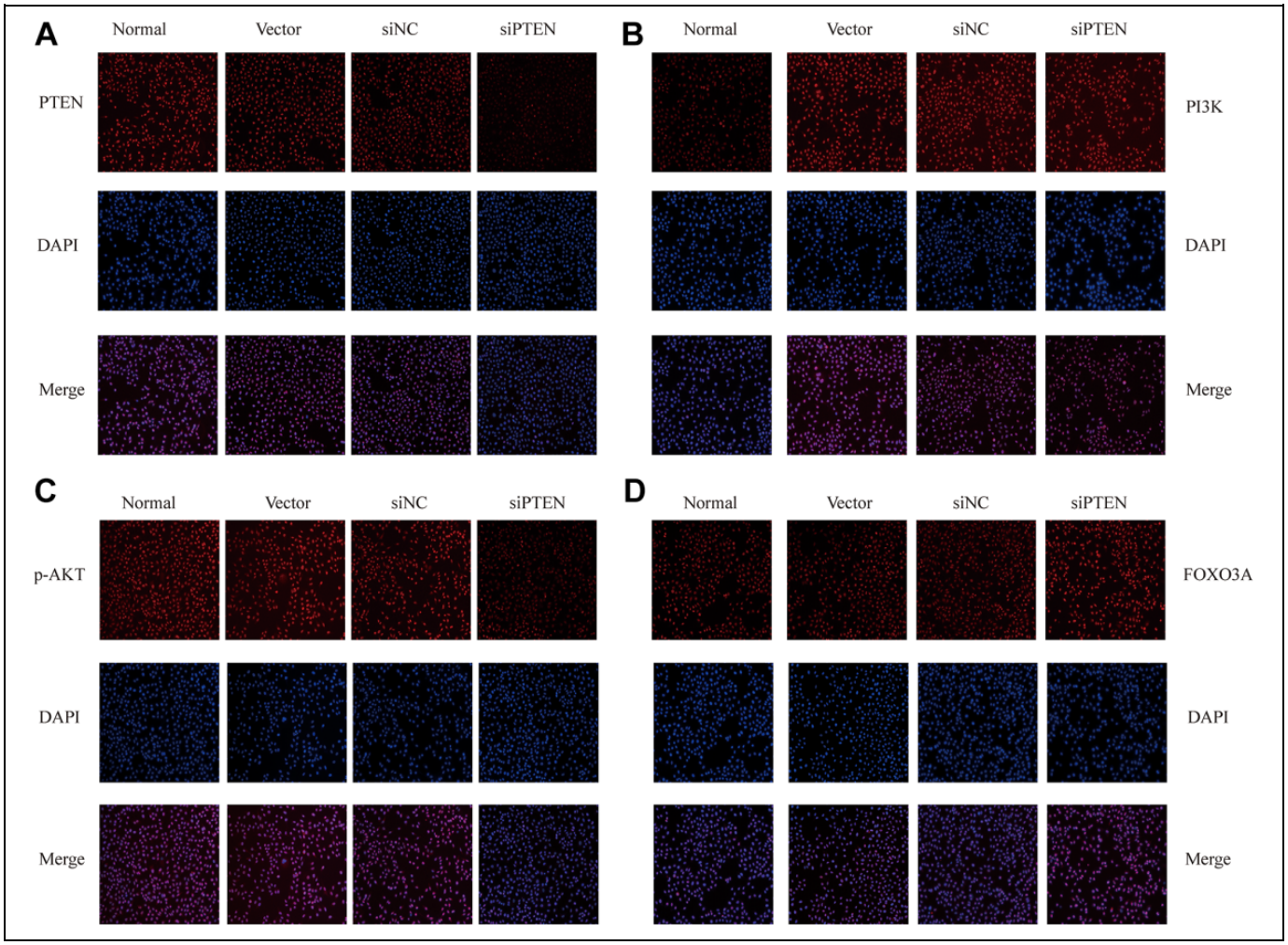

Figure 4. After *PTEN* gene was interfered, the expression of *PTEN* (A), *PI3K* (B), and *p-Akt* (C) protein decreased significantly in the cytoplasm and increased significantly in the nucleus, that is, from the cytoplasm to the nucleus. *FoxO3a* (D) protein was transferred from the nucleus to the cytoplasm.

Discussion

The occurrence of cancer is usually the result of a combination of several factors, such as genetics, environment, viral infection, diet, and so on. Nasopharyngeal carcinoma, as a malignant tumor with strong geographical distribution, is more affected by genetic factors than other cancers. Because NPC is highly sensitive to radiation therapy, the current standard treatment for this disease is still radiotherapy. Patients with stage III and IV need to undergo both radiotherapy and chemotherapy,[5] but according to relevant data,[6] using radiotherapy and chemotherapy for NPC, only short-term local control can be achieved and long-term prognosis is not effective. It is prone to adverse symptoms such as recurrence and metastasis, resulting in a decrease in patient survival rate. With the advancement of medical science and technology, molecular biology-based gene-level research has elevated the understanding of diseases to a new stage. The focus of NPC research has gradually shifted to related gene modification to control the development of the disease from a genetic perspective.

