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Verteporfin Suppresses Vascular Smooth Muscle Cell Growth-Associated Responses and Modulates YAP Nuclear Localization-Related Signaling

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Abstract

Intimal hyperplasia and restenosis following vascular interventions are major clinical challenges, with vascular smooth muscle cell (VSMC) proliferation playing a critical role in their development. Yes-associated protein (YAP), a key effector of the Hippo signaling pathway, regulates VSMC phenotypic modulation and proliferation, and its activity is closely associated with nucleocytoplasmic localization. Although verteporfin (VP) has been reported to inhibit YAP-associated signaling, its effects on VSMCs and the potential involvement of YAP nuclear localization remain incompletely understood. In this study, primary rat VSMCs were stimulated with platelet-derived growth factor-BB (PDGF-BB) to induce a proliferative phenotype. The effects of VP on cellular metabolic activity and YAP-associated signaling were investigated using CCK-8 assays, Western blotting, immunofluorescence, subcellular protein fractionation, and quantitative real-time PCR.

PDGF-BB stimulation increased YAP expression and was accompanied by changes in VSMC phenotypic markers. VP treatment reduced CCK-8 signals in a concentration-dependent manner, and 3 μM VP was selected for subsequent experiments. VP decreased YAP protein expression and altered the expression and subcellular distribution of several YAP-associated regulatory proteins, including angiomotin (AMOT), protein tyrosine phosphatase non-receptor type 14 (PTPN14), α-catenin, and zonula occludens-2 (ZO-2). Furthermore, VP reduced the mRNA expression of the YAP-associated transcriptional targets CTGF and CYR61. These effects differed in several respects from those observed with the structurally related compound protoporphyrin IX (PPIX).

Collectively, these findings suggest that VP attenuates growth-associated responses in PDGF-BB-stimulated VSMCs and modulates YAP-associated signaling, potentially involving regulatory processes related to YAP nucleocytoplasmic localization. This study provides preliminary evidence

supporting further investigation of VP as a potential therapeutic candidate for vascular proliferative diseases.

Keywords: Intimal hyperplasia, nuclear localization, vascular smooth muscle cells, Verteporfin, YAP

Impact Statement

This study demonstrates that verteporfin attenuates PDGF-BB-induced growth-associated responses in vascular smooth muscle cells (VSMCs), accompanied by reduced YAP expression and alterations in YAP-associated regulatory proteins. Notably, changes in AMOT, PTPN14, α-catenin, and ZO-2 suggest a potential involvement of nucleocytoplasmic regulatory processes in verteporfin-associated YAP signaling. Comparison with protoporphyrin IX (PPIX) further reveals differences in their effects on selected signaling components. These findings provide new insights into the light-independent effects of verteporfin in VSMCs and support further investigation of its potential relevance to intimal hyperplasia and restenosis.

Introduction

The Hippo signaling pathway, first characterized in Drosophila melanogaster, is an evolutionarily conserved signaling cascade that regulates organ size and tissue homeostasis by controlling cell proliferation, survival, and apoptosis[1]. Yorkie (Yki) is a major downstream effector of the Hippo pathway in Drosophila[2], while its mammalian homolog, Yes-associated protein (YAP), plays a corresponding regulatory role in vertebrates[3]. In mammals, Hippo-YAP signaling is involved in various biological processes, including stem cell maintenance, tissue regeneration, and tumorigenesis[4].As a transcriptional co-regulator, YAP activity is closely associated with its subcellular localization, particularly its translocation between the cytoplasm and nucleus.

Intimal hyperplasia and restenosis following vascular interventions remain major clinical challenges, largely associated with the abnormal proliferation and phenotypic modulation of vascular smooth muscle cells (VSMCs). Following vascular injury, VSMCs can undergo a phenotypic transition from a contractile state to a synthetic, proliferative phenotype, contributing to neointimal formation and vascular restenosis. Therefore, understanding the molecular mechanisms underlying VSMC phenotypic modulation and proliferation is essential for identifying potential therapeutic targets[5]. Previous studies have demonstrated that YAP expression and activity are increased in VSMCs following vascular injury. YAP overexpression or activation of YAP/TAZ signaling by thromboxane A2 has been shown to promote VSMC proliferation in vitro. Conversely, YAP inhibition or depletion enhances the expression of contractile smooth muscle markers and attenuates injury-induced phenotypic switching and neointimal formation. These findings highlight YAP as an important regulator of VSMC phenotypic modulation and vascular remodeling6.

Verteporfin (VP), a second-generation photosensitizer approved by the US Food and Drug Administration for the treatment of neovascular age-related macular degeneration, was initially developed for photodynamic therapy. In addition to its established photodynamic effects, VP has been reported to exert biological activities under conditions without intentional photoactivation, including the modulation of autophagy and YAP-associated signaling.Beyond its established photodynamic effects, verteporfin (VP) has attracted increasing interest for its potential to modulate YAP-associated signaling under conditions without intentional photoactivation. Previous studies have suggested that VP may influence YAP signaling through mechanisms involving cytoplasmic retention and regulatory proteins such as 14-3-3σ[7]. The biological activity of YAP is regulated not only by its expression level but also by its nucleocytoplasmic localization. As a transcriptional co-regulator, YAP

generally exerts its transcriptional functions following nuclear accumulation, whereas cytoplasmic retention limits its nuclear transcriptional activity[8]. However, the effects of VP on YAP-associated regulatory proteins and their subcellular distribution in VSMCs remain insufficiently characterized.

