PLOD2 exacerbates cervical squamous cell carcinoma by suppressing p53 by binding to YAP1

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Abstract. Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2 (PLOD2) has been identified as an oncogene involved in the progression of several human cancers. However, its role in cervical squamous cell carcinoma (CESC) and its underlying mechanisms are not well understood. In the present study, several public databases, RT-qPCR and western blotting were employed to detect the expression of PLOD2 and the prognosis in CESC. Cell counting kit-8 assay, wound healing assay, Transwell assay, western blotting and flow cytometry were utilized to assess the proliferation, migration and cell apoptosis of CESC cells. Cellular senescence was examined by RT-qPCR and β-galactosidase staining. Prediction of PLOD2 binding to Yes-associated protein 1 (YAP1) was assessed using BioGrid, HDock and co-immunoprecipitation, and p53 and p21 signaling were assessed using immunofluorescence staining. The findings indicated that the expression of PLOD2 was elevated in CESC tissues and cell lines, and PLOD2 silencing caused the inhibition of CESC cell proliferation, migration and the promotion of apoptosis and senescence of CESC cells. PLOD2 was predicted to be bound to YAP1 and YAP1 overexpression reversed the effects of PLOD2 silencing on CESC cell proliferation, cell migration, apoptosis and senescence. In addition, PLOD2 facilitated CESC progression by regulating the P53 pathway through YAP1. PLOD2 exerted pro-oncogenic effects on CESC through the p53 pathway by binding to YAP1. These findings provide new perspectives for the future study of PLOD2-targeted therapy for CESC.

Introduction

Cervical cancer is one of the leading causes of cancer deaths in women (1). The age-standardized incidence rates worldwide is 13.1/100,000 women (2). Epidemiological investigations report 445,000 new cases of cervical cancer and 236,000 deaths annually (3), with ~90% of the deaths occurring in developing countries and low and middle income countries (2). The burden of cervical cancer remains high in numerous parts of the world, with incidence rates in most countries above the thresholds agreed by the WHO Cervical Cancer Elimination Initiative (4,5). Currently, the incidence and mortality rates of cervical cancer are progressively decreasing in developed countries (6). In underdeveloped countries, however, cervical cancer is still one of the most common malignant tumors in women and is the leading cause of death from malignant tumors (7). Based on histopathology, cervical cancer can be divided into squamous cell carcinoma of the cervix, adenocarcinoma of the cervix and rare types such as adenosquamous carcinoma, neuroendocrine carcinoma and smooth muscle sarcoma (8). Among them, the most common type of cervical cancer is cervical squamous cell carcinoma (CESC), which accounts for ~80% of the total number of cases (9,10). It is now generally accepted that human papillomavirus (HPV) is the leading contributor to cervical cancer development and has been categorized into low-risk and high-risk strains on the basis of their oncogenic ability (11). Of the >40 established HPV species that can infect human genitalia, 18 HPV strains have been categorized as high-risk genotypes (12). Almost all cervical cancers are due to high-risk HPV, with serotypes 16 and 18 accounting for 70% of all cases (13). Patients with early-stage cervical cancer have favorable prognosis (14). However, for advanced cervical cancer, cisplatin-based chemotherapy is preferred, but its efficacy is unsatisfactory, with only 1/5 of patients responding to cisplatin-based chemotherapy modalities or radiotherapy-chemotherapy combinations (15). Therefore, searching for new prognostic molecules and effective target molecules for early diagnosis, and establishing new and effective therapeutic measures for CESC are urgent clinical problems.

Collagen, as the most abundant protein, provides a scaffold for the assembly of the extracellular matrix (ECM) and is considered a 'highway' for cancer cell migration and invasion (16). The extent of collagen lysine hydroxylation influences the stability of intermolecular collagen cross-links (17). Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2 (PLOD2), also termed lysyl hydroxylase 2, is a key enzyme that mediates the formation of stable collagen cross-links by catalyzing the

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hydroxylation of lysine (18). Studies have shown that PLOD2 is significantly overexpressed in head and neck squamous cell carcinoma and promotes cancer cell proliferation, migration and invasion (19,20). RNA sequencing identified PLOD2 as a key gene marker for HPV-associated oropharyngeal squamous cell carcinoma (21). In addition, database analysis revealed that PLOD2 was significantly upregulated in cervical esophageal carcinoma (CESC), which is a potent prognostic marker and associated with immune infiltration in HPV-associated CESC (22). It is worth noting that all of the aforementioned cancers can be caused by HPV infection (23,24). Therefore, it was hypothesized that there is an association between PLOD2 and HPV infection. Several studies have revealed that PLOD2 is overexpressed in bone carcinoma, hepatocellular carcinoma, pancreatic carcinoma and squamous cell carcinoma of the head and neck, accompanied by promoted proliferation, migration and invasion of cancer cells (19,25). Nevertheless, studies on PLOD2 in CESC have not yet been published. Hence, the objective of the present study was to characterize the role of PLOD2 in CESC cells and the mechanism by which PLOD2 impedes the progression of CESC.