RNA interference technology refers to the effective degradation and silencing gene expression of homologous host mRNA by double-stranded RNA. After transcription, specific double-stranded RNA is processed into siRNA duplexes and loaded into RNA-induced silencing complexes. Small interfering RNA recognizes target mRNA by base pairing, for direct cleavage and degradation.[7] RNA interference technology can precisely block specific gene expression and produce gene knockout effects,[8] providing a new method for the majority of scholars to analyze gene function. In this study, the expression of *PTEN* gene in human NPC HK-1 cell line was inhibited by RNAi. The results of qRT-PCR and Western blot showed that the expression levels of *PTEN* mRNA and protein in the siPTEN group were significantly lower than those in normal and siNC group, indicating that RNAi technology successfully inhibited *PTEN* gene expression in human NPC HK-1 cell line. *PTEN*, also known as mutated in multiple advanced cancer, transforming growth factor (TGF)-regulated and epithelial cell-enriched phosphatase (TEP1), is a novel cancer suppressor

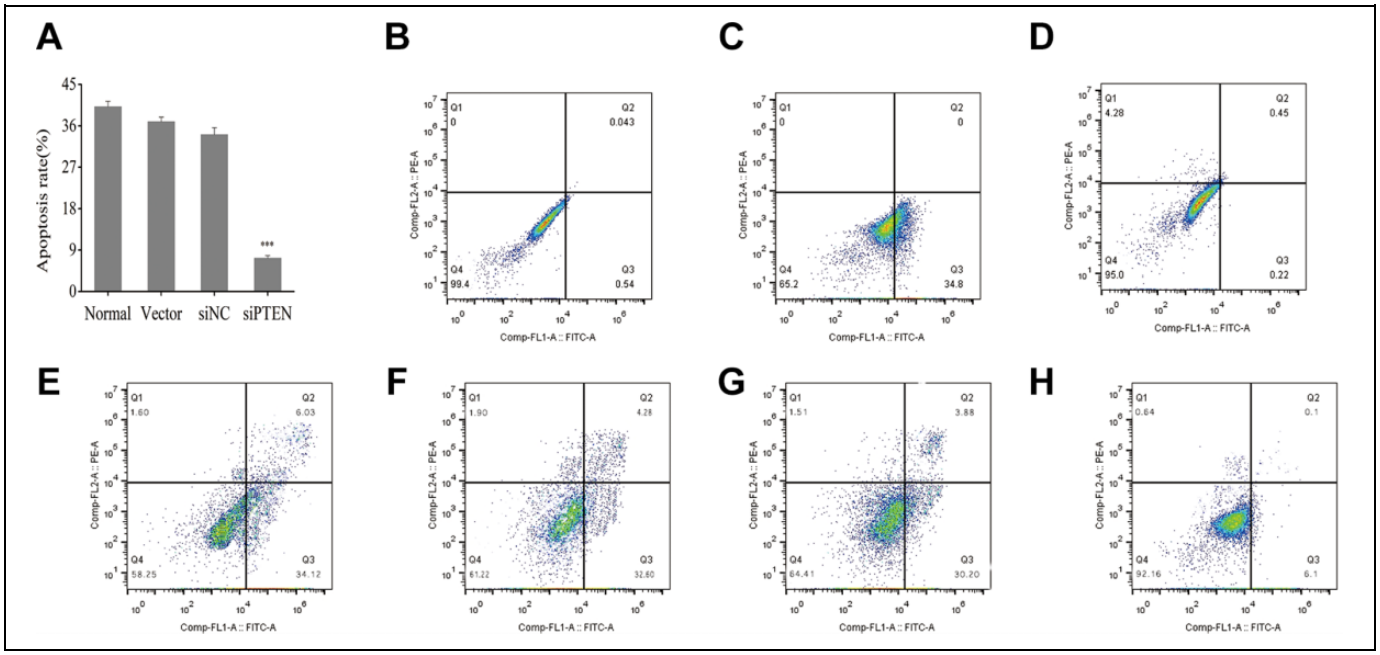

Figure 5. Flow cytometry shows that the proportion of HK-1 cells in the early stage of apoptosis in the siPTEN group was 6.1%, and the total apoptosis rate of HK-1 cells in the early stage of apoptosis was 7.2%, which was significantly lower than that in the siNC group. ***$P < .001$. A, Apoptotic rate. B, No fluorescein isothiocyanate (FITC) and propidium iodide staining. C, Only FITC staining. D, Only propidium iodide staining. E, Normal group. F, Vector group. G, The siNC group. H, The siPTEN group.