Therefore, the present study investigated the effects of VP on PDGF-BB-induced VSMC growth-associated responses, with particular emphasis on YAP expression and proteins involved in its nucleocytoplasmic regulation. By examining AMOT, PTPN14, α-catenin, ZO-2, and the YAP-associated transcriptional targets CTGF and CYR61, we sought to explore the potential relationship between VP treatment and YAP nuclear localization-related signaling in VSMCs.

Materials and Methods

Isolation and Culture of Primary VSMCs

Primary VSMCs were isolated from the thoracic aortas of adult Sprague–Dawley rats (200 ± 20 g; Beijing Vital River Laboratory Animal Technology Co., Ltd., Beijing, China). Rats were anesthetized with sodium pentobarbital and euthanized according to the institutionally approved animal protocol. Following removal of the thoracic aorta, the surrounding connective tissue and adventitia were carefully dissected, and the endothelial layer was mechanically removed. The isolated vascular tissues were washed with phosphate-buffered saline (PBS) containing penicillin–streptomycin and minced into approximately 1–2 mm^3 fragments. The tissue fragments were enzymatically digested at 37°C for approximately 45–90 min, and the isolated cells were subsequently collected and cultured under standard conditions. Cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2.

Induction and Characterization of VSMCs Phenotypic Modulation

To establish an in vitro model of VSMC phenotypic modulation, primary VSMCs were stimulated with

platelet-derived growth factor-BB (PDGF-BB; 20 ng/mL; MedChemExpress, HY-P7278). Phenotypic changes were evaluated by examining the expression of contractile markers, including SM22α, smooth muscle myosin heavy chain (SMMHC), and calponin, together with the proliferation-associated protein cyclin D. Protein expression and cellular staining patterns were assessed using Western blotting and immunofluorescence staining, respectively.

Verteporfin Treatment and CCK-8 Assay

The effects of verteporfin (VP) on VSMC metabolic activity were evaluated using a Cell Counting Kit-8 (CCK-8) assay. PDGF-BB-stimulated VSMCs were seeded into 96-well plates at a density of 1×10^4 cells/well and allowed to adhere overnight. Cells were subsequently treated with VP at concentrations of 0, 0.3, 1, 3, 6, or 10 μM for 24 h.

After treatment, 10 μL of CCK-8 reagent was added to each well, followed by incubation for 4 h at 37°C. Absorbance was measured at 450 nm using a microplate reader. Each concentration was tested in triplicate wells, and untreated cells served as controls.

Based on the concentration-response results, 3 μM VP was selected for subsequent experiments. For comparative analyses, VSMCs were treated with 0.1% dimethyl sulfoxide (DMSO), 3 μM VP, or 3 μM protoporphyrin IX (PPIX) for 24 h.

Western Blot Analysis and Nuclear–Cytoplasmic Protein Fractionation

Western blotting was performed to assess the expression of YAP and proteins associated with its nucleocytoplasmic regulation. For subcellular protein analysis, nuclear and cytoplasmic fractions were prepared using the NE-PER Nuclear and Cytoplasmic Extraction Reagents Kit (Thermo Fisher Scientific) according to the manufacturer's instructions.

Briefly, cells were collected and lysed using cytoplasmic extraction reagents (CER I and CER II).

Following centrifugation, the cytoplasmic supernatant was collected, and the remaining nuclear pellet was extracted using nuclear extraction reagent (NER). The resulting protein fractions were subjected to Western blot analysis to determine the expression of angiomotin (AMOT), protein tyrosine phosphatase non-receptor type 14 (PTPN14), α-catenin, and zonula occludens-2 (ZO-2).

Protein band intensities were quantified using Quantity One software (Bio-Rad, USA). Lamin B1 and GAPDH were used as nuclear and cytoplasmic markers, respectively, subject to verification against the original experimental records.

Immunofluorescence Staining

Immunofluorescence staining was performed to evaluate the expression and subcellular distribution of YAP-associated regulatory proteins in VSMCs. Cells were washed with PBS and fixed with 4% paraformaldehyde for 15 min, followed by permeabilization with 0.5% Triton X-100 for 20 min at room temperature.

After blocking with 10% normal goat serum for 30 min, cells were incubated with the corresponding primary antibodies overnight at 4°C. Following washing with PBST, cells were incubated with an appropriate fluorophore-conjugated secondary antibody for 1 h at room temperature in the dark. Nuclei were counterstained with DAPI, and fluorescence images were acquired using an Olympus BX51 fluorescence microscope.

Quantitative Real-Time PCR

Total RNA was extracted from VSMCs treated with DMSO (0.1%), VP (3 μM), or PPIX (3 μM) for 24 h using TRIzol reagent (Invitrogen). Complementary DNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems).

Quantitative real-time PCR (qPCR) was performed using SYBR Green Master Mix (Applied

Biosystems) to assess the mRNA expression of the YAP-associated transcriptional targets connective tissue growth factor (CTGF) and cysteine-rich angiogenic inducer 61 (CYR61). GAPDH was used as the reference gene, and relative expression levels were calculated using the $2^{-\Delta\Delta}$Ct method. All reactions were performed in triplicate.

