

Polyphyllin VII inhibits triple-negative breast cancer via THOP1 modulation

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Abstract

Objective: We assessed the role of polyphyllin VII in triple-negative breast cancer (TNBC) and investigated the potential molecular mechanism. **Methods:** Cellular proliferation was measured using the CCK-8 assay. To evaluate colony formation and cell migration, we conducted colony formation assays and transwell analyses. Apoptotic protein levels were determined through Western blot. The in vivo anti-TNBC efficacy of polyphyllin VII were evaluated using xenograft tumor models.

Results: Polyphyllin VII exhibited inhibitory effects on cellular proliferation, colony formation, migration, and also induced apoptosis. Polyphyllin VII downregulated THOP1 expression in TNBC. THOP1 overexpression noticeably reversed the aforementioned effects of polyphyllin VII. In vivo, polyphyllin VII demonstrated inhibition of TNBC tumor growth and THOP1 expression, while simultaneously upregulating cleaved caspase 3. **Conclusion:** These results demonstrated that polyphyllin VII induced apoptosis by regulating THOP1 in TNBC. THOP1 is therefore proposed as a potential novel target of polyphyllin VII for combating TNBC.



1 Introduction

Breast cancer stands as one of the prevailing malignancies affecting women globally, with an excess of 300,000 newly reported cases in 2023 [1]. Breast cancer, characterized by its significant heterogeneity, can be categorized into four distinct subtypes: Her2-enriched, luminal A, luminal B, and triple-negative [2]. Triple-negative breast cancer (TNBC), comprising approximately 15%-20% of all breast cancer cases, is well-known for its aggressive characteristics, including high histologic grade, increased proliferation, and a poor prognosis [3]. The absence of specific treatment targets in TNBC is indeed unfortunate, posing a significant challenge and threat to clinicians and patients. Therefore, it is imperative to foster the advancement of innovative medications to address this pressing requirement.

Polyphyllin VII (PPVII) is a steroidal saponins derived from the rhizome of *Paris polyphylla* var. *yunnanensis*. It has strong anticancer effects against many cancers, such as lung cancer, oral cancer, nasopharyngeal cancer, and hepatoblastoma cancer [4-7]. In previous researches, PPVII has been implicated in a range of anticancer mechanisms, encompassing cell cycle arrest, inhibition of migration, induction of apoptosis and autophagy, as well as modulation of specific cell signaling pathways [8]. Recently, PPVII has also been shown to promote apoptosis in breast cancer through downregulation of SOS1 and inhibition of the MAPK/ERK pathway [9], and to protect against breast cancer-induced osteolysis by suppressing osteoclastogenesis [10].

Nevertheless, the specific activity of PPVII in TNBC and its potential molecular target remain largely unexplored. To the best of our knowledge, no prior study has systematically identified a candidate molecular target of PPVII in TNBC, which represents a significant gap in understanding its therapeutic potential for this aggressive subtype. Moreover, the

precise molecular mechanism through which PPVII exerts its cytotoxic effects in TNBC is yet to be elucidated. To identify the potential molecular target of PPVII in TNBC, we performed quantitative proteomics analysis and found that THOP1 (thimet oligopeptidase) was significantly downregulated by PPVII. THOP1 is a zinc-dependent metallopeptidase that is widely distributed in the cytoplasm of mammalian cells and has been implicated in the degradation of bioactive peptides such as bradykinin. Therefore, identifying a potential target of PPVII in TNBC is essential for clarifying its mechanism of action and supporting its further development as a potential therapeutic agent for TNBC. In this study, we investigated the impact of PPVII on TNBC and explored the underlying molecular mechanism.

2. Methods

2.1 Materials

PPVII (molecular formula: $C_{51}H_{82}O_{21}$, molecular weight: 1031.2, purity=99.15%, #68124-04-9) was purchased from ShangHai PureOne Biotechnology (China). PPVII was dissolved in dimethyl sulfoxide (DMSO) (Meilunbio, China) and stored in dark at $-20^{\circ}C$. Antibody against THOP1 (#ab233243) was from Abcam (UK). Antibodies against cleaved caspase 3 (#ET1602-47), cleaved caspase 9 (#ET1603-27) and the secondary antibodies were from Huaan Biotechnology Co., Ltd (China). Antibody against cleaved caspase 8 (#AF5267) were from Affinity Biosciences (Australia), Bcl-2 (#12789-1-AP) and Bax (#50599-2-Ig) antibodies were from Proteintech (China). The HRP-conjugated secondary antibodies, including goat anti-rabbit IgG and goat anti-mouse IgG, were from Huaan Biotechnology Co., Ltd (China). For immunohistochemistry, xylene and ethanol were from Sigma-Aldrich (USA), and citrate buffer (pH 6.0), hydrogen peroxide (H_2O_2), bovine serum albumin (BSA), 3,3'-diaminobenzidine (DAB), and hematoxylin were from Servicebio (China).

2.2 Cell culture and construction of TNBC cell lines with THOP1 overexpression

Human TNBC cell lines MDA-MB-231 and MDA-MB-453 were obtained from American Type Culture Collection (ATCC, USA). All cells were cultured in RPMI-1640 medium (Gibco, USA) supplemented with 10% Fetal Bovine Serum (FBS) (Biological Industries, Israel), 100 U/mL penicillin, and 100 µg/mL streptomycin (Gibco, USA). They were incubated at 37 °C with 5% CO₂ in a humidified incubator.

TNBC cells were transfected with PGMLV-CMV-MCS-3-THOP1 plasmids and the corresponding control vectors (Genomeditech Biotechnology Co., Ltd., China) using Lipofectamine™ 3000 and P3000™ reagents (ThermoFisher Scientific, USA) following the manufacturer's instructions. Two transfected cell lines were generated, designated as the vector group and the OE-THOP1 group. The expression efficiency of THOP1 was validated by Western blot analysis.

