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*Original Article*

# Verteporfin induced SUMOylation of YAP1 in endometrial cancer

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**Abstract:** Yes-associated protein (YAP or YAP1) has been proposed to function as an oncogene in most cancers, with nuclear localization of YAP1 correlating with poor prognosis. Photosensitizer Verteporfin has been proven as an inhibitor of YAP1 through preventing the combination of YAP1 with TEA domain transcription factor (TEAD). We showed previously that the total and phospho-levels of YAP1 were related to the clinical characteristics and outcomes in endometrial cancer (EC) patients, and that YAP1 promoted the proliferation and metastasis of EC cells in vitro cell line studies and in animal models. We also reported that Verteporfin inhibited cell growth and induced cell death through inhibiting YAP1 in EC in our previous study. However, the mechanism of how Verteporfin inhibits the function of YAP1 remains unclear. In this study, we analyzed the global effects of Verteporfin on cell function by using Reverse Phase Protein Arrays (RPPA) and Ingenuity Pathway Analysis (IPA). Furthermore, we demonstrated that Verteporfin induced the SUMOylation of YAP1 for the first time. Interestingly, we found that the SUMOylation of YAP1 was regulated by YAP1 phosphorylation. Together, our study revealed a novel mechanism by which Verteporfin inhibits the function of YAP1 through regulating YAP1 SUMOylation. Our study may provide a rationale for the clinical use of Verteporfin in endometrial cancer by targeting YAP1.

**Keywords:** SUMOylation, YAP1, Verteporfin, endometrial cancer

## Introduction

The Yes-associated protein (YAP or YAP1) is the key downstream effector in the HIPPO signaling cascade [1], which emerged as a major contributor to cancer pathophysiology. By associating with TEA domain transcription factor (TEAD) family of transcription factors, YAP1 promotes cell growth and inhibits apoptosis [2, 3]. In many cancers, the nuclear localization of YAP1 is correlated with poor prognosis [4]. Thus, the YAP oncogene have been identified as a target for the development of new cancer drugs [5] and small compounds that disrupt the YAP-TEAD complex formation have been tested for therapeutic potentials [6].

Components of the HIPPO tumor suppressor pathway are mutated in 30% of ECs (endometrial cancers) and, strikingly, in 54% of ECs with microsatellite instability [7]. YAP1 is a potent oncogene and is frequently amplified in various human cancers. We have shown previously for the first time that YAP1 regulates the biological processes of EC cells through activating PI3K/Akt/mTOR pathway via up-regulating GAB2 [8]. Verteporfin is a second-generation photosensitizer approved by the Food and Drug Administration in 2000 for the treatment of age-related macular degeneration [9]. It has been identified as an inhibitor of TEAD-YAP association [6]. Since then, Verteporfin has been used as an inhibitor of YAP in oncologic research. This effect has been proven in colon cancer [10]. We also observed that Verteporfin mimicked the effects of siYAP on EC cells and inhibited EC tumor growth in vivo in our previous study [8]. However, the molecular mechanisms underlying Verteporfin inhibition of YAP remain elusive.

SUMOylation is a reversible and dynamic protein post-translational modification that regulates the function of target proteins by affecting

their intracellular location, activity and stability [11, 12]. In this study, we present for the first time that Verteporfin induced a SUMOylation of YAP1, which may contribute to the inhibitory effect of Verteporfin in endometrial cancer. Hence, the data from our study suggested a potential therapeutic approach of targeting YAP1 SUMOylation for the treatment of endometrial cancer or other diseases.

## Materials and methods

### *Reagents and materials*

Antibodies from Cell Signaling Technology. Plasmids were from Gordon B Mills's previous Lab., Ju-Seog Lee' Lab. of MD Anderson Cancer Center (MDACC), Houston, TX. X-tremeGENE HP DNA Transfection Reagent was from Roche Diagnostics.

### *Cell culture, transient transfection and assays*

KLE, EFE184 and NOU-1 were from the MDACC Characterized Cell Line Core Facility and propagated as monolayer cultures with, respectively, DMEM F12, RPMI1640 and DMEM, supplemented with 5% heat-inactivated fetal bovine serum (FBS), except 10% FBS for NOU-1 at 37°C in a humidified incubator containing 5% $CO_2$. All cell lines are validated by STR in the Core before distribution.

### *Western blot*

Cells were washed twice in ice-cold phosphate-buffered saline (PBS) and then lysed in RIPA Lysis Buffer (Santa Cruz Biotechnology) for 20 min at 4°C. The resulting suspension was centrifuged for 15 min, 14,000 rpm at 4°C. The supernatant was then collected, and the protein concentration was determined using a bicinchoninic acid protein assay kit (Thermo Scientific). Cell lysates were incubated with 6×SDS sample buffer for 5 min at 100°C and then were run on SDS-PAGE gels, transferred to polyvinylidene fluoride membranes, and probed with the appropriate primary and secondary antibodies. Protein bands were detected by enhanced chemiluminescence (ECL, GE Healthcare).