Materials and methods

Data mining using public databases. The CESC-related dataset GSE64217 was screened and downloaded from the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/) and used to mine PLOD2 gene expression in patients with CESC samples (26). The differentially expressed genes of $P<0.05$ were screened out. UALCAN database (https://ualcan.path.uab.edu) analyzed PLOD2 expression in CESC tissues from the TCGA database (https://portal.gdc.cancer.gov/) and the prognostic impact of PLOD2 in patients with CESC (27,28). In addition, the BioGrid v4.4 website (https://thebiogrid.org) (29) predicted the molecules that PLOD2 may bind to and HDock (http://hdock.phys.hust.edu.cn/) was utilized to verify the binding of PLOD2 to Yes-associated protein 1 (YAP1).

Cell culture. Human cervical endometrial cell line End1/E6E7 and CESC cell lines C33A, SiHa, HT-3 and MS751 were obtained from Cellverse Bioscience Technology Co., Ltd. Cells were incubated in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin/streptomycin at 37˚C under 5% CO_2.

Cell transfection. The specific siRNA targeting PLOD2 (siRNA-PLOD2#1/2) and the corresponding control siRNA (siRNA-NC), the pc-DNA3.1 vector containing the whole length of YAP1 (Ov-YAP1) and the empty vector (Ov-NC) were synthesized by GenePharma. The sequence information of siRNAs used for cell transfection are shown in Table I. Using Lipofectamine® 2000 reagent, 100 nM vectors was transfected into SiHa cells following a typical protocol at 37˚C for 48 h (30). Cells were harvested 48 h after transfection for subsequent experimentation.

Cell counting kit-8 (CCK-8) assay. SiHa cells were inoculated into 96-well plates, followed by transfection with siRNA-PLOD2 with or without Ov-YAP1 for 48 h. After which, 10 μl WST-8 (Beyotime Institute of Biotechnology) was added to each well to grow the cells for 2 h, and the absorbance was calculated at 450 nm with a microplate reader (Bio-Rad Laboratories, Inc.).

Wound healing assay. Transfected SiHa cells were initially inoculated into a six-well plate. After the cells reached 90% confluence, the cell monolayers were scratched with a white pipette tip (31). Following 24 h of incubation (without serum), the migratory rate was determined by a light microscope. Image J v1.50 (National Institutes of Health) was used to quantify the wound healing percentage. The formula was as follows: Initial wound size-healing wound size/initial wound size x100%.

Transwell assay. Cell suspension was prepared by serum-free medium and the transfected SiHa cell suspension ($2x10^5$ cells/ml) was loaded into the upper chamber. Then the medium containing 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) was added to the lower chamber. After incubating at 37˚C for 24 h, the bottom of the cell chamber was fixed with 100% methanol and stained with 0.1% crystal violet at room temperature for 10 min (31). The number of migrating cells was counted by a fluorescent microscope.

Cell apoptosis analysis. Transfected SiHa cells were washed with precooled PBS. The cells were then stained with Annexin V-FITC for 15 min, followed by the addition of propidium iodide (10 mg/ml) for 5 min at room temperature in darkness. Apoptosis was analyzed by CytoFLEX flow cytometer (Beckman Coulter, Inc.) recognized using FlowJo software v10.8 (Tree Star, Inc.).

SA-β staining. After treatment, SiHa cells were washed three times with HBSS and treated with 1 ml β-galactosidase fixative for 15 min at room temperature. After which, the cell fixative was separated and the cells were allowed to incubate with the staining solution overnight at 37˚C (32). The plates were viewed under an inverted microscope.

Co-immunoprecipitation (co-IP). Total protein was extracted from SiHa cells by IP lysate (NCM Biotech; cat. no. P70100) containing protease inhibitor and was incubated with rabbit IgG (1 μg; cat. no. Sc-2027, Santa Cruz Biotechnology, Inc.) or IP-indicating antibody (1 μg) at 4˚C overnight, while an appropriate amount of the extracted protein was used as an input control. The following antibodies were used: PLOD2 (Abcam; cat. no. ab313765) and YAP1 (Abcam; cat. no. ab52771). Protein A/G PLUS-Agarose (20 μl) was applied and incubated at 4˚C for 2 h to form the immune mixture, and then centrifuged at 1,000 x g at 4˚C for 3 min to isolate the complexes. After washing 4 times with 1 ml cold lysis buffer and boiling for 5 min in the appropriate protein sample buffer, the supernatant was collected in a new tube to carry out western blotting as mentioned below to analyze the immuno-complexes.