2.3 Cell viability and colony formation assay

Cell viability was evaluated by Cell Counting Kit-8 (CCK-8) (Multisciences, China) assay. TNBC cells were seeded into 96-well plates at a density of 0.5×10^4 cells/well and incubated for 24 hours. Afterwards, TNBC cells were treated with various concentrations of PPVII for 24, 48, and 72 hours. Following the treatment, CCK-8 solution was added to each well. After incubation for 3 hours, the absorbance at 450 nm was measured using a spectrophotometer, and the cell viability curve was plotted based on the recorded absorbance values.

TNBC cells were seeded in 6-wells plates with a density of 1,000 cells per well, treated with different concentrations of PPVII (0, 0.5, 1, and 2 µmol/L) for 48 hours, and then incubated for 10 days. TNBC cells were fixed with 4% paraformaldehyde solution and

stained with 0.1% crystal violet. The colonies consisting of more than 50 cells were quantified and documented using an optical microscope.

2.4 Transwell migration experiments

TNBC cells were placed on top of a porous transwell membrane and treated with different concentration of PPVII (0, 0.5, 1, and 2 µmol/L) for 48 hours. RPMI-1640 medium supplemented with 10% FBS was added to the lower chamber. After removing non-migrated cells on the upper side of the membrane, the migrated cells were fixed, stained with 0.1% crystal violet, and subsequently quantified under an optical microscope.

2.5 Annexin V/PI staining assay

Apoptotic TNBC cells were estimated by Annexin V/PI staining. TNBC cells were exposed to PPVII (0, 0.5, 1, and 2 µmol/L) for 48 hours and washed with Phosphate-Buffered Saline (PBS). Afterward, TNBC cells were suspended in the binding buffer, incubated with 5 µL Annexin V-FITC and 10 µL Propidium Iodide for 15 minutes at room temperature in dark, and then analyzed using the flow cytometry.

2.6 Western blot analysis

The TNBC cells were lysed with ice-cold radioimmunoprecipitation assay (RIPA) buffer. The protein concentration of the extracted sample was determined using the BCA protein assay (Beyotime Biotechnology, China). The protein samples were loaded on SDS-PAGE (Beyotime Biotechnology, China) and then transferred to PVDF (Merck, USA) membranes. The membranes were blocked with 5% fat-free milk in Tris-buffered saline and then incubated with primary antibodies at 4°C overnight. The primary antibodies were used at the following dilutions: THOP1 (1:1000), cleaved caspase 3 (1:1000), cleaved caspase 8 (1:1000), cleaved caspase 9 (1:1000), Bcl-2 (1:1000), Bax (1:1000), and β-actin (1:5000). After washing, the membranes were incubated with

HRP-conjugated secondary antibodies (goat anti-rabbit IgG or goat anti-mouse IgG; Huaan Biotechnology, China) at 1:5000 for 1 hour at room temperature. Finally, the blots were detected using the enhanced chemiluminescent detection system ChemiDoc XRS+ (BioRad, USA). The band intensities were quantified using Image Lab software (Bio-Rad, USA) and normalized to β -actin. Relative protein expression levels were calculated as fold change relative to the control group.

2.7 TNBC xenograft experiment

The animal experiments were approved by Institutional Animal Care and Use Committee of Zhejiang Cancer Hospital. Healthy female BALB/c-nu mice (5 weeks old) were purchased from Hangzhou Hangsi Biotechnology Co., LTD (China). The mice were housed in standard cages at room temperature and provided with a standard animal diet and water ad libitum. MDA-MB-231 cells were subcutaneously inoculated in mice at a density of 1×10^6 cells. Following subcutaneous injection, the mice were randomly assigned to four groups ($n = 5$ per group): control group, PPVII 0.5 mg/kg group, PPVII 1 mg/kg group, and PPVII 2 mg/kg group. The doses of PPVII were determined through our pilot experiments, in which this dose range showed effective antitumor activity with acceptable safety in mice. The mice were administered the drug via intraperitoneal injection once daily for 21 days. Tumor volume was measured every 3 days with a caliper and calculated as $V = (\text{length} \times \text{width}^2) / 2$. Mice were monitored daily, and humane endpoints were predefined as body weight loss $> 20\%$, severe lethargy, or inability to access food and water. Subsequently, the mice were euthanized 22 days after treatment initiation by CO_2 inhalation followed by cervical dislocation, and the tumors were surgically excised. Tumor weight and volume were measured.

Paraffin-embedded tumor tissues were cut into $4 \mu\text{m}$

sections, deparaffinized in xylene, rehydrated through a graded ethanol series, and subjected to antigen retrieval in citrate buffer (pH 6.0) by microwave heating for 20 min. After blocking endogenous peroxidase with 3% H_2O_2 for 10 min and non-specific binding with 5% bovine serum albumin (BSA) for 30 min at room temperature, the sections were incubated with primary antibodies against cleaved caspase 3 (1:200), Ki67 (1:200), and THOP1 (1:200) at 4°C overnight. The sections were then incubated with HRP-conjugated goat anti-rabbit IgG (1:200; Huaan Biotechnology, China) for 30 min at room temperature, developed with DAB, counterstained with hematoxylin, dehydrated, and mounted. The representative images were captured by the optical microscope (Nikon, Japan).

2.8 Proteomics analysis

To identify potential molecular targets of PPVII in TNBC, MDA-MB-231 cells were treated with PPVII or vehicle control for 48 hours. Cells were lysed in ice-cold RIPA buffer supplemented with protease and phosphatase inhibitors. Protein concentration was determined using the BCA protein assay. Equal amounts of protein were reduced with dithiothreitol, alkylated with iodoacetamide, and digested with trypsin overnight at 37°C . Peptides were desalted and analyzed by nano-LC-MS/MS using an Orbitrap Fusion Lumos mass spectrometer coupled to an EASY-nLC system. The mass spectrometer was operated in data-dependent acquisition mode. Raw data were searched against the UniProt Human database using MaxQuant software. Carbamidomethylation of cysteine was set as a fixed modification, and oxidation of methionine was set as a variable modification. The false discovery rate (FDR) was set to $< 1\%$ at both peptide and protein levels. Differentially expressed proteins were defined as those with a fold change > 1.5 or < 0.67 and $P < 0.05$.