Cells were lysed in IP lysis buffer supplemented with protease and phosphatase inhibitors. Protein lysate was then incubated with corresponding antibody overnight followed by incubation with protein A/G agarose beads (Santa Cruz Biotechnology). Centrifuge and keep the pellet, wash with pre-chilled washing buffer. Collect the supernatant to proceed to *Western Blot.*

### *Cytotoxicity*

Cells on chamber slides were washed twice with a warm PBS solution and stained with a LIVE/DEAD Viability/Cytotoxicity Kit (Molecular Probes, Life Technologies). The optimal concentration of ethidium homodimer-1 dye was 0.4 mM and that of calcein dye 0.4 mM. The samples were incubated for 30 min at 37°C and viewed under a fluorescence microscope. Dead cells were stained red; live cells, green.

### *Reverse phase protein arrays (RPPA)*

KLE and EFE184 cells were treated with Verteporfin 3 μM, MG132 10 μM, and DMSO for 24 hours (groups arrangement see notes in **Figure 1**). Protein lysis were collected and sent it to MDACC CCSG core for performance of RPPA. RPPA was performed in the MDACC CCSG core as described at https://www.mdanderson.org/research/research-resources/core-facilities/functional-proteomics-rppa-core.html.

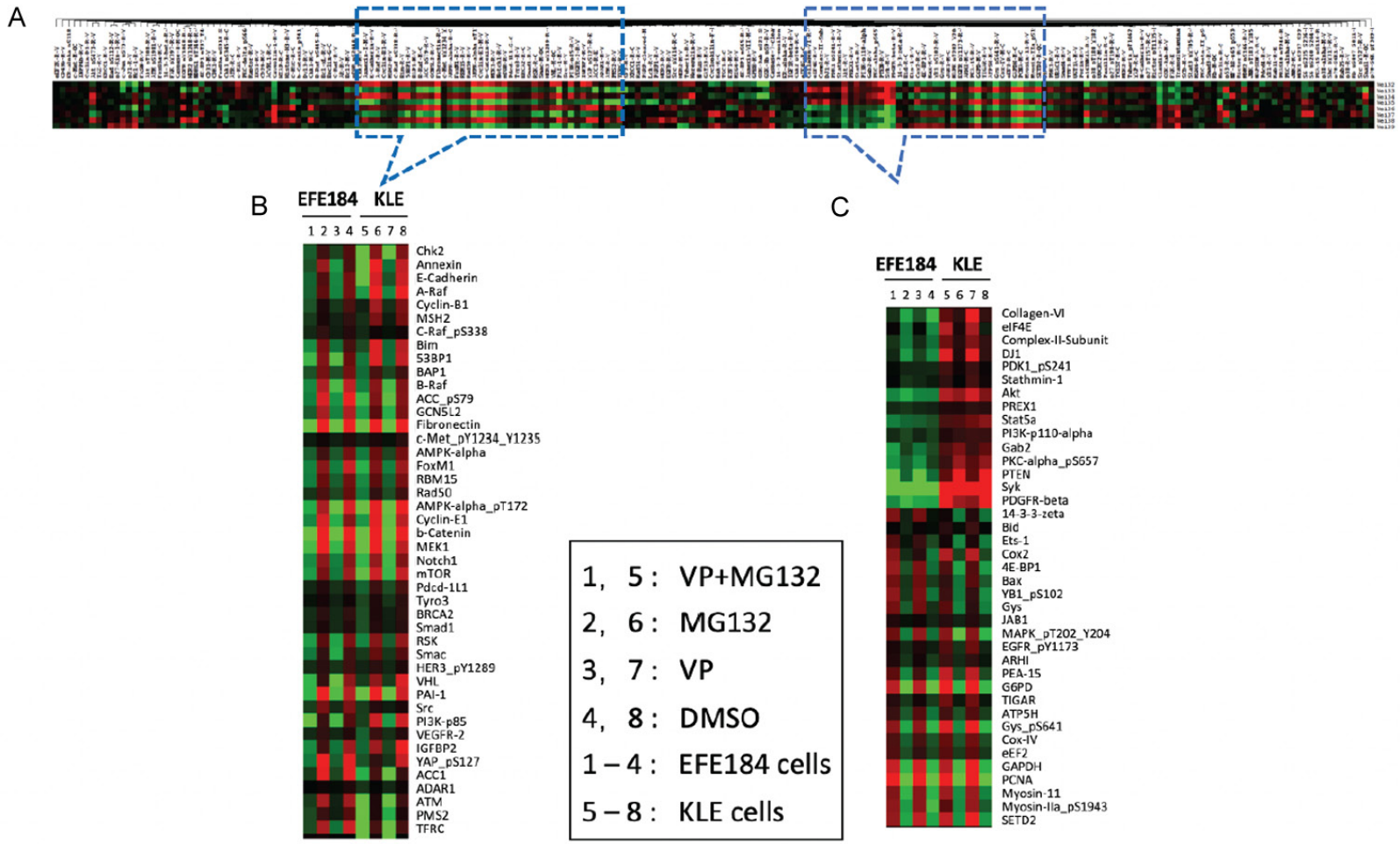
### *Statistical analysis*

The robustness and generalizability of the results are supported by repeating the studies across multiple relevant cell lines.