Immunofluorescence staining. SiHa cells were fixed with 4% polyoxymethylene at room temperature for 10 min and permeabilized with 0.5% Trition-X100. Following blocking with 10% BSA (Biofroxx; neoFroxx) in PBS for 1 h at room temperature,

Table I. Sequence information of siRNAs used for cell transfection.

si-RNA	Direction	Sequence (5'-3')
siRNA-PLOD2#1	Sense	ACUAUACGGUUGACAUAUGGA
	Antisense	CAUAUGUCAACCGUAUAGUUC
siRNA-PLOD2#2	Sense	AUCGAAUUCACAAAGAGUGCA
	Antisense	CACUCUUUGUGAAUUCGAUAC
siRNA-NC	Sense	UUCUCCGAACGUGUCACGUTT
	Antisense	ACGUGACACGUUCGGAGAATT

NC, negative control; PLOD2, procollagen-lysine 2-oxoglutarate 5-dioxygenase 2.

the cells were incubated with primary antibodies (1:100) overnight at 4˚C and secondary antibodies (1:500) for 2 h at room temperature, and stained with DAPI at room temperature for 10 min. The following antibodies were used: p21 (Proteintech Group, Inc.; cat. no. 67362-1-Ig), p53 (Proteintech Group, Inc.; cat. no. 60283-2-Ig) and CoraLite488-conjugated Donkey Anti-IgG (H+L; Proteintech Group, Inc.; cat. no. SA00013-5). The samples were visualized under a confocal microscope.

RT-qPCR. The total RNA used in the present study was isolated from SiHa cells using Trizol® reagent. The cDNA was synthesized using a cDNA reverse transcription kit (Applied Materials, Inc.) at 37˚C for 15 min. qPCR was performed using the SYBR Green PCR Master Mix (Applied Biosystems, cat. no. 4367659) and MiniOpticon qPCR detection System (Bio-Rad Laboratories, Inc.). Thermocycling conditions used for qPCR comprised a preincubation step at 95˚C for 60 sec, followed by 40 cycles: 95˚C for 15 sec for denaturation; 63˚C for 25 sec for annealing and extension. The results were estimated based on the $2^{-\Delta\Delta Cq}$ method (33) with GAPDH used as the internal reference gene. The primers used are shown in Table II.

Western blotting. Total proteins from SiHa cells were extracted using RIPA lysis buffer (NCM Biotech; cat. no. WB3100). The protein samples of each group were quantified by BCA kit (NCM Biotech) and 20 μg/lane protein samples were loaded on 10% SDS-PAGE gel. After 10% SDS-PAGE separation, equal amounts of proteins were transferred to a PVDF membrane. After blocking with 5% BSA (Biofroxx; neoFroxx) for 1 h at room temperature, the membranes were cultured with primary antibodies PLOD2 (1:1,000; Abcam; cat. no. ab313765), MMP2 (1:3,000; Proteintech Group, Inc.; cat. no. 66366-1-Ig), MMP9 (1:500; Proteintech Group, Inc.; cat. no. 27306-1-AP), Bcl-2 (1:1,000; Affinity Biosciences; cat. no. AF6139), Bax (1:1,000; Affinity Biosciences; cat. no. AF0120), caspase 3 (1:1,000; CST Biological Reagents Co., Ltd.; cat. no. 9662), cleaved caspase 3 (1:1,000; CST Biological Reagents Co., Ltd.; cat. no. 9661), YAP1 (1:5,000; Abcam; cat. no. ab52771) and GAPDH (Proteintech Group, Inc.; cat. no. 60004-1-Ig; 1:5,000) overnight at 4˚C and with secondary antibodies HRP-conjugated Goat Anti-Mouse IgG (H+L; 1:2,000; Proteintech Group, Inc.; cat. no. SA00001-1) or HRP-conjugated Goat Anti-Rabbit IgG (H+L; 1:2,000; Proteintech Group, Inc.; cat. no. SA00001-2) at 37˚C for 2 h. The ELC A solution was mixed with the B solution in equal proportions and visualization of the protein bands was achieved using the ECL detection system (Thermo Fisher Scientific, Inc.) in accordance with standard protocols (34), and analysis of protein density was assessed by Image J software v1.50 (National Institutes of Health).

Statistical analysis. Statistical analyses were performed using SPSS 22.0 (IBM Corp.) and GraphPad Prism 6 software (Dotmatics). Data are presented as the mean ± standard deviation of three independent experiments. Results were obtained using one-way ANOVA followed by Bonferroni post hoc tests for multiple comparisons. $P<0.05$ was considered to indicate a statistically significant difference.

Results

PLOD2 expression is upregulated in CESC tissues and cell lines, and inhibition of PLOD2 decreases CESC cell proliferation. To determine whether PLOD2 plays a role in CESC tumorigenesis, bioinformatic analyses were performed. PLOD2 expression was markedly enhanced in CESC tissues in public databases, as shown in Fig. 1A and B. High expression of PLOD2 was associated with poor prognosis in CESC tissues (Fig. 1C). In addition, by comparative analysis it was found that PLOD2 levels were specifically increased in CESC cell lines compared with the End1/E6E7 cell line. Among them, the expression of PLOD2 was strongest in SiHa cells, so it was selected for subsequent experiments (Fig. 1D and E). Next, PLOD2 expression was silenced and the transfection efficiency was shown in Fig. 1F and G. The findings exhibited that the transfection efficiency of siRNA-PLOD2-1 was higher, hence siRNA-PLOD2-1 (termed siRNA-PLOD2) was selected for the subsequent experiments. CCK-8 assay indicated that silencing PLOD2 significantly decreased the proliferation of SiHa cells when compared with the control group (Fig. 1H).