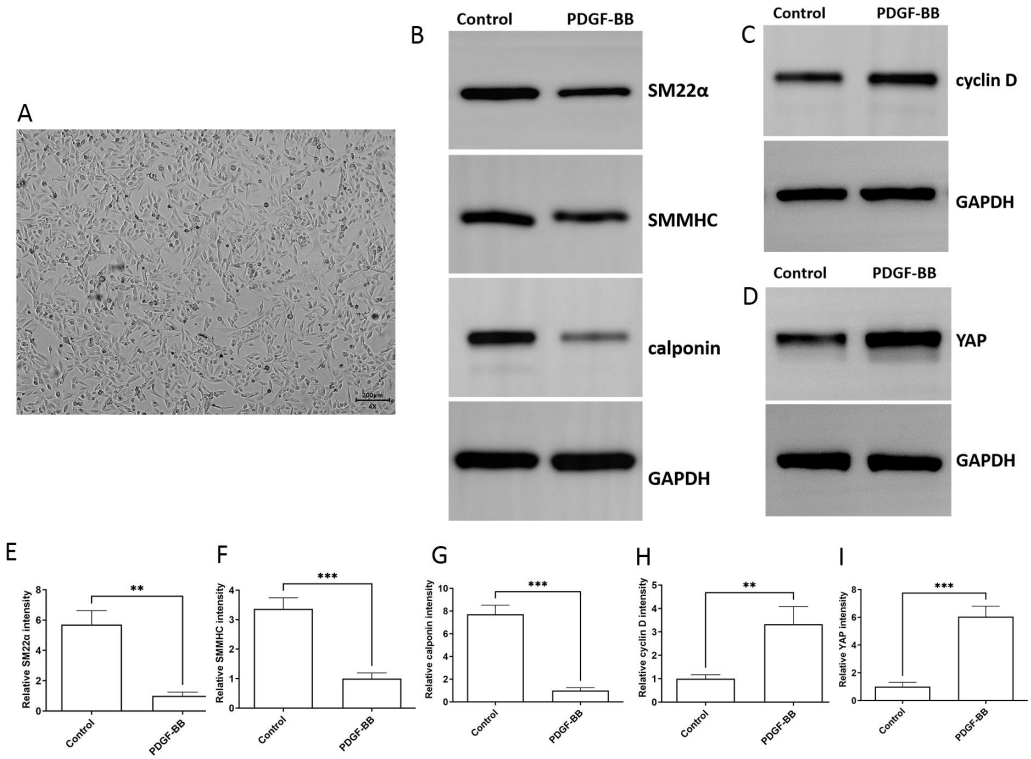
Statistical Analysis

Numerical data were expressed as mean ± SD from at least three independent biological replicates (n = 3). For comparisons between two groups, Student's t-test was applied. For comparisons among three or more groups, one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used. All statistical analyses were performed using GraphPad Prism 9 (GraphPad Software, Inc., USA). $P < 0.05$ was considered statistically significant.

Results

PDGF-BB Induces Phenotypic Modulation and Increases YAP Expression in VSMCs

Following stimulation with PDGF-BB, primary VSMCs exhibited changes in phenotypic marker expression consistent with a transition toward a synthetic phenotype (Figure 1A). Western blot and immunofluorescence analyses demonstrated significant reductions in the expression of the contractile markers SM22α, smooth muscle myosin heavy chain (SMMHC), and calponin (Figure 1B, E–G; Figure 2A–C, E–G). In contrast, the expression of the proliferation-associated protein cyclin D was increased (Figure 1C, H; Figure 2D, H). Furthermore, YAP protein expression was significantly higher in PDGF-BB-stimulated VSMCs than in untreated cells ($P \leq 0.001$; Figure 1D, I).



181

182 **Figure1 PDGF-BB induces phenotypic switching and YAP upregulation in primary VSMCs.** Primary

183 vascular smooth muscle cells (VSMCs)(A). The western blot results of VSMCs contractile marker

184 proteins (SM22α, SMMHC, and calponin)(B,E,F,G) and synthetic marker protein (cyclin D) (C,H) for

185 control and PDGF-BB group. The western blot result of YAP control and PDGF-BB group(D,I) Data

186 are mean ± SD (n = 3 independent experiments).