2.9 Statistical analysis

Quantification was assessed based on three independent experiments. The data were presented as the mean $\pm$ standard deviation. Normality was assessed using the Shapiro-Wilk test, and homogeneity of variance was assessed using Levene's test. For comparisons among more than two groups, one-way ANOVA followed by Tukey's HSD post hoc test was used. For longitudinal tumor volume data, repeated measures ANOVA was performed to evaluate the effects of treatment, time, and their interaction, followed by Tukey's post hoc test. All statistical analyses were performed with R (version 4.2.0). A significance level of $P < 0.05$ was considered statistically significant (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

3 Results

3.1 Inhibitory effects of PPVII on TNBC

The cytotoxicity of PPVII on the proliferation of two TNBC cell lines (MDA-MB-231 and MDA-MB-453) was assessed using CCK-8 assay. PPVII significantly inhibited cell viability in a dose-dependent and time-dependent manner (Figure 1A-1B). According to the experimental results mentioned above, subsequent experiments were conducted using PPVII at concentrations of 0.5, 1, and 2 $\mu\text{mol/L}$. The results of colony formation assays further showed PPVII inhibited colony formation in both MDA-MB-231 (Figure 1C-1D, $P < 0.05$) and MDA-MB-453 (Figure 1E-1F, $P < 0.05$) cells in a dose-dependent manner. Transwell assays were performed to evaluate the cell migration in response to PPVII treatment. As depicted in Figure 1G-1J, PPVII exhibited concentration-dependent inhibitory effects on the cell migration ability in two TNBC cell lines ($P < 0.05$).

To investigate the potential mechanism underlying the suppressive effect of PPVII on TNBC cells, flow cytometry was employed to evaluate cell apoptosis in TNBC cells treated with PPVII (0, 0.5, 1, 2 $\mu\text{mol/L}$) for 48 hours. The number of apoptotic TNBC cells

exhibited a significant dose-dependent increase in both MDA-MB-231 (Figure 2A-2B, $P < 0.05$) and MDA-MB-453 (Figure 2C-2D, $P < 0.05$) cells following PPVII treatment. Additionally, a western blot assay was conducted to evaluate the protein levels of apoptosis-related markers. In MDA-MB-231, the expression levels of cleaved caspase 3, 8, 9, and Bax proteins were significantly up-regulated, while the Bcl-2 protein was markedly down-regulated (Figure 2E and 2G, $P < 0.05$). A similar trend in the expression of apoptosis-related proteins was observed in the MDA-MB-453 cells as well (Figure 2F and 2H, $P < 0.05$).

3.2 PPVII suppresses TNBC potentially through modulating THOP1

To identify potential molecular targets of PPVII in TNBC, we performed quantitative proteomics analysis on MDA-MB-231 cells treated with PPVII or vehicle control. Among the differentially expressed proteins, THOP1 was significantly downregulated by PPVII (Supplementary Figure S1A). In order to investigate the role of THOP1 in TNBC cells treated with PPVII, we constructed two TNBC cell lines overexpressing THOP1. The transfection efficiency was assessed and confirmed by western blot. It revealed that the THOP1 expression level in the THOP1 overexpression group was significantly higher compared to the control and vector groups (Figure 3A-3D, $P < 0.05$). No considerable difference in THOP1 expression was observed between the vector and control groups. Subsequently, the TNBC cells were divided into four groups: the Vector group, Vector+PPVII group, OE-THOP1 group, and OE-THOP1+PPVII group. PPVII demonstrated the ability to suppress colony formation in both TNBC cells transfected with the vector and those overexpressing THOP1 plasmid. It indicated that THOP1 overexpression could reverse the inhibitory effect of PPVII in MDA-MB-231 (Figure 3E-3F, $P < 0.05$) and MDA-MB-453 (Figure 3G-3H, $P < 0.05$). Moreover,

PPVII suppressed cell migration ability in the vector group ($P < 0.05$). In the TNBC cells overexpressing

THOP1, PPIII did not have an impact on cell migration (Figure 31-3L, $P > 0.05$).