PLOD2 silencing suppresses the migration, and promotes apoptosis and senescence in SiHa cells. As illustrated in Fig. 2A and B, PLOD2 knockdown diminished cell migration. Silencing PLOD2 resulted in suppression of the protein levels of MMP2 and MMP9 (Fig. 2C). In addition, flow cytometry analysis demonstrated a notable increase in apoptosis following transfection with siRNA-PLOD2 (Fig. 2D), which was consistent with the Western blotting results whereby the level of Bcl-2 was declined in cells silenced with PLOD2,

Table II. Primer sequences used in the RT-qPCR.

Gene name	Direction	Sequence (5'-3')
PLOD2	Forward	GACAGCGTTCTCTTCGTCCTCATC
	Reverse	ACCACCTCCCTGAAAGTCTTCTCC
YAP1	Forward	CGTCATGGGTGGCAGCAACTC
	Reverse	TCAGCCGCAGCCTCTCCTTC
IL-6	Forward	GGTGTTGCCTGCTGCCTTCC
	Reverse	GTTCTGAAGAGGTGAGTGGCTGTC
IL-1β	Forward	GGACAGGATATGGAGCAACAAGTGG
	Reverse	CAACACGCAGGACAGGTACAGATTC
IL-8	Forward	GGACCACACTGCGCCAACAC
	Reverse	CCCTCTGCACCCAGTTTTCCTTG
CCL20	Forward	TGCTGTACCAAGAGTTTGCTCCTG
	Reverse	CTTCTGATTCGCCGCAGAGGTG
GAPDH	Forward	GTGGACCTGACCTGCCGTCTAG
	Reverse	GAGTGGGTGTCGCTGTTGAAGTC

PLOD2, procollagen-lysine 2-oxoglutarate 5-dioxygenase 2.