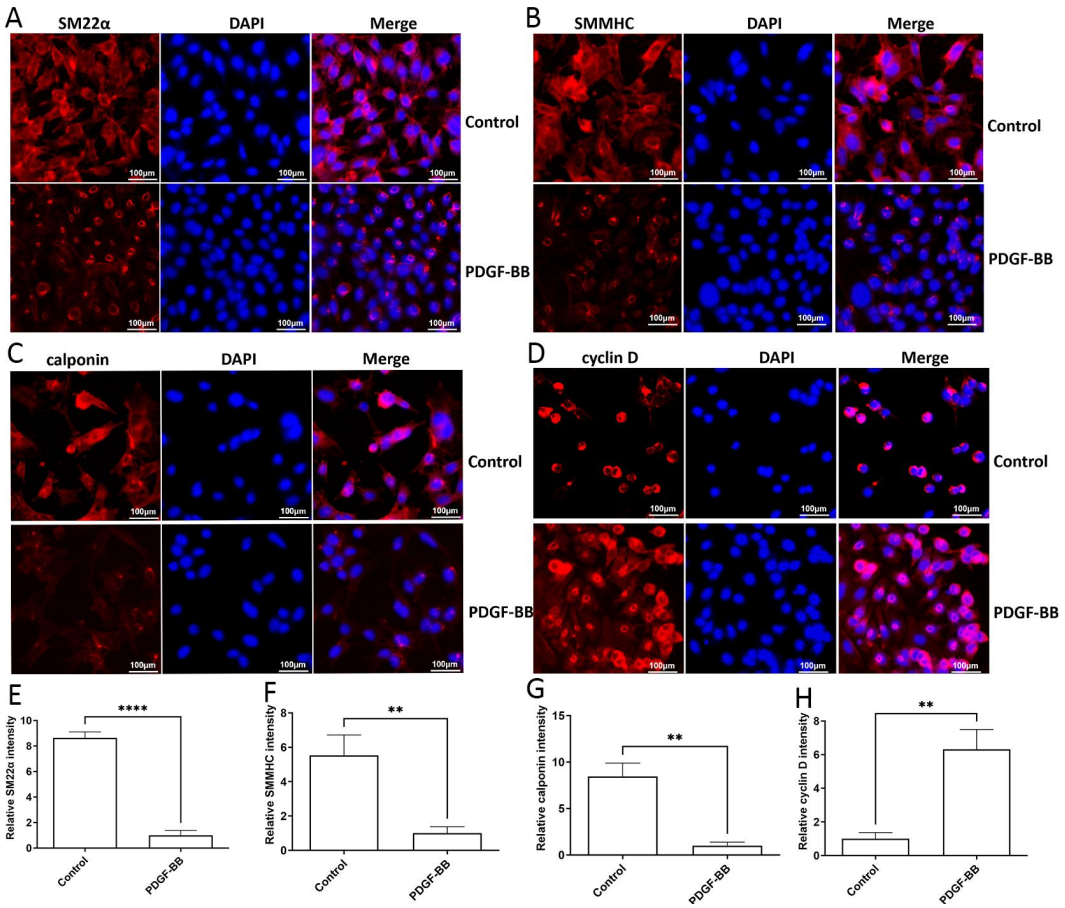


Figure2 Immunofluorescence validation of VSMC phenotypic switching. The immunofluorescence results of VSMCs contractile marker (SM22α, SMMHC, and calponin) (A,B,C,E,F,G) and synthetic marker (cyclin D) (D,H) for control and PDGF-BB group. Data are mean ± SD (n = 3 independent experiments).

Verteporfin Reduces VSMC Metabolic Activity and YAP Protein Expression

VP treatment resulted in a concentration-dependent reduction in CCK-8 signals in PDGF-BB-stimulated VSMCs (Figure 3A). The cytotoxicity-related measurements reported in Figure 3B also increased with VP concentration. Based on these findings, 3 μM VP was selected for subsequent experiments as a concentration associated with a marked reduction in CCK-8 signals and relatively limited cytotoxicity under the tested conditions.Compared with the DMSO and PPIX groups, VP treatment produced a greater reduction in the measured growth-associated response (Figure 3C).

Western blot analysis further demonstrated that YAP protein expression was significantly lower in the VP group than in the DMSO and PPIX groups ($P < 0.001$; Figure 3D, E).

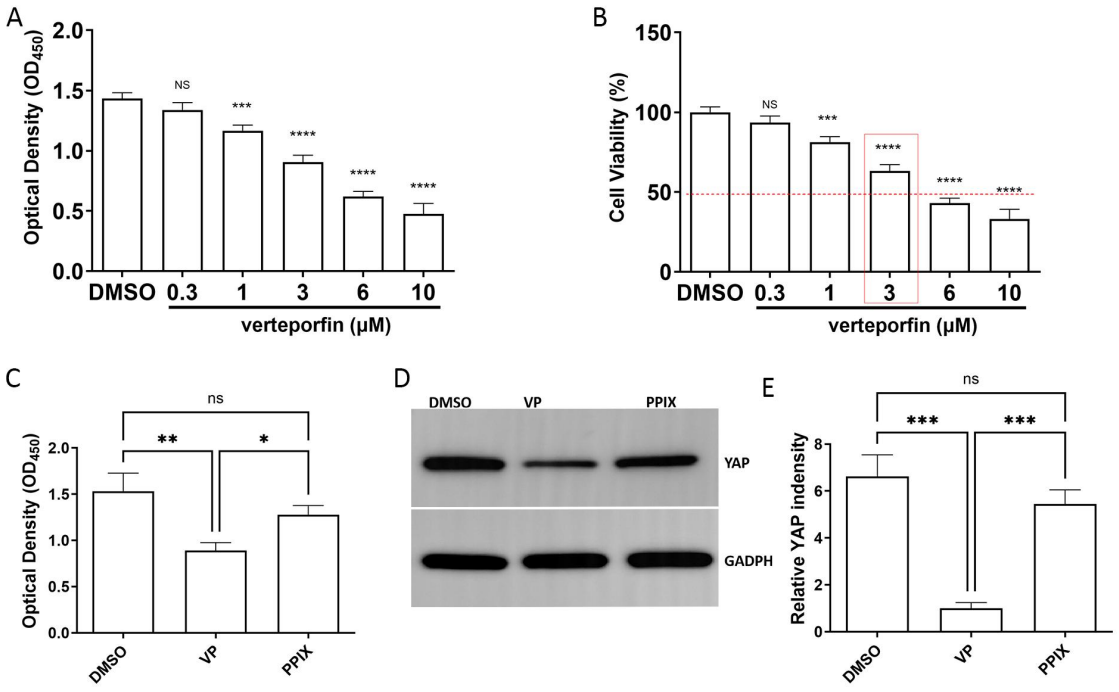


Figure3 VP selectively inhibits proliferation and reduces YAP expression in synthetic VSMCs. Inhibitory effect(A) and cytotoxicity(B) of different concentrations of VP on the proliferation of synthetic VSMCs. Inhibitory Effects(C) and YAP Expression Analysis(D,E) in VSMCs: DMSO, PPIX, and VP Groups. Data are mean ± SD (n = 3 independent experiments).