## Results

### *Global effect of Verteporfin on cellular function through ingenuity pathway analysis (IPA) with RPPA data*

Since Verteporfin has been shown to inhibit the growth of EC cells in vitro and inhibited the EC tumor growth in vivo in our previous study, to understand the molecular mechanisms underlying Verteporfin function, we conducted Ingenuity Pathway Analysis with RPPA data to investigate the effects of Verteporfin in KLE cells and EFE184 cells, which are endometrial cancer cells without mutations in PTEN, PIK3CA and AKT genes. The overall heatmap made by RPPA data was shown in **Figure 1A**. Proteins


A
B
EFE184
KLE
1 2 3 4 5 6 7 8
Chk2
Annexin
E-Cadherin
A-Raf
Cyclin-B1
MSH2
C-Raf_pS338
Bim
53BP1
BAP1
B-Raf
ACC_pS79
GCN5L2
Fibronectin
c-Met_pY1234_Y1235
AMPK-alpha
FoxM1
RBM15
Rad50
AMPK-alpha_pT172
Cyclin-E1
b-Catenin
MEK1
Notch1
mTOR
Pdcd-1L1
Tyro3
BRCA2
Smad1
RSK
Smac
HER3_pY1289
VHL
PAI-1
Src
PI3K-p85
VEGFR-2
IGFBP2
YAP_pS127
ACC1
ADAR1
ATM
PMS2
TFRC
1, 5 : VP+MG132
2, 6 : MG132
3, 7 : VP
4, 8 : DMSO
1 – 4 : EFE184 cells
5 – 8 : KLE cells
C
EFE184
KLE
1 2 3 4 5 6 7 8
Collagen-VI
eIF4E
Complex-II-Subunit
DJ1
PDK1_pS241
Stathmin-1
Akt
PREX1
Stat5a
PI3K-p110-alpha
Gab2
PKC-alpha_pS657
PTEN
Syk
PDGFR-beta
14-3-3-zeta
Bid
Ets-1
Cox2
4E-BP1
Bax
YB1_pS102
Gys
JAB1
MAPK_pT202_Y204
EGFR_pY1173
ARHI
PEA-15
G6PD
TIGAR
ATP5H
Gys_pS641
Cox-IV
eEF2
GAPDH
PCNA
Myosin-11
Myosin-IIa_pS1943
SETD2

D

**IPA with RPPA data of treatment with VP and DMSO in KLE cells**

**Log Ratio up-regulated**

| Molecules | Exp. Value | Exp. Chart |
|---|---|---|
| PCNA | ↑4.788 | |
| G6PD | ↑4.582 | |
| SETD2 | ↑2.101 | |
| GYS1* | ↑2.008 | |
| GAPDH | ↑1.990 | |
| TP53 | ↑1.787 | |
| CDKN2A | ↑1.497 | |
| MDM2 | ↑1.353 | |
| MYH11 | ↑1.331 | |
| PEA15* | ↑1.218 | |

**Log Ratio down-regulated**

| Molecules | Exp. Value | Exp. Chart |
|---|---|---|
| CTNNB1* | ↓-4.245 | |
| FN1 | ↓-3.256 | |
| SERPINE1 | ↓-2.495 | |
| PRKAA1* | ↓-2.405 | |
| CHEK2* | ↓-1.967 | |
| CCNE1 | ↓-1.967 | |
| MAP2K1 | ↓-1.909 | |
| ACACA | ↓-1.854 | |
| IGFBP2 | ↓-1.836 | |
| MTOR* | ↓-1.819 | |

E

**IPA with RPPA data of treatment with VP and DMSO in EFE184 cells**

**Log Ratio up-regulated**

| Molecules | Exp. Value | Exp. Chart |
|---|---|---|
| PCNA | ↑3.997 | |
| G6PD | ↑2.728 | |
| GAPDH | ↑1.868 | |
| TP53 | ↑1.673 | |
| SETD2 | ↑1.658 | |
| MYH9 | ↑1.623 | |
| GYS1* | ↑1.525 | |
| CDKN2A | ↑1.516 | |
| MYH11 | ↑1.489 | |
| CASP7 | ↑1.275 | |

**Log Ratio down-regulated**

| Molecules | Exp. Value | Exp. Chart |
|---|---|---|
| CTNNB1* | ↓-4.284 | |
| FN1 | ↓-3.181 | |
| PRKAA1* | ↓-2.298 | |
| BRAF* | ↓-1.870 | |
| MAP2K1 | ↓-1.869 | |
| SERPINE1 | ↓-1.868 | |
| VHL | ↓-1.843 | |
| TP53BP1 | ↓-1.540 | |
| FOXM1 | ↓-1.538 | |
| YAP1* | ↓-1.507 | |