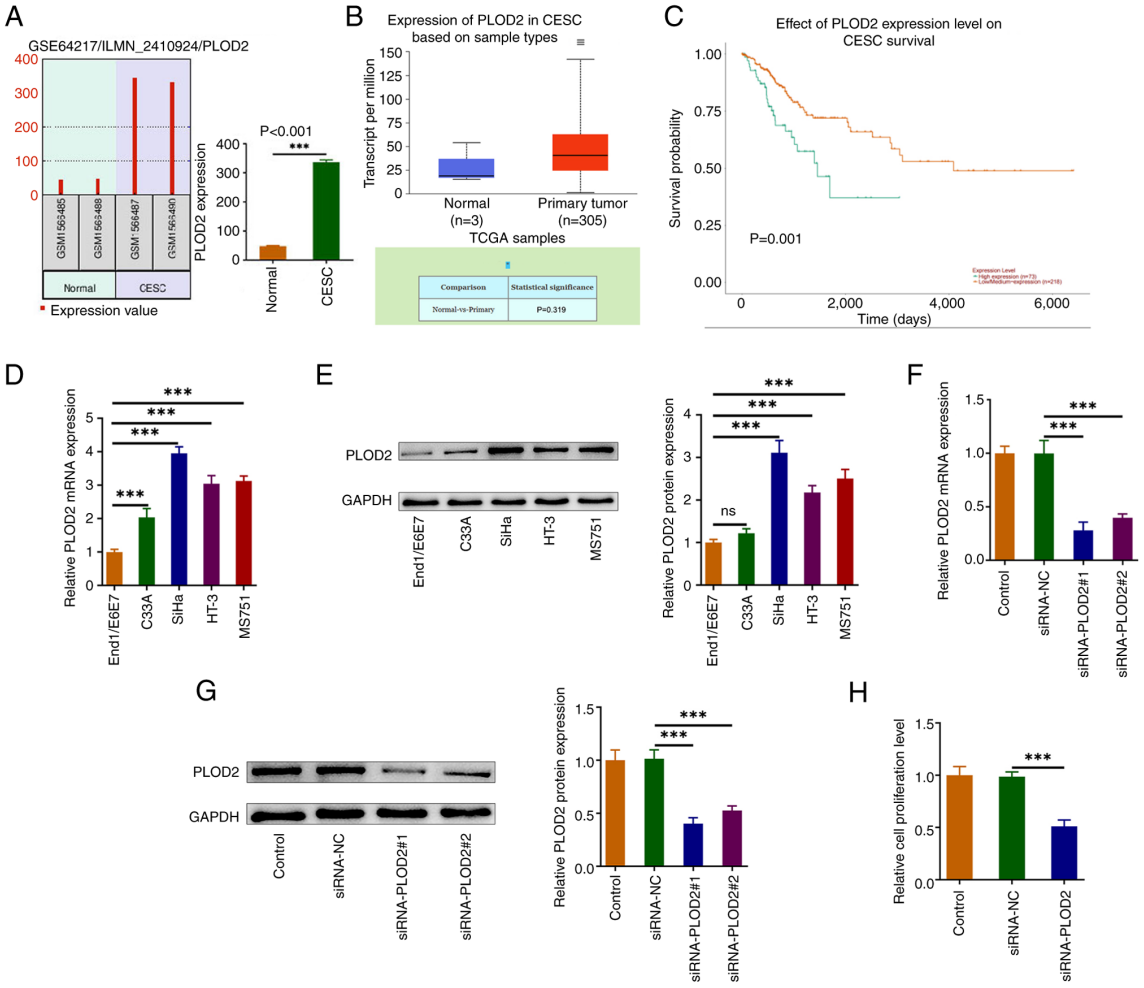

Figure 1. PLOD2 expression is upregulated in CESC tissues and cell lines, and inhibition of PLOD2 reduces CESC cell proliferation. Analysis of the expression of PLOD2 in patients with CESC with the (A) GSE64217 dataset and (B) UALCAN database. (C) The association between PLOD2 and the prognosis of patients with CESC assessed by the UALCAN database. (D) mRNA and (E) protein level of PLOD2 in CESC cell lines were detected by RT-qPCR and western blotting. (F) mRNA and (G) protein level of PLOD2 in SiHa cells after PLOD2 silencing were detected by RT-qPCR and western blotting. (H) CCK-8 assay was used to measure the cell proliferation of SiHa cells. Results are the mean ± SD. ***P<0.001. PLOD2, procollagen-lysine 2-oxoglutarate 5-dioxygenase 2; CESC, cervical esophageal carcinoma; NC, negative control; ns, not significant.