Verteporfin Alters the Subcellular Distribution of YAP-Associated Regulatory Proteins

To explore potential regulatory processes associated with YAP nucleocytoplasmic localization, we examined the nuclear and cytoplasmic expression of AMOT, PTPN14, α-catenin, and ZO-2 following VP treatment.

Western blot analysis showed that VP treatment was associated with increased cytoplasmic and decreased nuclear levels of AMOT and PTPN14 (Figure 4A, C, D, G, H). Compared with the DMSO and

PPIX groups, VP significantly increased cytoplasmic AMOT expression ($P < 0.01$ and $P < 0.05$, respectively) and decreased nuclear AMOT expression (both $P < 0.01$). Similarly, cytoplasmic PTPN14 expression was significantly increased (both $P < 0.05$), whereas its nuclear expression was decreased (both $P < 0.01$) in the VP group.

For α-catenin, VP treatment was associated with a nonsignificant increase in cytoplasmic expression, whereas nuclear expression was significantly decreased compared with the DMSO and PPIX groups ($P < 0.01$ and $P < 0.05$, respectively; Figure 4A, E, I).

ZO-2 exhibited a different expression pattern. VP treatment significantly reduced cytoplasmic ZO-2 expression compared with the DMSO and PPIX groups ($P < 0.001$ and $P < 0.01$, respectively). Nuclear ZO-2 expression was also reduced in the VP group, with a significant difference compared with DMSO ($P < 0.01$), but not compared with PPIX. In addition, PPIX treatment decreased both cytoplasmic and nuclear ZO-2 expression compared with DMSO (both $P < 0.05$; Figure 4B, F, J).

Immunofluorescence staining further demonstrated increased AMOT, PTPN14, and α-catenin fluorescence signals, predominantly in the cytoplasmic compartment, following VP treatment. Quantitative analysis showed significant differences between the VP group and the control groups, whereas no significant differences were observed between the DMSO and PPIX groups (Figures 5 and 6A, B).

In contrast, ZO-2 fluorescence intensity was significantly reduced in both the VP and PPIX groups compared with DMSO ($P < 0.001$ and $P < 0.01$, respectively). A significant difference was also observed between the VP and PPIX groups ($P < 0.05$; Figure 6C, D).

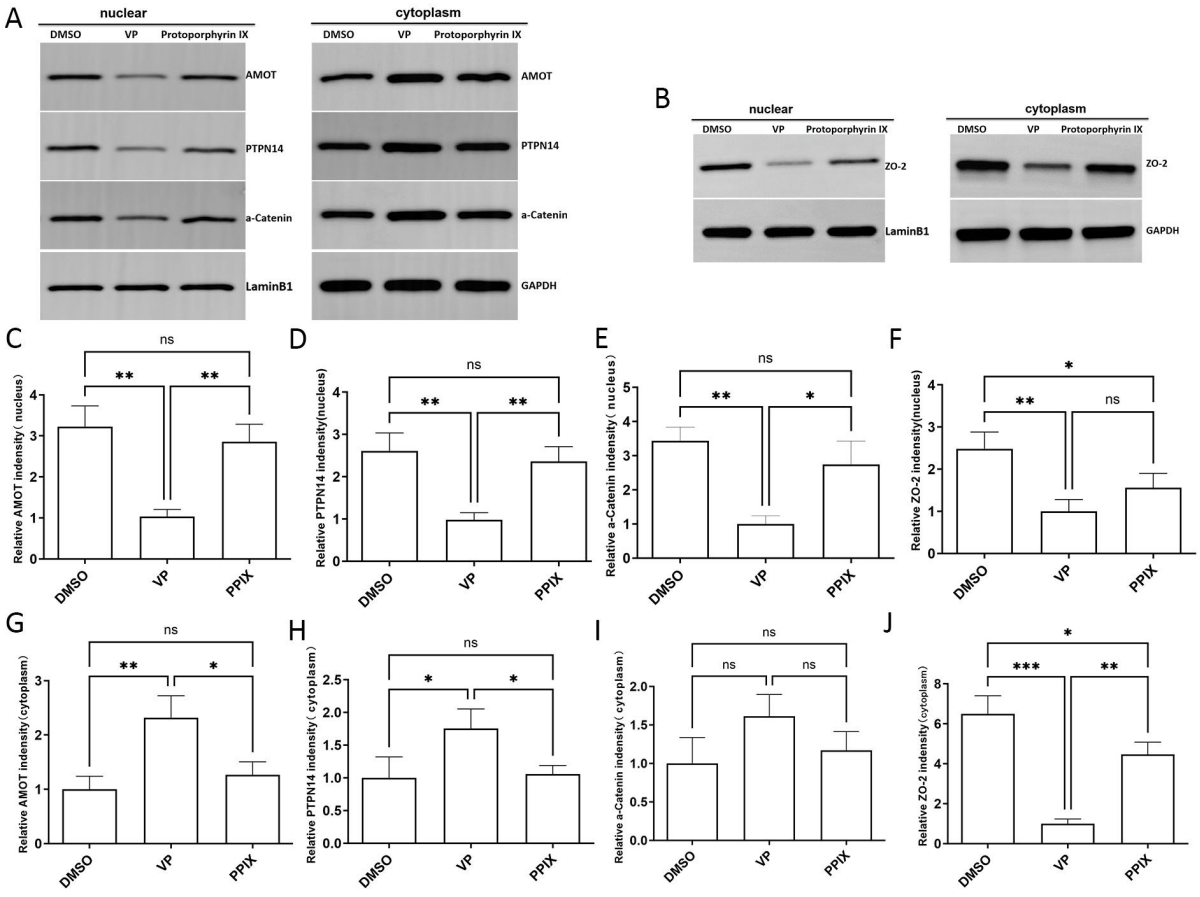
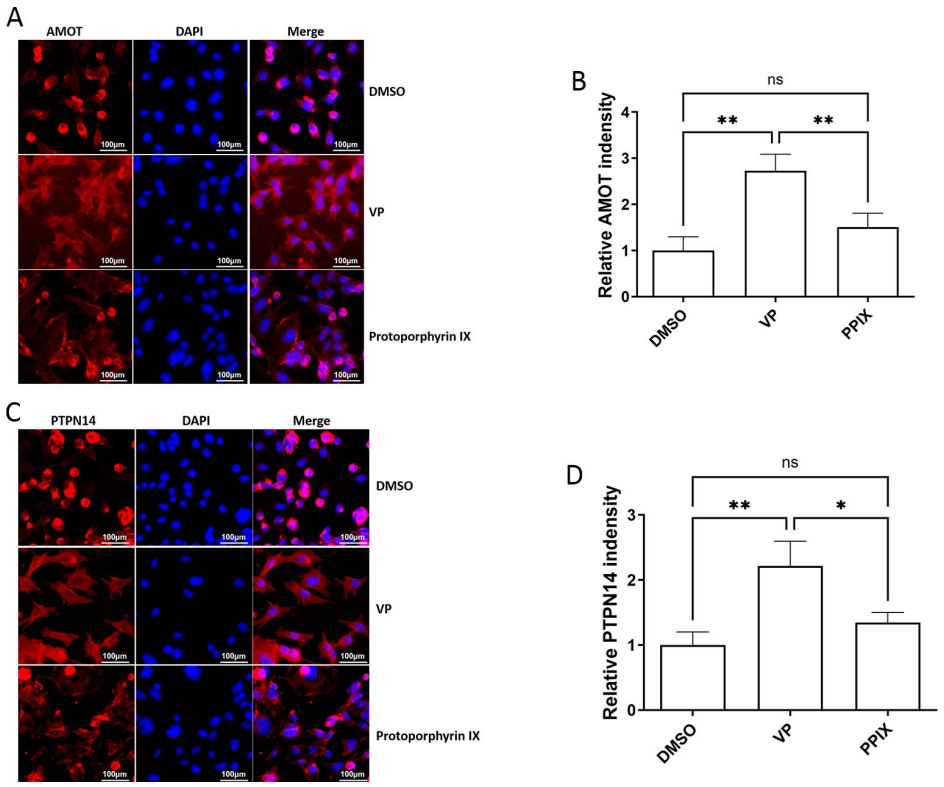