F

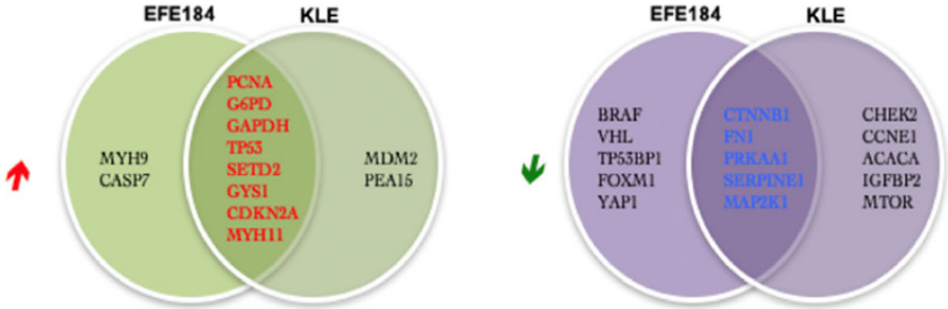



**Figure 1.** Ingenuity Pathway Analysis (IPA) with RPPA data. EFE184 and KLE cells were treated with DMSO, 3 uM Verteporfin, 10 uM MBG132, 3 uM Verteporfin and 10 uM MBG132 respectively for 24 h. Cell lysates were collected and processed for RPPA. (A) The heatmap with RPPA data. Two screenshots within blue dashed boxes were magnified in (B and C). (B) An enlarged picture of one dashed box showing proteins decreased obviously by Verteporfin. (C) An enlarged picture of one dashed box showing proteins increased obviously by Verteporfin. (D, E) IPA was conducted with RPPA data. The top 10 up-regulated molecules and the top 10 down-regulated molecules caused by Verteporfin treatment were listed, respectively. (F) The common proteins that were increased or decreased by Verteporfin in two cell lines were listed. The red arrow represents proteins with the increased amount, whereas green arrow represents proteins with the decreased amount.

that were down-regulated by Verteporfin were shown in **Figure 1B**, while those that were up-regulated were shown in **Figure 1B**, **1C**. According to the IPA, the top 10 up-regulated molecules and the top 10 down-regulated molecules are shown in **Figure 1D** and **1E**. They included PCNA, G6PD, GAPDH, TP53, SETD2, GYS1, CDKN2A, MYH11, CTNNB1, FN1, PRKAA1, SERPINE1, MAP2K1 and so on. These proteins are involved in many major signaling networks such as in carbohydrate metabolism, cellular growth and proliferation, cell death and survival, cellular movement, DNA replication, recombination, and repair. Proteins that exhibited the same changes in these two cell lines were shown in **Figure 1F**. The information obtained from this analysis will provide the basis for our further investigation on the function of Verteporfin.

*Verteporfin decreased cell growth and increased cell death in endometrial cancer cells*

Verteporfin is an FDA-approved drug and has been shown to inhibit cell growth in colon cells. Since our interest is on the effect of Verteporfin in endometrial cancer, we examined the effect of Verteporfin on endometrial cancer cell EFE184, and we found that Verteporfin inhibited EFE184 cell growth in a dose-dependent manner (**Figure 2A**) with clear cell morphological changes. Cells were round up and began to die within 1 day after treatment (**Figure 2B**).

*Verteporfin decreased the expression level of total YAP1 protein and phospho-YAP1 protein*

We further investigated the functional mechanism of Verteporfin on cell growth. Verteporfin has been reported to inhibit the complex formation between TEAD-YAP. We then focused our study on the effect of Verteporfin in YAP1 function. Interestingly, we found that Verteporfin decreased YAP1 protein level in a dose-dependent manner in EC cell line, NOU-1 cells (**Figure 3A**). Verteporfin treatment at 3 μM for 24 h led to a complete inhibition in the expression of YAP1 protein. Furthermore, the protein level of GAB2, a downstream target gene of YAP1, was also inhibited by Verteporfin treatment (**Figure 3A**), though this inhibition required higher concentration of Verteporfin (10 μM). This decrease in YAP1 and GAB1 protein by Verteporfin treatment was not the result of inhibition at YAP1 and GAB1 gene transcription as when we transfected YAP1 and GAB1 expression plasmids under the control of MTV promoter, we still observed the reduction of YAP1 and GAB1 protein (**Figure 3B**). In both experiments we used Erk2 as a specificity control to indicate the specific function of Verteporfin on YAP1 and GAB2 since Erk2 protein level was not affected by Verteporfin treatment (**Figure 3A** and **3B**). If the change in YAP1 and GAB2 protein level after Verteporfin treatment was not the result of transcriptional regulation, does Verteporfin treatment affect the protein stability/degradation of YAP1 and GAB2? To test this, we treated the EC cancer cell lines KLE and EFE184 cells with proteasome inhibitor MG132 together with Verteporfin to block protein degradation, and we found that in both cell lines MG132 treatment could not rescue the effect of Verteporfin in decreasing YAP1 and GAB2 protein level (**Figure 3C**), suggesting protein degradation is not the cause for the reduction in YAP1 and GAB2 protein level. Since YAP1 protein level and activity is mainly regulated by the phosphorylation of YAP1 at Serine 127, which regulates YAP1 cytoplasmic retention and degradation, we were curious about the effect of Verteporfin on the phospho-YAP1 protein level. We found that the phospho-YAP1 protein level was also decreased by Verteporfin treatment in both KLE and EFE184 cells (**Figure 3D**). Erk2 western blot was used as sample control. Consistent with the observation with YAP1 total protein, MG132 treatment also failed to rescue this effect (**Figure 3D**). Similar inhibition was also observed in phospho-GAB2 protein level; Verteporfin treatment led to a decrease in phospho-GAB2 protein level, and this reduction could not be rescued by MG132 treatment (**Figure 3E**).