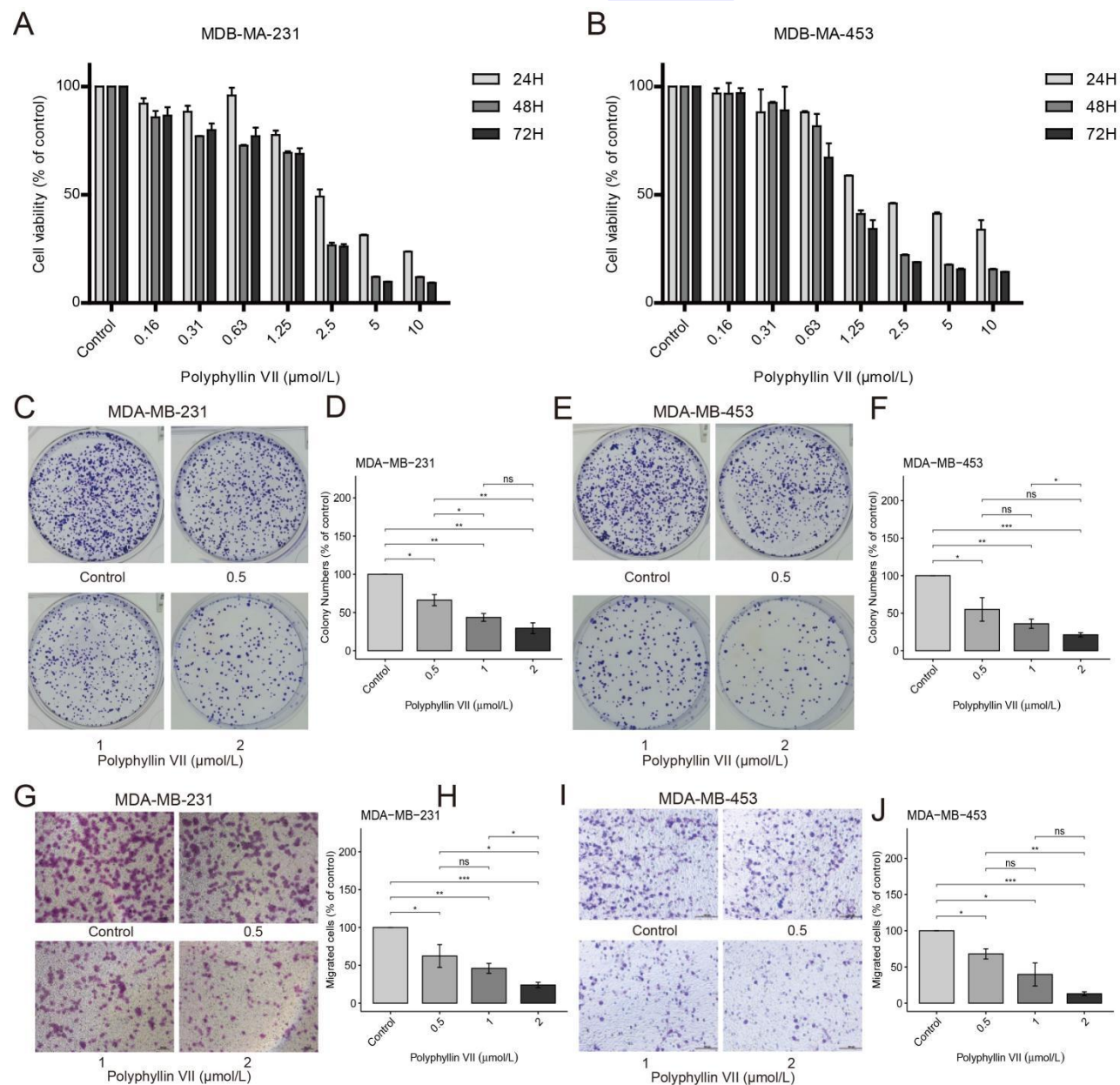


Figure 1 Effect of polyphyllin VII on cell proliferation, colony formation, and migration of TNBC cells. The cell viability of MDA-MB-231 (A) and MDA-MB-453 (B) cells treated with polyphyllin VII for 24, 48, and 72 h. Colony formation was observed in MDA-MB-231 (C, D) and MDA-MB-453 (E, F) cells treated with polyphyllin VII. The inhibitory effects of polyphyllin VII on cell migration of MDA-MB-231 (G, H) and MDA-MB-453 (I, J) cells. (***) $P < 0.001$, ** $P < 0.01$, * $P < 0.05$).

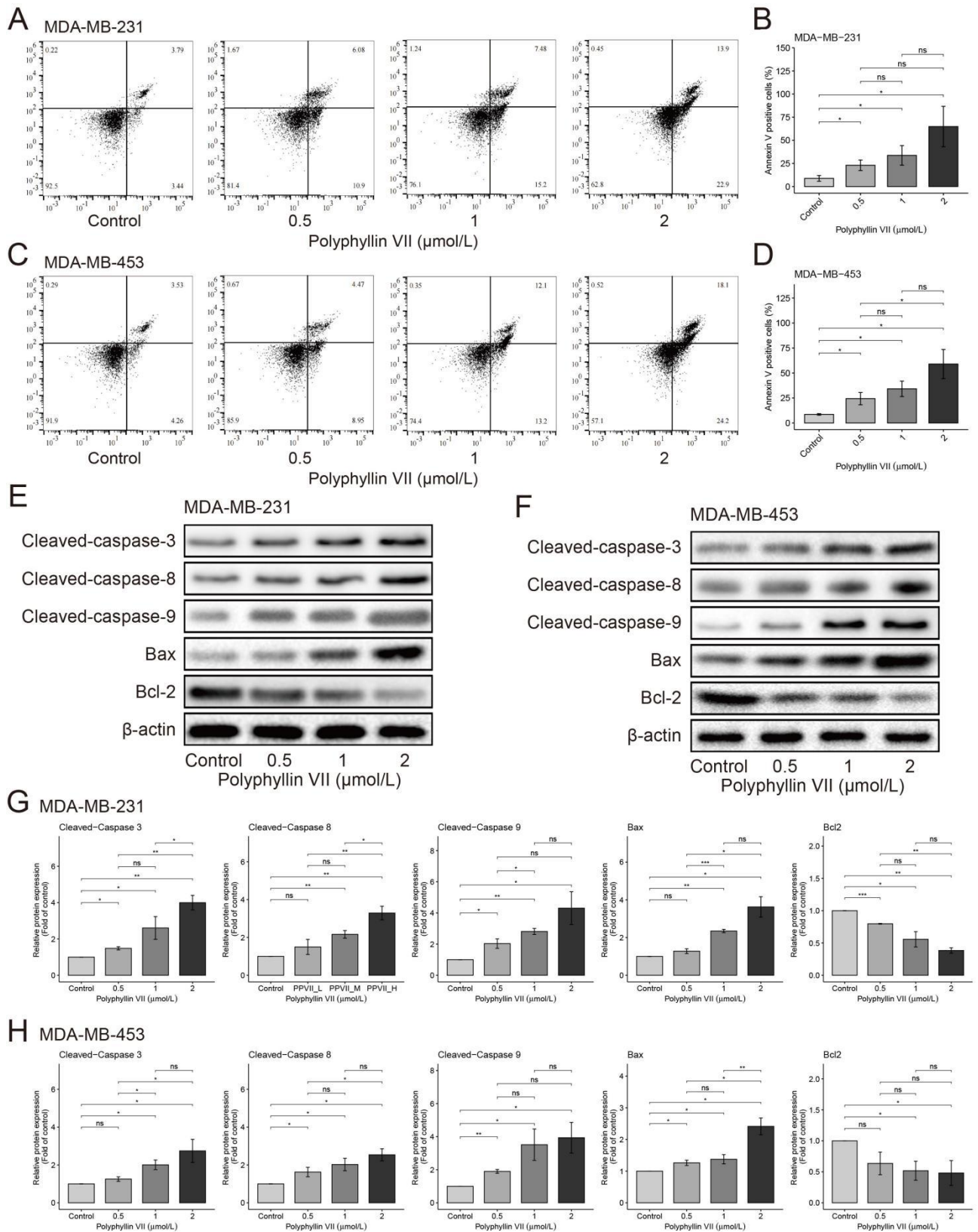


Figure 2 Polyphyllin VII induces apoptosis in TNBC cells. Cell apoptosis of MDA-MB-231 (A, B) and MDA-MB-453 (C, D) cells treated with Polyphyllin VII at 0.5 μ M, 1 μ M and 2 μ M for 48h was analyzed by flow cytometry. Western blot analysis was performed to examine apoptosis-related proteins using antibodies against cleaved caspase 3, cleaved caspase 8, cleaved caspase 9, Bax, and Bcl-2 in MDA-MB-231 (E, G) and MDA-MB-453 (F, H). β -actin was used as internal control. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