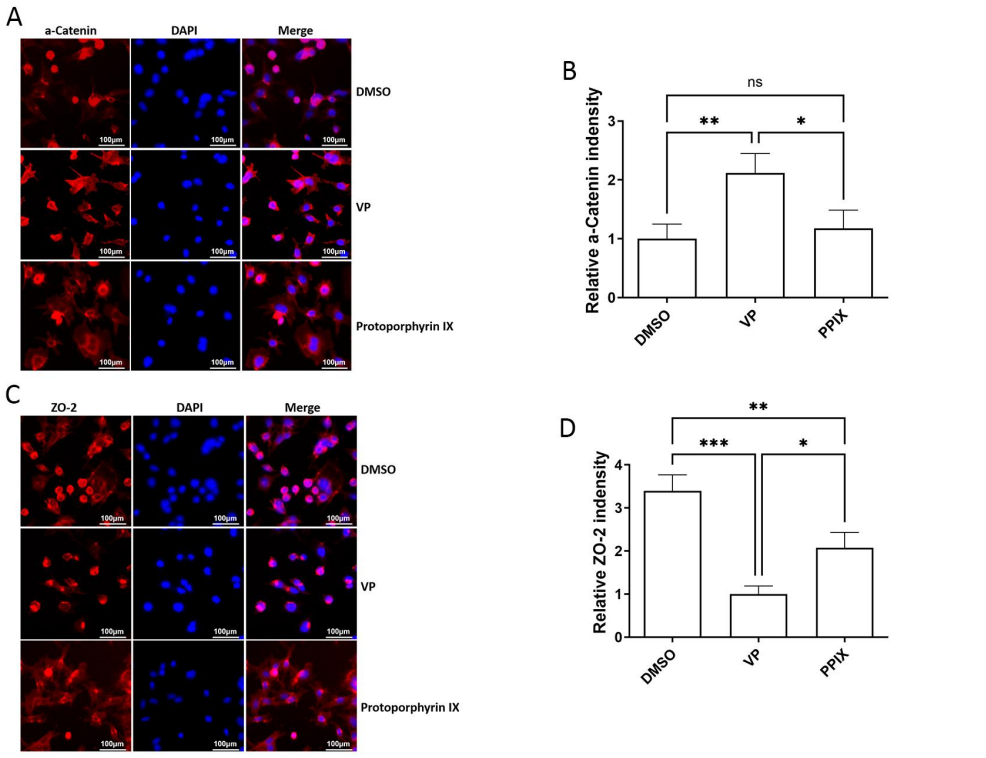
Figure 4 Quantitative analysis of the western blot results for AMOT, PTPN14, α-Catenin, (A,C,D,E,G,H,I)and ZO-2(B,F,J) in the cytoplasmic and nuclear fractions of the DMSO, VP, and PPIX groups. Data are mean ± SD (n = 3 independent experiments).