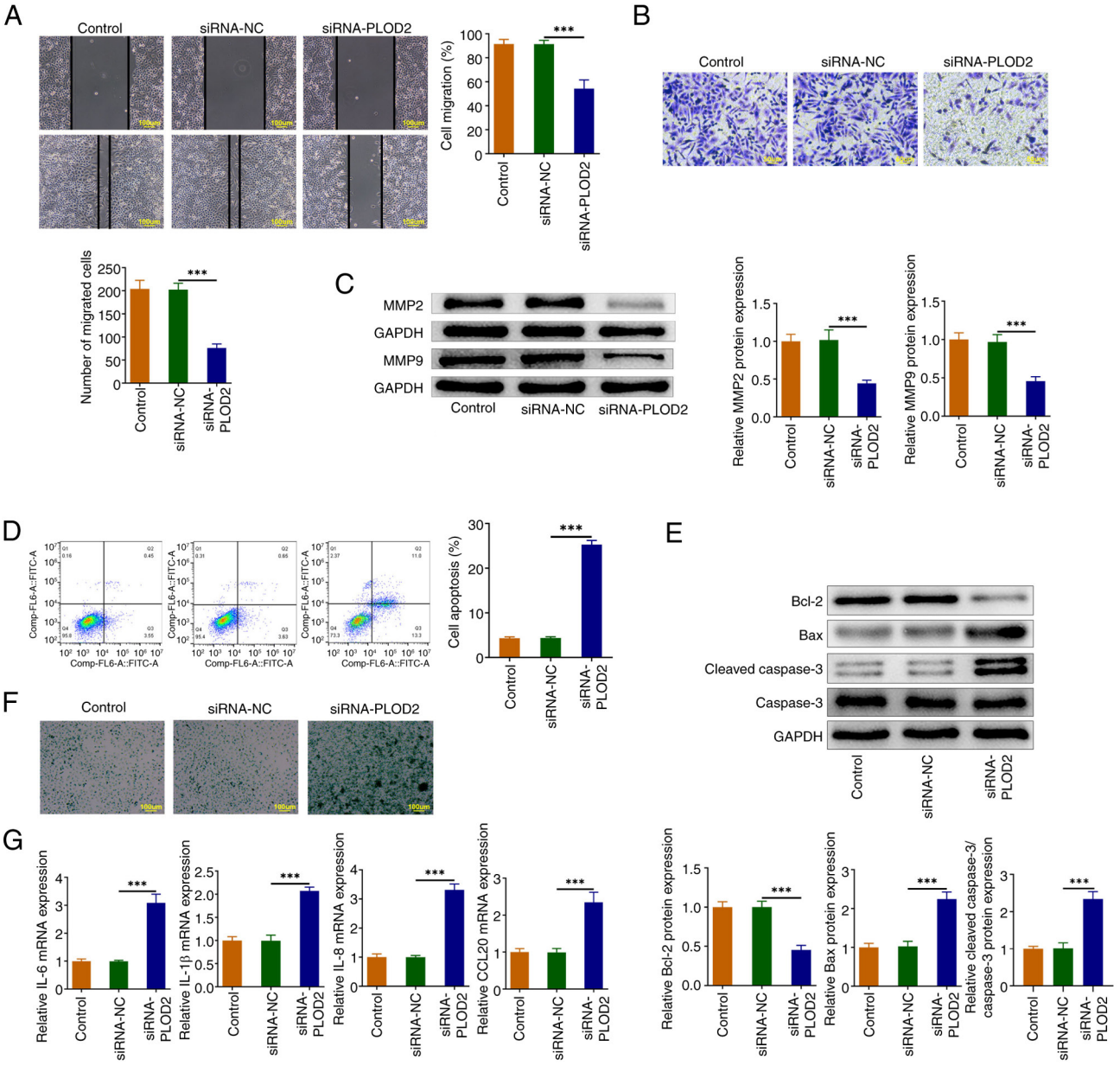

Figure 2. PLOD2 silencing suppresses the migration, and promoted apoptosis and senescence in SiHa cells. (A) Cell migration was measured by wound healing assay at 0 h and the ending timepoint and (B) Transwell assay at the ending timepoint. (C) Western blotting was performed to assess the levels of invasion-associated proteins (MMP2 and MMP9). (D) Cell apoptosis was examined by flow cytometric analysis. (E) Western blotting was used to assess apoptotic-related protein (Bcl-2, Bax, cleaved caspase 3 and caspase 3) levels in SiHa cells. (F) The expression level of SA-β-Gal was detected by SA-β-Gal staining. (G) The levels of senescence-associated secretory phenotype genes (IL-6, IL-1β, IL-8 and CCL20) were identified by RT-qPCR. Results are the mean ± SD. ***P<0.001. PLOD2, procollagen-lysine 2-oxoglutarate 5-dioxygenase 2; NC, negative control; SA-β-Gal, β-galactosidase.

but the levels of Bax and cleaved caspase 3 were augmented (Fig. 2E). Moreover, the knockdown of PLOD2 facilitated the levels of senescence-associated β-galactosidase (SA-β-Gal) and mRNA expressions of senescence-associated secretory phenotype (SASP) genes IL-6, IL-1β, IL-8 and CCL20 (Fig. 2F and G).

PLOD2 binds to YAP1. The possible mechanism of PLOD2 in CESC was then explored. Using the BioGrid website, PLOD2 was predicted to bind to YAP1 (Fig. 3A). The combination of PLOD2 and YAP1 was further verified by HDock software (Fig. 3B). RT-qPCR and western blotting results revealed that YAP1 expression was evidently increased in CESC cell lines when compared with the control End1/E6E7 cells (Fig. 3C and D). The data also revealed that PLOD2 silencing significantly reduced the protein level of YAP1 compared with the negative control group (Fig. 3E and F). The co-IP experiment verified that PLOD2 could combine with YAP1 (Fig. 3G).

PLOD2 regulates YAP1 to promote the proliferation and migration of SiHa cells. To explore the biological roles of YAP1 in SiHa cells, YAP1 was overexpressed and transfection efficiency was shown in Fig. 4A and B. CCK-8 assay indicated that YAP1 elevated the cell proliferation of PLOD2-modulated SiHa cells (Fig. 4C). The migration of SiHa cells was enhanced