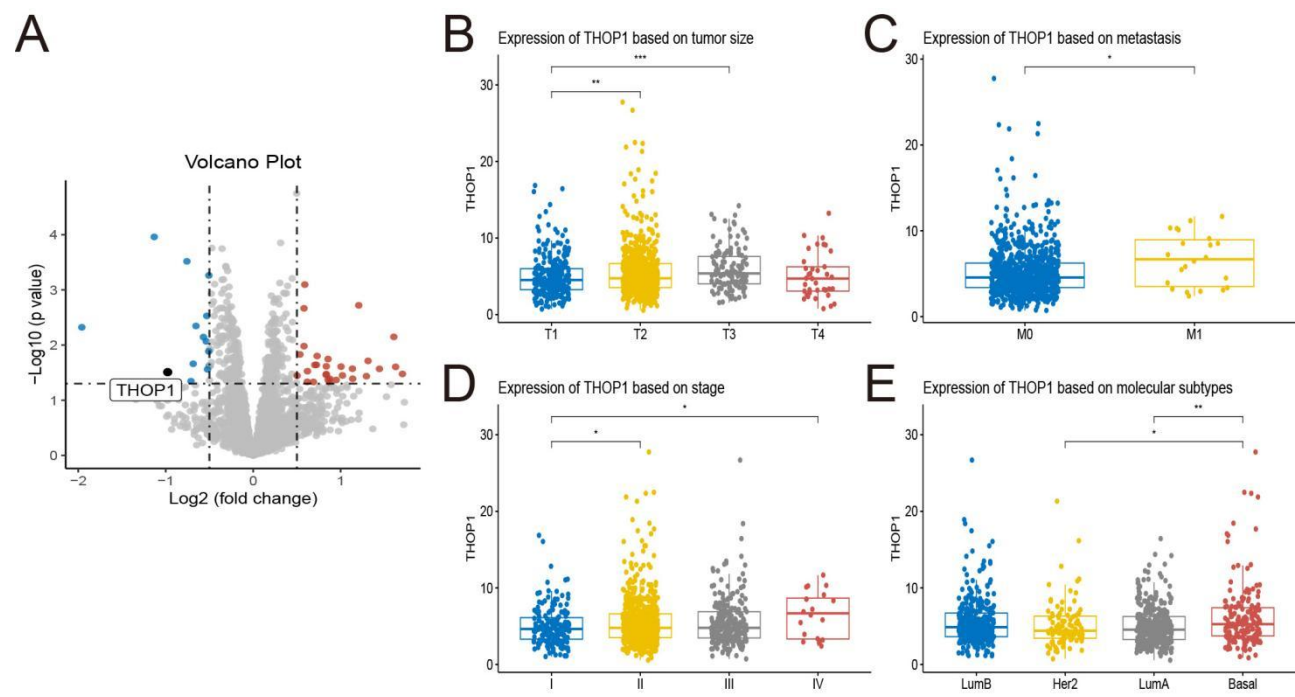


Figure S1 The clinical importance of THOP1. (A) Volcano plot showing differentially expressed proteins identified by pharmaceutical proteomics after PPVII treatment. THOP1 was significantly downregulated and is indicated in the plot. The correlation between THOP1 expression and several adverse clinicopathological factors, including tumor size (B), metastasis (C), and stage (D). THOP1 expression in four breast cancer subtypes (E).