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238 **Figure5** Immunofluorescence results and quantitative analysis of AMOT(A,B) and PTPN14 (C,D) in

239 the DMSO, VP, and PPIX groups. Data are mean ± SD (n = 3 independent experiments).



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Figure6 Immunofluorescence results and quantitative analysis of α-Catenin(A,B) and ZO-2 (C,D) in the DMSO, VP, and PPIX groups. Data are mean ± SD (n = 3 independent experiments).

Verteporfin Downregulates YAP-Associated Target Gene Expression in VSMCs

To further evaluate changes in YAP-associated transcriptional signaling following VP treatment, we measured the mRNA expression of two established YAP-responsive genes, connective tissue growth factor (CTGF) and cysteine-rich angiogenic inducer 61 (CYR61), using quantitative real-time PCR.

VP treatment significantly reduced the mRNA expression of both CTGF and CYR61 compared with the DMSO and PPIX groups ($P < 0.0001$; Figure 7). No significant differences were observed between the DMSO and PPIX groups ($P > 0.05$).

These findings are consistent with reduced YAP-associated transcriptional output following VP treatment, although they do not establish whether this effect is directly mediated by altered YAP nuclear localization.

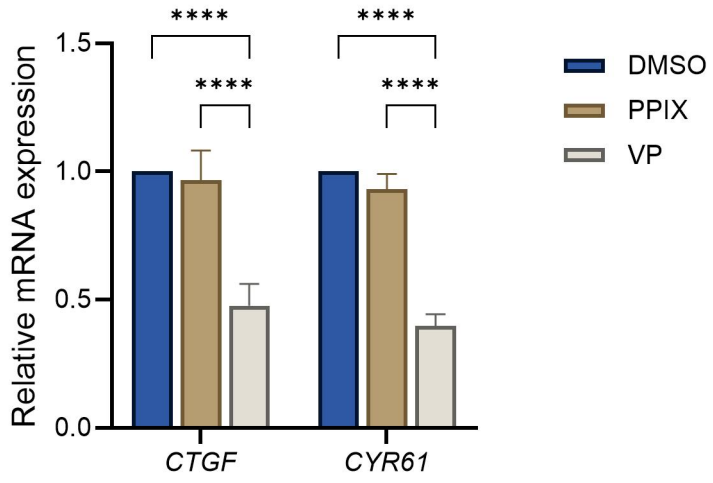


Figure 7 qPCR analysis of CTGF and CYR61 mRNA levels(D). Data are mean ± SD (n = 3 independent experiments).

Discussion

This study investigated the effects of verteporfin (VP) on PDGF-BB-induced vascular smooth muscle cell (VSMC) phenotypic modulation and YAP-associated signaling. Our findings showed that VP treatment reduced VSMC metabolic activity and YAP protein expression, accompanied by alterations in the expression and subcellular distribution of several YAP-associated regulatory proteins, including AMOT, PTPN14, α-catenin, and ZO-2. Furthermore, VP significantly decreased the mRNA expression of the YAP-responsive genes CTGF and CYR61. These findings suggest that VP influences growth-associated responses and YAP-related signaling in VSMCs, potentially involving regulatory processes associated with YAP nucleocytoplasmic localization.

From an evolutionary conservation perspective, the Hippo pathway is highly conserved from Drosophila to mammals. YAP, the functional homolog of Drosophila Yki, plays a central role in regulating cell proliferation, apoptosis, and the maintenance of stem cell pluripotency[9]. Previous studies have demonstrated that YAP contributes to VSMC phenotypic modulation and neointimal formation following vascular injury[10]. Consistent with these findings, our results showed that PDGF-BB stimulation was associated with increased YAP expression and reduced expression of contractile markers, including SM22α, SMMHC, and calponin.VP treatment subsequently reduced YAP protein expression in PDGF-BB-stimulated VSMCs. Since YAP activity is influenced by both protein abundance and nucleocytoplasmic localization, these observations provide a rationale for examining regulatory proteins potentially involved in YAP subcellular trafficking. However, changes in total YAP expression alone do not establish altered YAP nuclear localization.

An important finding of this study was that VP treatment altered the subcellular distribution of several proteins implicated in YAP nucleocytoplasmic regulation. Western blot analysis showed

increased cytoplasmic and decreased nuclear expression of AMOT and PTPN14 following VP treatment. A reduction in nuclear α-catenin expression was also observed, although the increase in its cytoplasmic expression did not reach statistical significance. Angiomotin (AMOT), a component of tight junction, interacts with YAP/ TAZ through its PPxY motif and induces cytoplasmic sequestration of YAP/TAZ[11,12]. Similarly, non-receptor tyrosine phosphatase 14 (PTPN14), one of adherens junction protein, also interacts with YAP via PPxY–WW domains, thereby restricting its nuclear localization. In addition to regulating subcellular trafficking, PTPN14 dephosphorylates YAP at tyrosine 357, which attenuates YAP's co-transcriptional activity[13]. α-Catenin is another junction-associated protein involved in YAP regulation, potentially through interactions with 14-3-3 proteins and mechanisms affecting YAP phosphorylation[14]. In contrast to AMOT and PTPN14, α-catenin does not bind YAP directly. Instead, it associates with S127-phosphorylated YAP through 14-3-3 chaperone proteins, forming a trimeric complex that promotes YAP cytoplasmic retention. This complex additionally interferes with PP2A-mediated dephosphorylation of YAP, further enhancing its cytoplasmic sequestration[14]. In the present study, the observed changes in AMOT, PTPN14, and α-catenin are consistent with alterations in proteins associated with YAP localization control. Nevertheless, their direct contribution to VP-induced changes in YAP signaling remains to be established.