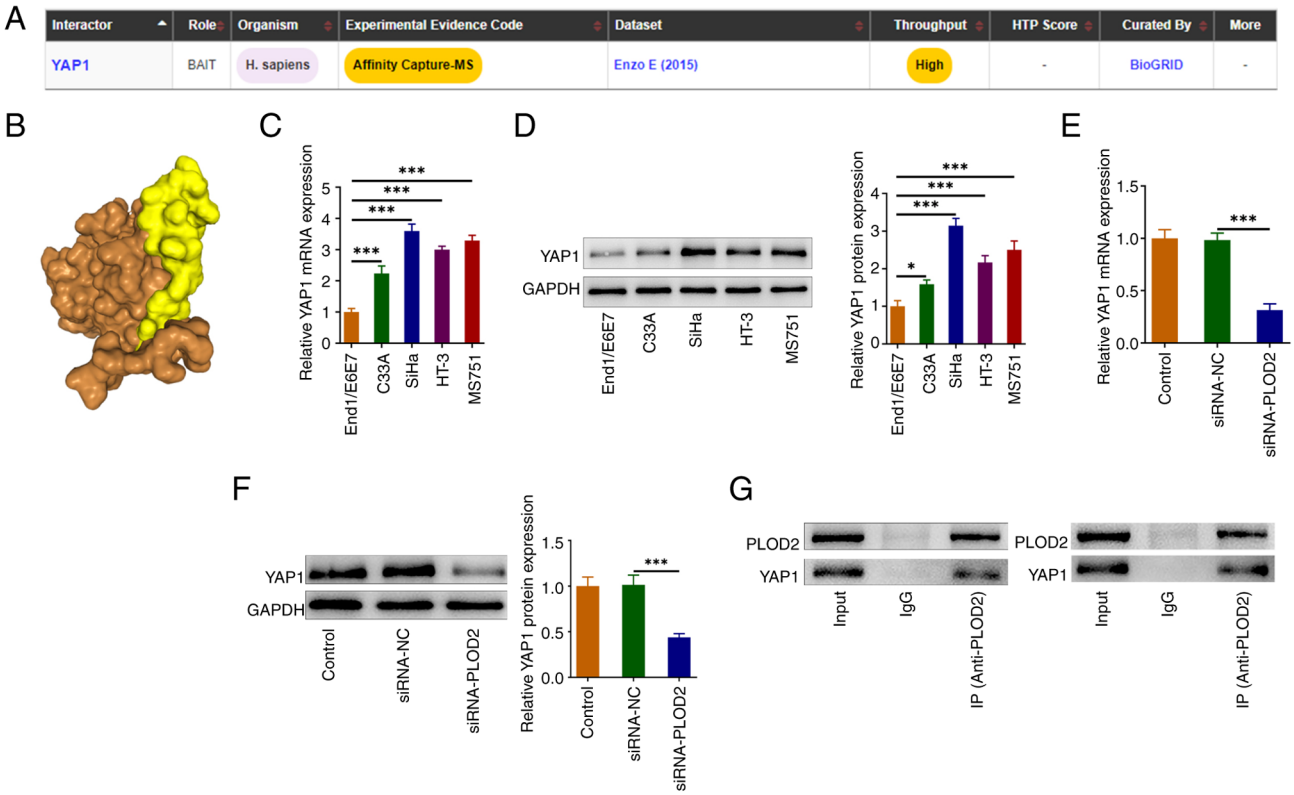

Figure 3. PLOD2 binds to YAP1. (A) PLOD2 was predicted to bind to YAP1 using BioGrid v4.4 website (https://thebiogrid.org). (B) The combination of PLOD2 and YAP1 was verified by HDock software (http://hdock.phys.hust.edu.cn/). (C) mRNA and (D) protein levels of YAP1 in CESC cell lines were detected by RT-qPCR and western blotting. (E) mRNA and (F) protein levels of YAP1 in SiHa cells after PLOD2 silencing were detected by RT-qPCR and western blotting. (G) co-IP experiment verified that PLOD2 could combine with YAP1. Results are the mean ± SD. *P<0.05 and ***P<0.001. PLOD2, procollagen-lysine 2-oxoglutarate 5-dioxygenase 2; CESC, cervical esophageal carcinoma; NC, negative control; YAP1, Yes-associated protein 1; IgG, immunoglobulin G; IP, immunoprecipitation,

after YAP1 overexpression (Fig. 4D and E). Moreover, the upregulation of YAP1 reversed the decreased levels of MMP2 and MMP9 in PLOD2-silenced SiHa cells (Fig. 4F).

PLOD2 silencing promotes apoptosis and senescence in SiHa cells by binding to YAP1 and regulates the p53 pathway. As shown in Fig. 5A, the rate of cell apoptosis in YAP1-overexpressed SiHa cells was significantly reduced compared with the PLOD2-silenced SiHa cells alone, which was consistent with the western blotting results where Bcl-2 levels were increased and the protein levels of cleaved caspase 3 and Bax were reduced (Fig. 5B). YAP1 overexpression in turn alleviates the level of SA-β-Gal and SASP genes (Fig. 5C and D). Furthermore, PLOD2 silencing increased the levels of p21 and p53 in SiHa cells, which was reversed by YAP1 overexpression (Fig. 5E and F).

Discussion

Present studies have illustrated that apoptosis and cellular senescence are considered to be the two primary mechanisms for preventing the development of cancer (35). Cellular senescence is currently defined as the cell cycle arrest in the G1 phase (36), and senescent cells are characterized by an increase in SA-β-gal, a flattened cellular morphology, and large and vacuolated cell size (37). Goodwin *et al* (38) demonstrated that E6 and E7 proteins, which are strongly expressed in cervical cancer cells, actively prevent cervical cancer cell senescence. Consequently, activation of the endogenous senescence pathway in cancer cells has been proposed as a therapy for cervical cancer, including CESC. In the present study, it was confirmed that suppression of PLOD2 resulted in repression of CESC cell proliferation, migration and exacerbation of apoptosis and senescence. It was discovered that PLOD2 binds to YAP1 and plays a modulatory role in SiHa cells, which may be related to p53 signaling.