*Verteporfin treatment led to a mobility shift of YAP1 protein due to sumo modification of YAP1*

From western blot experiments above, we observed that the expression of YAP and phospho-YAP1 was decreased by Verteporfin treatment. This effect was not the result of transcriptional regulation and protein degradation induced by Verteporfin treatment. However, intriguingly, when we carefully examining the expression of phospho-YAP1 protein after Verteporfin treatment by immunofluorescence staining, we could still detect the signal of YAP1 protein in cells (data not shown, and reference

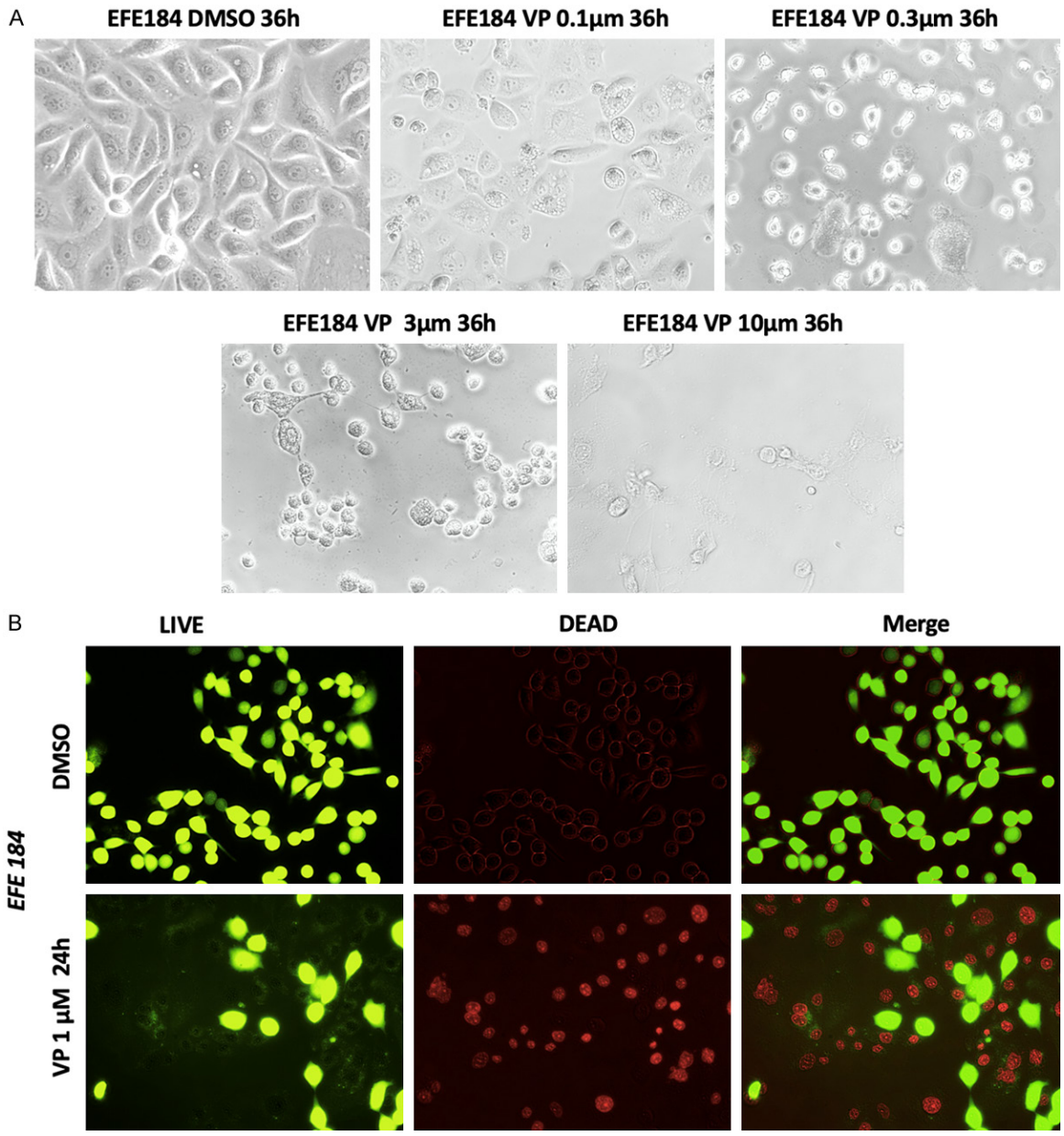



**Figure 2.** Verteporfin decreased cell growth and increased cell death in endometrial cancer cells. A. EFE184 cells were treated with 0.1 μM, 0.3 μM, 3 μM, 10 μM Verteporfin for 36 hours. The pictures are showing the morphologic change under microscope (* 20). B. EFE184 cells were treated with 1 μM Verteporfin for 24 h. Cells were processed for LIVE/DEAD assay. Red color refers to dead cells; green color refers to live cells.

[13]). To understand this discrepancy, we carefully re-examined our western blot results, and found that there was phospho-YAP1 protein signal at the molecular weight higher than 70 kDs of the expected molecular size of YAP1 after Verteporfin treatment (**Figure 4A**). This shift in molecular size of phospho-YAP1 was observed in a Verteporfin dose-dependent manner in both NOU-1 and EFE184 cells (**Figure 4A** and **4B**).