In contrast to the changes observed in AMOT and PTPN14, VP treatment was associated with reduced ZO-2 expression in both nuclear and cytoplasmic fractions. ZO-2 is a tight junction-associated protein that has been implicated in YAP subcellular localization[15,16]. While many junctional proteins facilitate cytoplasmic retention of YAP, ZO-2 exhibits a contrasting function. Unlike AMOT, PTPN14, and α-catenin, ZO-2 promotes nuclear accumulation of YAP. Earlier work by Oka et al. proposed that ZO-2 binds YAP via its PDZ domain and facilitates its nuclear translocation through a

nuclear localization signal (NLS)[17]. However, a subsequent study demonstrated that YAP nuclear localization persists even after ZO-2 knockdown, suggesting that the mechanism by which ZO-2 regulates YAP trafficking requires further clarification[18]. Importantly, PPIX treatment also reduced ZO-2 expression, indicating that this response was not exclusive to VP. Moreover, the difference in nuclear ZO-2 expression between the VP and PPIX groups was not statistically significant. These findings suggest that ZO-2 may participate in the cellular response to porphyrin-related compounds, although its specific contribution to VP-associated YAP signaling in VSMCs remains uncertain.

It is particularly noteworthy that although both VP and PPIX are structurally porphyrin-based compounds, VP exhibits significantly specific regulatory effects. In comparison, VP exerts more specific effects on AMOT and PTPN14. For α-Catenin, VP primarily suppresses its nuclear localization, while its impact on cytoplasmic α-Catenin appears less specific. Regarding ZO-2, both VP and PPIX demonstrate inhibitory effects with no significant difference between them. These findings suggest that the two compounds differentially influence YAP nuclear localization-associated proteins. Previous studies have also confirmed that VP and PPIX exhibit differential effects in inhibiting the proliferation of liver cancer cells[19]. This specificity may originate from: the unique molecular structure of VP, which may confer higher binding affinity to target protein domains[20]; VP's potential activation of upstream signaling events not triggered by PPIX; and more favorable intracellular distribution properties of VP, enabling more efficient delivery to its sites of action. These insights may inform future structure-based drug design efforts targeting specific YAP regulatory complexes.

The reduction in CTGF and CYR61 mRNA expression following VP treatment provides additional evidence of altered YAP-associated transcriptional signaling. CTGF and CYR61 are widely used as transcriptional readouts associated with YAP/TAZ activity. In the present study, both transcripts were

significantly decreased in VP-treated VSMCs compared with the DMSO and PPIX groups.These findings are consistent with reduced YAP-associated transcriptional output and complement the observed decrease in YAP protein abundance. Nevertheless, because CTGF and CYR61 can also be regulated by other signaling pathways, their downregulation cannot be attributed exclusively to YAP nuclear exclusion based on the present data.

From a translational perspective, the modulation of VSMC growth-associated responses and YAP signaling may be relevant to vascular proliferative disorders. YAP plays a critical role in vascular remodeling disorders such as atherosclerosis[21], pulmonary hypertension[22], and transplant vasculopathy[23]. As an approved photosensitizing agent, VP represents an existing compound whose biological activities are being explored beyond its established clinical indication. Our findings provide preliminary in vitro evidence supporting further investigation of VP in the context of VSMC-associated vascular remodeling. However, its potential application in preventing restenosis or as a component of drug-eluting vascular devices requires additional evaluation of efficacy, local toxicity, drug delivery, and safety in relevant experimental models.

Limitation

Several limitations should be acknowledged. First, the effects of VP on VSMC growth were primarily evaluated using the CCK-8 assay, which reflects cellular metabolic activity rather than directly measuring cell proliferation. Second, although changes in YAP expression, YAP-associated regulatory proteins, and downstream target genes were observed, direct evidence linking these alterations to YAP nucleocytoplasmic trafficking and establishing a causal mechanism remains limited. Third, the present study was restricted to an in vitro VSMC model, and the relevance of these findings to vascular injury and restenosis in vivo remains to be determined. Further studies are

therefore warranted to clarify the underlying mechanisms and evaluate the potential translational significance of VP in vascular proliferative disease.

Conclusion

In conclusion, verteporfin attenuated growth-associated responses in PDGF-BB-stimulated VSMCs and reduced YAP protein expression. These effects were accompanied by alterations in the expression and subcellular distribution of YAP-associated regulatory proteins, including AMOT, PTPN14, α-catenin, and ZO-2, as well as decreased expression of the YAP-responsive genes CTGF and CYR61. Collectively, these findings suggest that VP modulates YAP-associated signaling in VSMCs, potentially involving regulatory processes related to YAP nucleocytoplasmic localization. This study provides preliminary evidence supporting further investigation of VP in vascular proliferative diseases, particularly intimal hyperplasia and restenosis

Author contributions

Zhixiang Su: Designed and performed the experiments, organized the data, and wrote the manuscript.

Shibiao Liu and Qi Wu: Assisted with the experiments.

Jiazheng Li, Zhi Fan, Shuang Guo, and Rongrong Zhu: Provided constructive input and assistance regarding experimental design, data analysis, and manuscript preparation.

Weiwei Wu: Oversaw the entire project, managed the progress, analyzed experimental data, and provided critical guidance and suggestions.

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Conflict of interest

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

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request

Declarations Ethics approval and consent to participate

The study adhered to the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of the *Beijing Tsinghua Changgung Hospital*

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