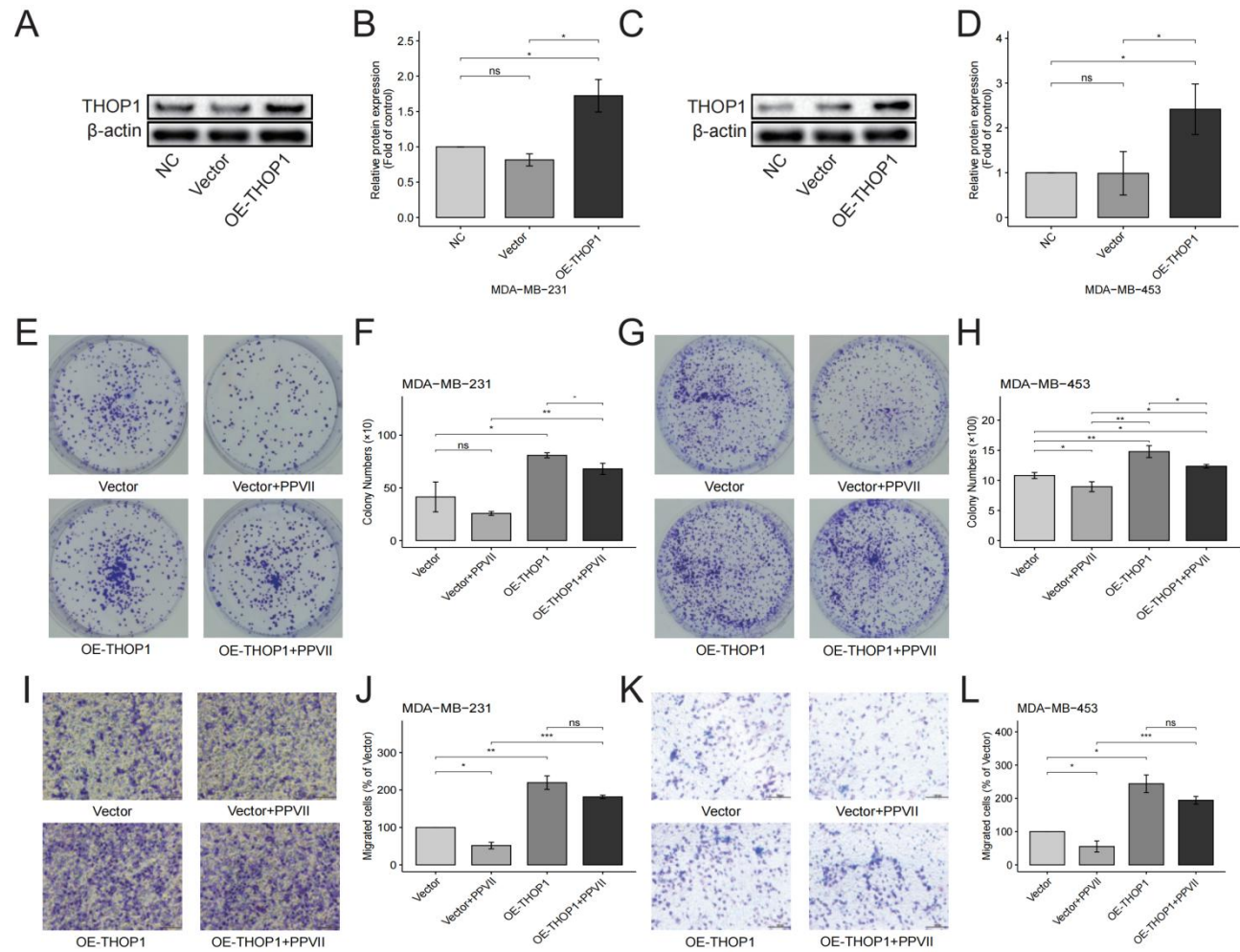


Figure 3 Polyphyllin VII exerts inhibitory effects on colony formation and migration of TNBC cells through the regulation of THOP1. THOP1 expression in MDA-MB-231 (A, B) and MDA-MB-453 (C, D) cells transfected with THOP1 overexpression plasmid. TNBC cells were divided into four groups, the vector group, vector+polyphyllin VII group, OE-THOP1 group, and OE-THOP1+polyphyllin VII group. TNBC cells were treated with Polyphyllin VII for 48h for colony formation and migration assays. Colony formation of MDA-MB-231 (E, F) and MDA-MB-453 (G, H) cells was assessed in these four groups. Migration of MDA-MB-231 (I, J) and MDA-MB-453 (K, L) cells was observed in these four groups. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Furthermore, we observed no notable difference in the proportion of apoptotic cells between the vector and OE-THOP1 groups, as well as between the OE-THOP1 and OE-THOP1+PPVII groups. PPVII could induce apoptosis in TNBC cells transfected vector. The proportion of apoptotic cells in the vector+PPVII groups was substantially higher compared to the OE-THOP1+PPVII group in MDA-MB-231 (Figure 4A-4B) and MDA-MB-453 (Figure 4C-4D, $P < 0.05$) cells. Besides, western blot analysis was utilized to evaluate apoptosis-related proteins in the four aforementioned groups. PPVII upregulated the expression of cleaved caspase 3, 8, 9, and Bax proteins while downregulating Bcl-2 in TNBC cells transfected with vector ($P < 0.05$). No prominent changes were observed in the expression of apoptosis-related proteins between the OE-THOP1 and OE-THOP1+PPVII groups. In MDA-MB-231 (Figure 4E and 4G) and MDA-MB-453 (Figure 4F and 4H) cells treated with PPVII, THOP1 overexpression resulted in a decrease in the expression of cleaved caspase 3, 8, 9, and Bax proteins, while increasing Bcl-2 expression ($P < 0.05$). Therefore, these findings suggested that THOP1 overexpression could counteract the ability of PPVII to induce apoptosis in TNBC cell lines.

3.3 PPVII inhibits TNBC in vivo

In order to validate the results obtained from in vitro experiments and evaluate the in vivo antitumor efficacy of PPVII, a xenograft model of TNBC was established. As shown in Figure 5A, PPVII remarkably inhibited xenograft tumor, specially in PPVII 2mg/kg group. No discernible difference in xenograft body weight was observed among the control group and other three groups treated with PPVII (Figure 5B), indicating minimal lethal toxicity to the xenograft mice. We also revealed that PPVII led to a significant reduction in tumor volume and weight (Figure 5C-5D, $P < 0.05$). HE staining and immunohistochemistry analyses were conducted to further assess the impact of PPVII on morphological alterations. The quantitative analysis showed that PPVII treatment resulted in decreased immunoreactivity for Ki67 and increased immunoreactivity for cleaved caspase 3, while concurrently reducing THOP1 immunoreactivity (Figure 5E-H).

4 Discussion

In this study, we have successfully demonstrated the substantial inhibitory effect of PPVII on the proliferation of two TNBC cell lines using both the CCK-8 assay and colony formation assay. Additionally, through the Annexin V/PI staining assay, we have revealed that PPVII possesses the ability to induce apoptosis.