Based on the observation that Verteporfin treatment induced a mobility shift in phospho-YAP1, we speculated that Verteporfin treatment might cause protein modification of YAP1. Through a series of biochemical analysis, we confirmed that the mobility shift of YAP1 was the result of YAP1 sumo modification. As shown in **Figure 4C**, when we immunoprecipitated YAP1 from cells treated with or without Verteporfin, and immunoblotted the precipitat-

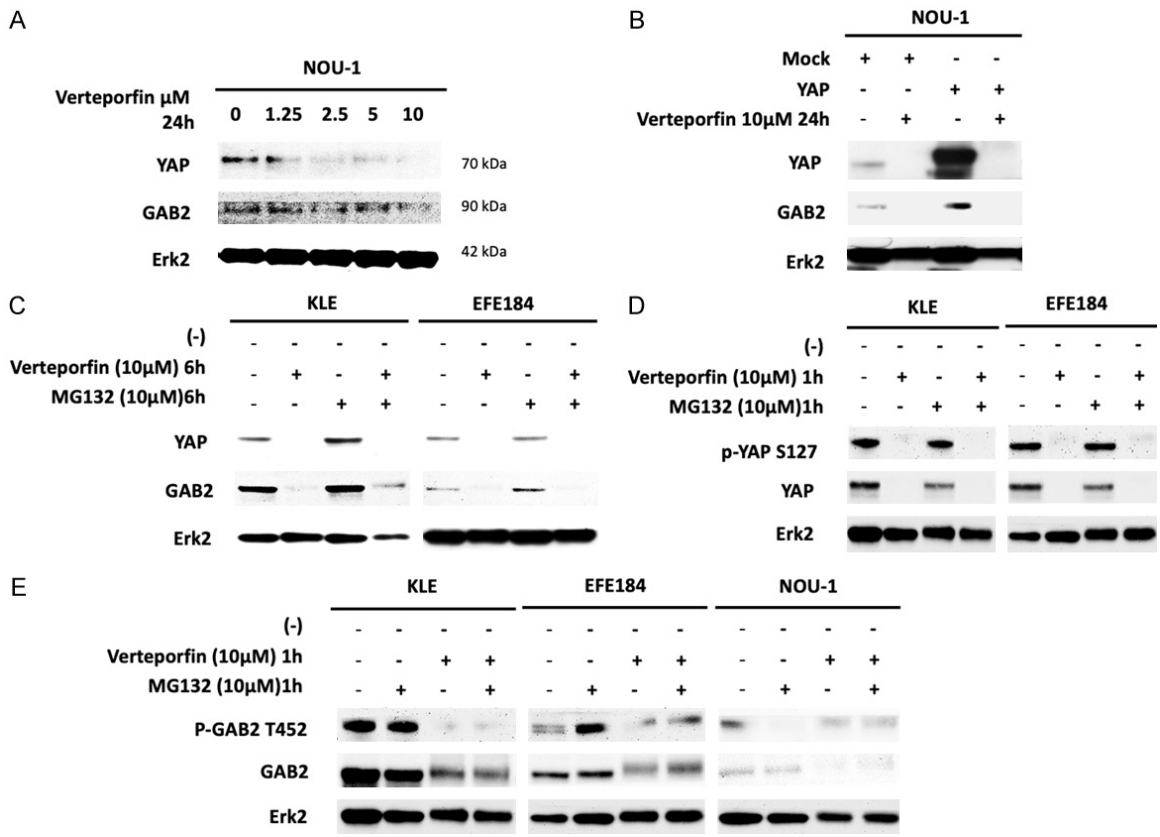



**Figure 3.** Verteporfin decreased the total and phospho-YAP1 protein level. (A) NOU-1 cells were treated with 0 μM, 1.25 μM, 2.5 μM, 5 μM, 10 μM Verteporfin for 24 h. Cell lysates were collected and subjected to western blot with anti-YAP1 and anti-GAB2 antibodies. Anti-Erk2 western blot was used as sample control. (B) YAP1-expressing plasmid was transfected in NOU-1 cells for 24 h, then. Cells were treated with DMSO or 10 μM Verteporfin for 24 h. Cell lysates were collected and western blot was performed as described in (A, C). KLE and EFE184 cells were treated with DMSO, 10 μM Verteporfin in the presence or absence of 10 μM MG132, respectively, for 6 h. Cell lysates were collected and western blot was performed as described in (A). (D) KLE and EFE184 cells were treated with DMSO, 10 μM Verteporfin in the presence or absence of 10 μM MG132, respectively, for 1 h. Cell lysates were collected and western blot was performed with indicated antibodies. (E) KLE, EFE184 and NOU-1 cells were treated with DMSO, 10 μM Verteporfin in the presence or absence of 10 μM MG132, respectively, for 1 h. Cell lysates were collected and western blot was performed with indicated antibodies.

ed products with anti-sumo antibody, sumo antibody recognized YAP1 protein at the higher molecular size only from the samples with Verteporfin treatment, suggesting Verteporfin treatment induced an sumo modification of YAP1. Furthermore, we believed that this sumo-modified form of YAP1 induced by Verteporfin treatment was mainly the Serine127-phosphorylated form of YAP1. As shown in **Figure 4D**, we immunoprecipitated YAP1 from cells treated with or without Verteporfin, and immunoblotted the precipitated products with anti-phospho-YAP1 antibody, phospho-YAP1 antibody recognized the higher molecular size of YAP1 only from Verteporfin treated cells. In **Figure 4E**, we used anti-YAP1 antibody to immunoblot the YAP1-immunoprecipitated product as our experimental control. The Verteporfin treatment induced sumo modification of YAP1 was further confirmed by using de-SUMOylation enzyme such as SUMO Specific Peptidase (SENP) in our experiments. As shown in **Figure 4F**, in NOU1 cells, the Verteporfin treatment-induced sumo modification was abolished by co-transfection of SENP1, but not by SENP2.