gene located on the human chromosome 10q23.3. It is encoded by 403 amino acid residues and was first discovered in 1997.[9] *PTEN* belongs to casein phosphatase family and also has lipophosphatase activity. It can inhibit cell proliferation, induce apoptosis, and enhance immune stability to achieve anticancer effect. *PTEN* has been used in the study of endometrial cancer, renal cancer, lung cancer, ovarian cancer, and other cancer diseases.[10] In this study, the results of qRT-PCR and Western blot showed that the expression levels of *FoxO3a* mRNA and protein in the siPTEN group were significantly lower than those in the siNC group. The expression level of *PI3K, p-Akt* mRNA, and protein in the siPTEN group was significantly higher than that of the siNC group, which suggested that inhibiting the expression of *PTEN* gene in HK-1 cells can upregulate the expression of *PI3K, p-Akt* mRNA, and protein and downregulate the expression of *FoxO3a* mRNA and protein. It also indicated that the *PTEN* gene could affect the expression of *PI3K, p-Akt,* and *FoxO3a*. *PI3K* is composed of a catalytic subunit and a regulatory subunit, which can be regulated by vascular endothelial growth factor, fibroblast growth factor, and other signal transducers, and autophosphorylation occurs in the medial side of the plasma membrane. It can catalyze phosphatidyl inositol 4,5-trisphosphate (PIP2) to produce phosphatidyl inositol 3,4,5-trisphosphate (PIP3).[11] *Akt* is a specific ser/thr protein kinase of the AGC family, which contains 3 domains of regulation, catalysis, and pH. On the one hand, Ser473 and Thr308, which are located on the regulatory and catalytic domain, respectively, can undergo phosphorylation under the regulation of *PI3K* to activate the *PI3K/Akt* signaling pathway. On the other hand, the PH domain of *Akt* has a natural affinity for PIP3 and thus binds to PIP3 to activate the *PI3K/Akt* signaling pathway.[12,13] Studies have shown that[14] *PI3K/Akt* signaling pathway is abnormally expressed in a variety of tumor diseases, including NPC, which can promote tumor cell proliferation, change the cell active, and participate in disease progression by phosphorylating a variety of related substrates. *FoxO3a* is located on human chromosome 6q21 and is encoded by 673 amino acids. It has a fork-head DNA-binding domain typical of the fork-shaped transcription factor family. It plays an important role in cell cycle regulation, DNA damage repair, and other cellular physiological processes, known as "tumor suppressor."[15,16] *FoxO3a* expression activity is closely related to subcellular localization. Under normal conditions, *FoxO3a* is localized in the nucleus, but in tumor cells, it can be phosphorylated by *Akt* stimulation in its upstream pathway, forming a complex with 14-3-3 protein and transferring to the cytoplasm, leading to inactivation.[17]

The results of immunofluorescence assay showed that after the PTEN gene was interfered, the expression of PTEN in the cytoplasm was significantly decreased and the expression in the nucleus was significantly increased. *PI3K* and *p-Akt* proteins first migrated to the plasma membrane region and then redisperse in the cytoplasm and nucleus. *FoxO3a* protein was mainly located in the nucleus, but after the *PTEN* gene was interfered, *FoxO3a* protein moved from the nucleus to the cytoplasm, suggesting that the accumulation of *FoxO3a* in the nucleus may be related to the inhibition of *PTEN*. The phosphatase activity of *PTEN* could transform PIP3 into PIP2, which reduces the phosphorylation of *PI3K* to *Akt* by blocking the level of PIP3 to block the *PI3K/Akt* signaling pathway and

promote the expression of *FoxO3a*.[18] When the *PTEN* gene was inhibited, the *PI3K/Akt* signaling pathway was enhanced, which promoted *PI3K* upregulation of *Akt* phosphorylation and inhibition of *FoxO3a* expression, resulting in decreased expression of *FoxO3a* mRNA and protein.

The results of MTT assay showed that the growth rate of HK-1 cells in the siPTEN group was significantly higher than that in the siNC group after 24 hours, and the difference was more obvious with time, suggesting that inhibition of *PTEN* gene expression could promote cell proliferation. The results of flow cytometry showed that in the siPTEN group, the proportion of HK-1 cells at the early stage of apoptosis was 6.1% and the total apoptotic rate of the siPTEN group was 7.2%, which was significantly lower than that in the siNC group, suggesting that inhibition of *PTEN* gene expression could inhibit apoptosis.

In conclusion, inhibition of *PTEN* gene expression in HK-1 cells upregulates *PI3K and Akt* protein and mRNA levels, activates *PI3K/Akt* signaling pathway, inhibits *FoxO3a* activity, and increases cell growth rate and slows down apoptotic rate, suggesting that *PTEN* can participate in the development of NPC disease by affecting the *PI3K/Akt/FoxO3a* signaling pathway.