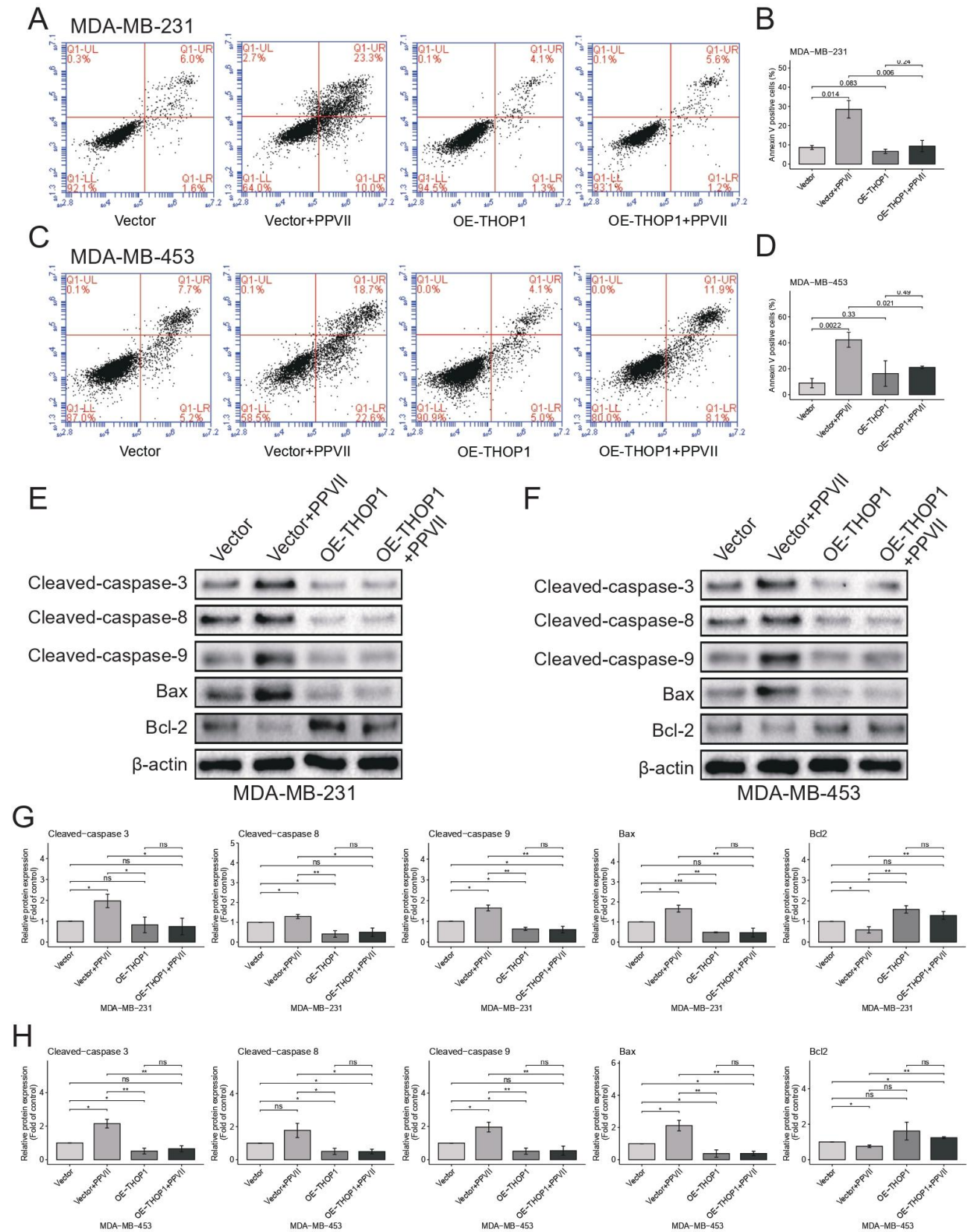


Figure 4 Polyphyllin VII induces apoptosis in TNBC cells by regulating THOP1. TNBC cells were divided into four groups, the vector group, vector+polyphyllin VII group, OE-THOP1 group, and OE-THOP1+polyphyllin VII group. TNBC cells were treated with Polyphyllin VII for 48 h for apoptosis analysis. The apoptosis of MDA-MB-231 (A, B) and MDA-MB-453 (C, D) was assessed in these four groups by flow cytometer. The apoptosis-related protein of MDA-MB-231 (E, G) and MDA-MB-453 (F, H) was observed in these four groups by Western blot analysis. (*