*Serine127 phosphorylation of YAP1 is important for YAP1 sumo modification induced by Verteporfin*

From our results in **Figure 4C** and **4D**, we believed that phospho-YAP1 is the mainly sumo modified form of YAP1 induced by Verteporfin

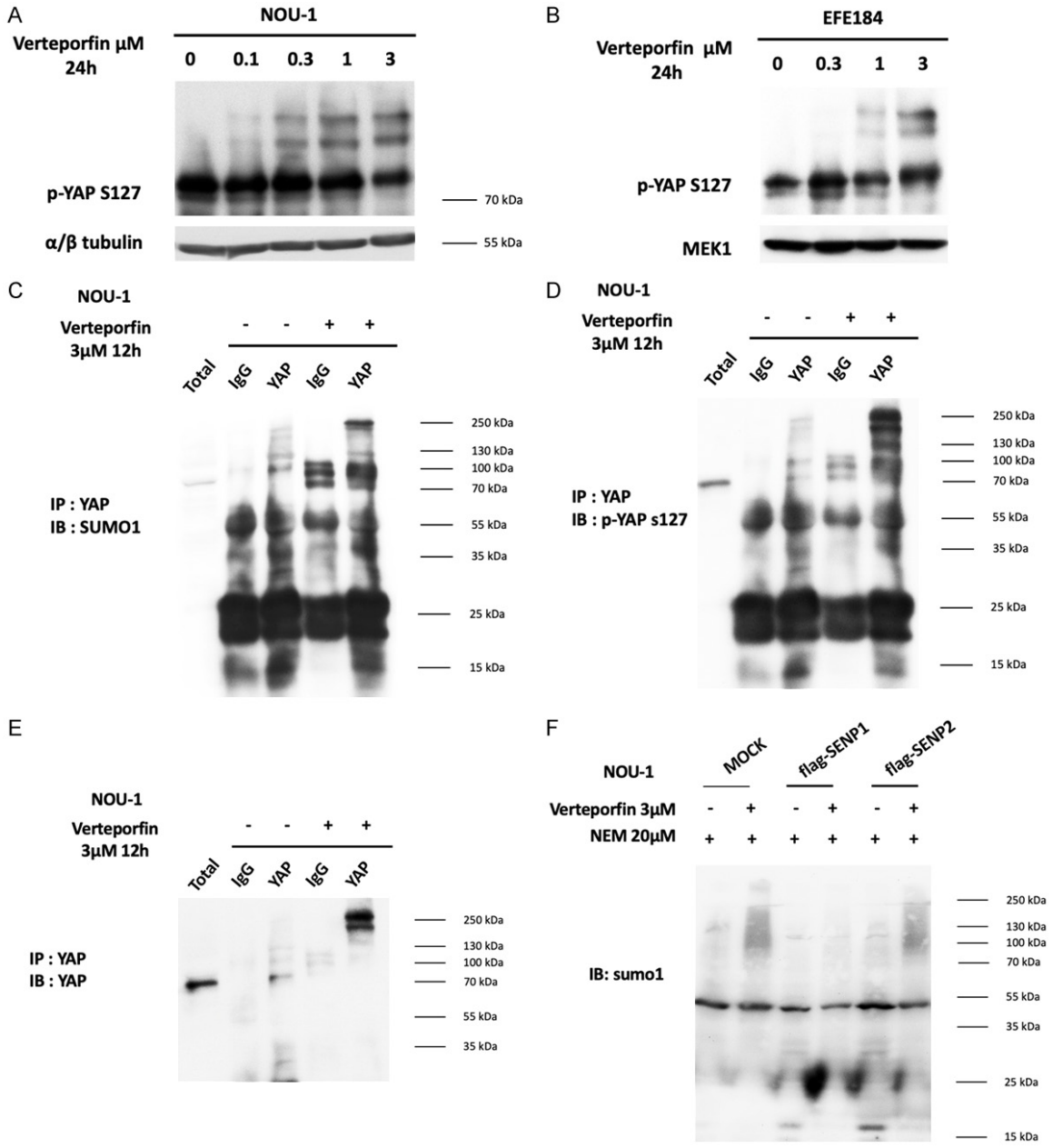



**Figure 4.** Mobility shift of YAP1 protein induced by Verteporfin. (A) NOU-1 cells were treated with Verteporfin (0, 0.1, 0.3, 1, 3 μM) for 24 h. Cell lysates were collected and subjected for western blot with anti-phospho-YAP1 antibody. (B) EFE184 cells were treated with Verteporfin (0, 0.3, 1, 3 μM) for 24 h. (C-E) NOU-1 cells were treated with 3 μM Verteporfin for 12 h. Cell lysates were subjected to immunoprecipitation with anti-YAP1 antibodies. The immunoprecipitated products were analyzed by western blot with anti-sumo1 antibody (C), anti-phospho-YAP1 antibody (D), and anti-YAP1 antibody (E). (F) NOU-1 cells were transfected with vector only, or SNEP1, or SENP2-expressing plasmid for 24 h. After that, cells were treated with 3 μM Verteporfin and 20 μM NEM for 24 h. Cell lysates were collected and subjected to western blot with anti-sumo1 antibodies.