Authors' Note

Our study did not require an ethical board approval because it did not contain human or animal trials

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID iD

Liang Zhong Yao https://orcid.org/0000-0001-5710-444X

References

1. Ramayanti O, Juwana H, Verkuijlen SA, et al. Epstein-Barr virus mRNA profiles and viral DNA methylation status in nasopharyngeal brushings from nasopharyngeal carcinoma patients reflect tumor origin. *Int J Can*. 2017;140(1):149-162.
2. Zhao L, Fong AHW, Liu N, Cho WCS. Molecular subtyping of nasopharyngeal carcinoma (NPC) and a microRNA-based prognostic model for distant metastasis. *J Biomed Sci*. 2018;25(1): 16-23.
3. Zhang X, Zhuang T, Liang Z, et al. Breast cancer suppression by aplysin is associated with inhibition of PI3K/AKT/FOXO3a pathway. *Oncotarget*. 2017;8(38):63923-63934.
4. Arun R P, Sivanesan D, Vidyasekar P, Verma RS. PTEN/FOXO3/AKT pathway regulates cell death and mediates morphogenetic differentiation of colorectal cancer cells under simulated microgravity. *Sci Rep*. 2017;7(1):5952-5960.
5. Lee AW, Ma BB, Ng WT, Chan AT. Management of nasopharyngeal carcinoma: current practice and future perspective. *J Clin Oncol*. 2015;33(29):3356-3364.
6. Marks JE, Bedwinek JM, Lee F, Purdy JA. Dose-response analysis for nasopharyngeal carcinoma: an historical perspective. *Cancer*. 2015;50(6):1042-1050.
7. Kedmi R, Veiga N, Ramishetti S, Goldsmith M. A modular platform for targeted RNAi therapeutics. *Nat Nanotech*. 2018;13(3): 1-5.
8. Panwar V, Jordan M, Mccallum B, Bakkeren G. Host-induced silencing of essential genes in *Puccinia triticina* through transgenic expression of RNAi sequences reduces severity of leaf rust infection in wheat. *Plant Biotech J*. 2018;16(5):1013-1023.
9. Pan H, Li T, Jiang Y, et al. Overexpression of circular RNA ciRS-7 abrogates the tumor suppressive effect of miR-7 on Gastric cancer via PTEN/PI3K/AKT signaling pathway. *J Cell Biochem*. 2018;119(1):440-446.
10. Arachchilage G M, Kharel P, Reid J, Basu S. Targeting of G-quadruplex harboring pre-miRNA 92b by LNA rescues PTEN expression in NSCL cancer cells. *Acs Chemical Biology*. 2018; 13(4):909-914.
11. Dieterle A M, Keppeler H, Alers S, et al. PDK1 controls upstream PI3 K expression and PIP3 generation. *Oncogene*. 2014;33(23): 3043-3053.
12. He Q, Ren X, Chen J, Li Y, Tang XR, Wen X. miR-16 targets fibroblast growth factor 2 to inhibit NPC cell proliferation and invasion via PI3K/AKT and MAPK signaling pathways. *Oncotarget*. 2016;7(3):3047-3058.
13. Etiology TM, Infection I, Reported HBW. Traditional herbal formula NPC01 exerts antiangiogenic effects through inhibiting the PI3K/Akt/mTOR signaling pathway in nasopharyngeal carcinoma cells. *Evid Based Complement Alternat Med*. 2018, 2018(1):5291517.
14. Jiang H, Gao M, Shen Z, et al. Blocking PI3K/Akt signaling attenuates metastasis of nasopharyngeal carcinoma cells through induction of mesenchymal-epithelial reverting transition. *Oncol Rep*. 2014;32(2):559-566.
15. Ying L, Xiang A, Wei D, et al. Critical role of FOXO3a in carcinogenesis. *Mol Cancer*. 2018;17(1):104.
16. Shou Z, Lin L, Liang J, Li JL, Chen HY. Expression and prognosis of FOXO3a and HIF-1α in nasopharyngeal carcinoma. *J Cancer Res Clin Oncol*. 2012;138(4):585-593.
17. Quan Q, Lin Y, Jingting P, Lili C, Hongyan C. FoxO3a drives proliferation and apoptosis through PI3K/Akt signaling pathway in human nasopharyngeal carcinoma CNE-1 and CNE-2 cells. *J Cell Sci*. 2012;125(pt 18):4253-4263.
18. Carnero A, Blancoaparicio C, Renner O, Link W, Leal JF. The PTEN/PI3K/AKT signalling pathway in cancer, therapeutic implications. *Curr Cancer Drug Targets*. 2008;8(3):187-198.