$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

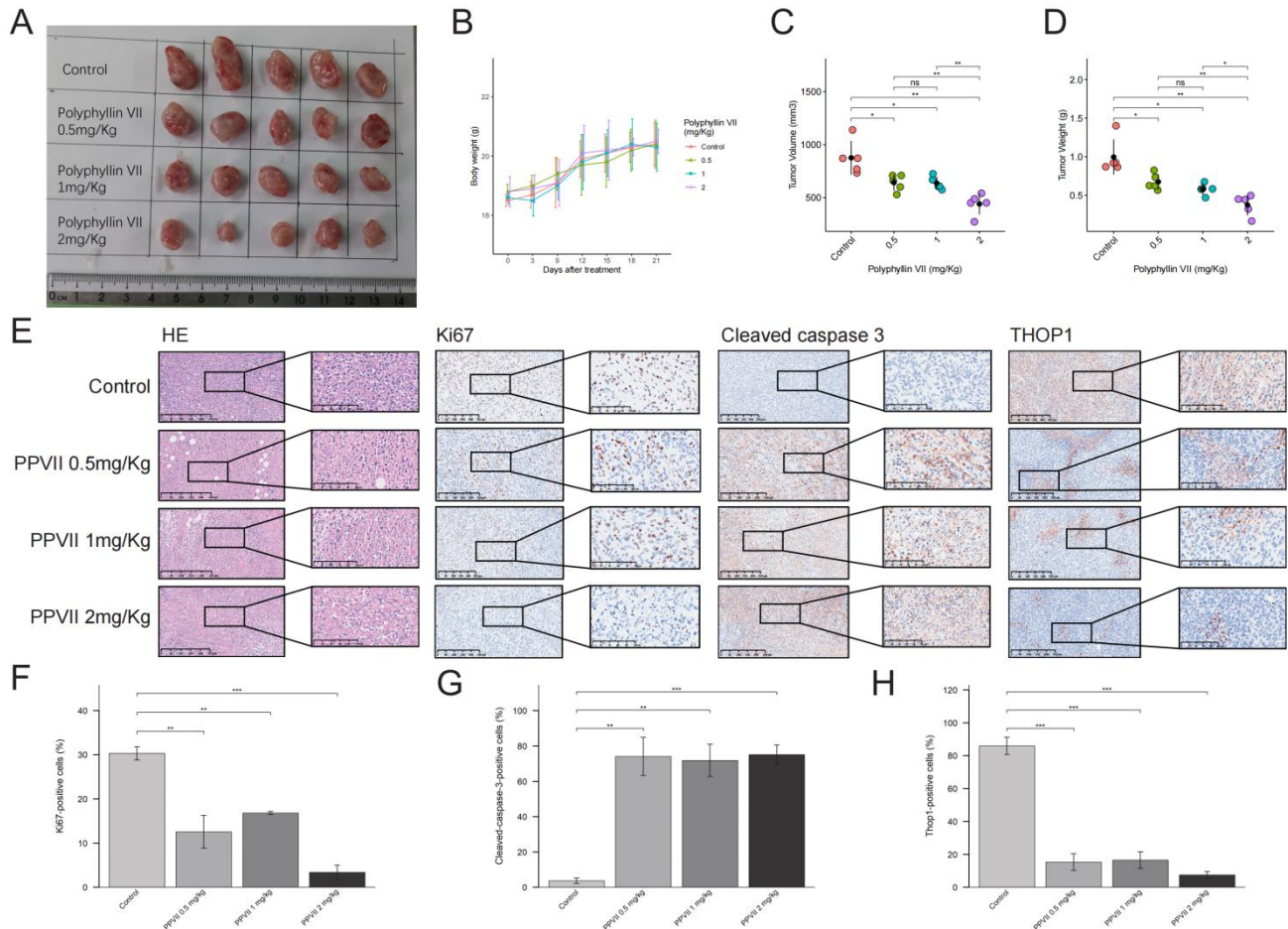


Figure 5 Polyphyllin VII exhibits inhibitory effects on TNBC in vivo. (A) MDA-MB-231 xenograft tumors were photographed. The mice weight (B), tumor volume (C), and tumor weight (D) were recorded. (E) Representative images of H&E staining and immunohistochemistry staining in the xenograft tumor were captured to examine the morphology as well as the expression of Ki67, cleaved caspase 3, and THOP1. Quantitative analysis of the percentage of positive cells for Ki67 (F), cleaved caspase 3 (G), and THOP1 (H) in each group. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Pharmaceutical proteomics was performed to investigate potential target of PPVII and explore probable mechanism (Supplementary data). Among the differentially expressed proteins, THOP1 was significantly downregulated by PPVII and was therefore selected for further validation (Supplementary Figure S1A). THOP1 (Thimet oligopeptidase, EC 3.4.24.15 EP24.15) is a metalloproteinase with a HEXXH zinc ion binding motif, ubiquitously distributed in the cytoplasm of mammalian cells and tumor cells [20,21]. THOP1 is implicated in the metabolism of diverse neuropeptides,

encompassing bradykinin and neurotensin [22,23]. Additionally, THOP1 exhibits intricate associations with the progression of numerous disorders, including obesity, depression, sepsis, diabetic retinopathy, and Alzheimer's disease [22,24-26]. Qi et al. suggested that the expression of THOP1 in non-small cell lung cancer (NSCLC) specimens was markedly elevated compared to that observed in normal lung tissues [27]. Moreover, reduced THOP1 expression was found to be a significant predictor of unfavorable 5-year disease-free survival and overall survival, highlighting its potential as a promising biomarker in patients with

NSCLC. In hepatocellular carcinoma, THOP1 expression in the background may be an independent prognostic factor [28]. Anti-angiogenesis has been proposed as one of the antitumor mechanisms attributed to THOP1 [29]. In breast cancer, the functional significance of THOP1 is likely linked to its role in degrading bradykinin (BK), a peptide that promotes breast cancer progression through multiple mechanisms. BK enhances breast cancer cell migration and invasion via the FAK/Src signaling pathway, and its receptors are overexpressed in breast cancer tissues [30]. As a kinase that inactivates BK, THOP1 may therefore function as an anti-angiogenic and anti-metastatic factor in breast cancer. In the context of TNBC, these functions suggest that THOP1 may serve as a tumor suppressor, and its downregulation by PPVII could contribute to apoptosis through both impaired BK clearance and altered peptide processing. In breast cancer, we demonstrated a significant correlation between elevated THOP1 expression and several adverse clinicopathological factors, including larger tumor size, increased metastasis, and advanced stage (Supplementary Figure S1B-D). THOP1 expression was notably elevated in basal-like breast cancer compared to other subtypes (Supplementary Figure S1E). In this study, we demonstrated that PPVII effectively inhibits cellular proliferation and induces apoptosis by partially downregulating THOP1 in vitro and in vivo. THOP1 is a potential target of PPVII.

However, this study has several limitations. First, although THOP1 overexpression reversed the effects of PPVII, direct binding between PPVII and THOP1 was not validated; therefore, THOP1 should be regarded as a potential target. Second, the mechanistic link between THOP1 downregulation and apoptosis was not directly tested, and caspase inhibition and THOP1 knockdown/rescue experiments were not performed. Third, only a subcutaneous xenograft model was used, which does not fully

recapitulate the tumor microenvironment or the metastatic process of TNBC. Fourth, the clinical relevance of THOP1 in TNBC patients and the pharmacokinetic properties of PPVII were not evaluated. Future studies should therefore focus on several translational directions, including validating THOP1 expression and its correlation with PPVII sensitivity in primary tumor tissues from TNBC patients, establishing orthotopic or metastasis models to evaluate the inhibitory effect of PPVII on TNBC metastasis in vivo, investigating the synergistic effects of PPVII in combination with standard chemotherapeutic agents such as paclitaxel or cisplatin, and performing pharmacokinetic assessments of PPVII.

In summary, this study demonstrates that the inhibition of TNBC by PPVII is associated with and potentially mediated by THOP1 modulation. THOP1 is proposed as a potential novel target of PPVII for combating TNBC. The findings provide a theoretical and experimental foundation for further research on the anti-TNBC properties of Chinese herbal medicine.

Acknowledgements

Not applicable.

Conflicts of Interest

The authors declare that there is no conflict of interests.

Author Contributions

The authors' responsibilities were as follows: Tian Lan designed the study, Tian Lan and Wangjin Xu extracted the data, performed the analyses, Xueyan Fu and Wangjin Xu interpreted the evidence and wrote the manuscript. Tian Lan revised the article. All authors agreed to be accountable for the work.

Ethics Approval and Consent to Participate

Not applicable.

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Availability of Data and Materials

The data presented in this study are available on request from the corresponding author.

Supplementary Material

Not applicable.

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