treatment. To directly prove this, we generated a YAP1 mutant in which the Serine127 site was mutated into Alanine (YAPS127A). We then transfected wild type YAP1 or YAPS127A mutant into EFE184 and NOU-1 cells, and examined the mobility shift of YAP1 or YAPS127A after Verteporfin treatment. As shown in **Figure 5**, we found that the Verteporfin treatment-induced mobility shift of YAP1 was abolished in YAP1S127A mutant, suggesting that the

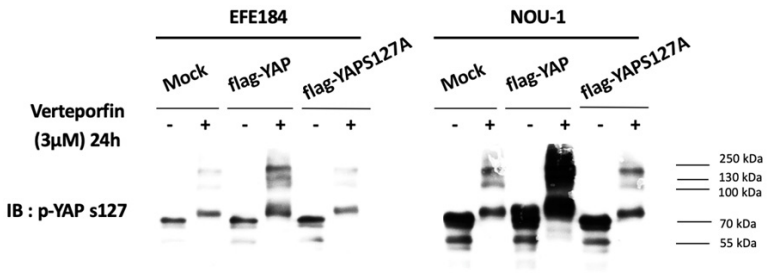



**Figure 5.** Phosphorylation of YAP1 affects its sumo modification induced by Verteporfin. EFE184 and NOU-1 cells were transfected with vector only, or wild type flag-YAP1, or phosphorylation mutant of flag-YAP1 S127A plasmids for 24 h. After that, cells were treated with 3 μM Verteporfin for 24 h. Cell lysates were collected and subjected to western blot with anti-phospho-YAP1 antibody.

Serine127 phosphorylation of YAP1 is important for the sumo modification of YAP1 induced by Verteporfin treatment.

## Discussion

Regulation of YAP1 has been extensively studied, especially on the role of phosphorylation in regulating YAP1 activity. YAP1 is phosphorylated by LATS1/2 in HIPPO tumor-suppressing pathway, leading to its cytosolic sequestration and resulting in subsequent proteasomal degradation [14]. When the activation of HIPPO pathway is inhibited, the de-phosphorylation of YAP1 will lead to the nuclear transportation to co-activated with TEAD [6, 15, 16], causing the tumorigenesis of cancers including colon cancer, gastric cancer, liver cancer, endometrial cancer [10, 13, 15, 17-20]. However, as one of the important posttranslational protein modification, SUMOylation of YAP1 has not been well characterized.

SUMOylation, as a dynamic post-translator, attributes to numerous cell functions such as nuclear-cytosolic transport, transcriptional regulation, apoptosis, gene repair, and progression through the cell cycle, as well as cancer suppression [11, 12, 21-23]. Though few studies have suggested the potential SUMO modification site on YAP1, a recent study has proved that there is SUMO-interacting motif (SIM) in YAP1 and TAZ [24]. We predicted that there exists possible SUMOylation site of YAP1 through the fourth-generation GPS algorithm [25], suggesting that there are interactions between YAP1 and SUMO. In this study, we showed that YAP1 is sumo-modified, and this SUMO modification is induced by Verteporfin treatment, Hence, our study provides the basis for the further characterization of YAP1 sumo-modification and on how sumo-modification of YAP1 is regulated. Sumo modification of YAP1 was further confirmed in our study as we found that the Verteporfin-induced SUMOylation of YAP1 was attenuated by the co-expression of SENP, especially by SENP1, but not by SENP2, suggesting a specific type of sumo modification is involved.

Verteporfin is a second-generation photosensitizer approved for the treatment of age-related macular degeneration by FDA [9]. Moreover, Verteporfin has been shown to regulate other cellular processed such altered functions associated with autophagy, cell proliferation, cell cycle and apoptosis [26-28]. Recently, it has been identified as an inhibitor of TEAD-YAP association and YAP induced liver overgrowth [6]. In our previous study, we proved at the first time that YAP1 activated PI3K/AKT signaling through altering expression of GAB2, which provided evidences that YAP1 played an oncologic role in endometrial cancer [8]. Moreover, we observed Verteporfin, as an inhibitor of YAP1-TEAD combination, exerted the inhibitory effects on tumor cell proliferation, cell migration, and the growth of EC cells in an orthotopic model. It indicated Verteporfin could be a promising drug targeted to YAP1 in endometrial cancer. But, how Verteporfin inhibit the interaction of YAP with TEAD has not been illustrated clear.

In this study, we also observed the SUMOylation-induced effect of Verteporfin on YAP1. This effect is Verteporfin-specific as other photosensitizers, such as Protoporphyrin and Hematoporphyrin, do not exhibit this effect (data not shown). Does Verteporfin induce SUMOylation of other proteins in addition to YAP1 protein? Our preliminary data showed that Verteporfin also induced the SUMOylation of other proteins (data not shown). In our previous work, we found that the intracellular translocation of YAP1 was induced by Verteporfin [13]. Does sumo modification of YAP1 regulate the intracellular location of YAP1? Moreover, how do YAP1 phosphorylation and sumo modification coordinate to regulate the function of

YAP1? We will explore these questions in our future study.

In summary, our finding of SUMOylation of YAP1 and the regulation of YAP1 SUMOylation by Verteporfin may provide the rationale for the potentially therapeutic targeting of YAP1 for cancer treatment. Our study will also help to understand the working mechanism of Verteporfin in treating patient. Future research will continue to delineate the relationships among YAP1, SUMOylation and Verteporfin.

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## Disclosure of conflict of interest

None.

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