PLOD2, a member of the PLOD family, is a critical enzyme in the process of forming collagen cross-links (39). PLOD2 fosters the aggressive progression of a number of tumors, including breast, hepatocellular and non-small-cell lung cancers (40). PLOD2 is positively associated with poor prognosis in cancers by acting on the morphologic changes in collagen fibers and facilitating the development of tumor metastatic 'highways' (41,42). The KEGG pathway and GO biological processes of PLOD2 as well as interacting genes revealed that PLOD2 is involved in protein digestion/absorption pathways and collagen fibre organization processes, which are closely associated with the ECM (22). This implies that PLOD2 expression may promote the migration and adhesion ability of cervical cancer cells by affecting the ECM. At present, the function and pathways involved in PLOD2 have only been assessed by bioinformatics methods and still need to be further validated by *in vitro* and *in vivo* experiments in future research (22). In the present study, PLOD2 expression was significantly higher in samples from patients with CESC compared with healthy

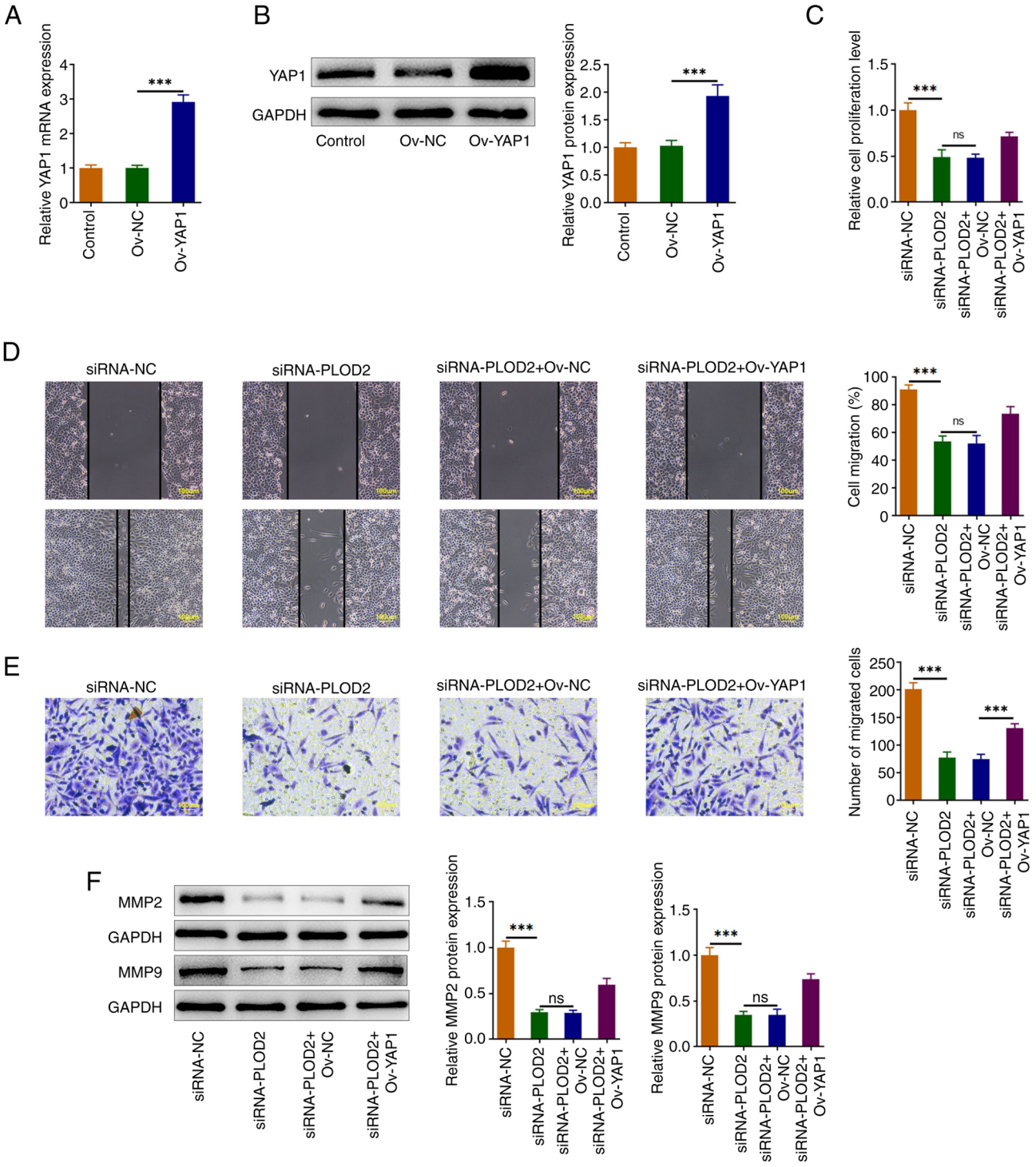

Figure 4. PLOD2 regulates YAP1 to promote proliferation, migration and invasion of SiHa cells. (A) mRNA and (B) protein levels of YAP1 in SiHa cells after YAP1 overexpression were detected by RT-qPCR and western blotting. (C) CCK-8 assay was used to measure the cell proliferation of SiHa cells. (D) Wound healing assay (at 0 h and the ending timepoint) and (E) Transwell assay (at the ending timepoint) were used to detect cell migration. (F) Western blotting was performed to assess the levels of invasion-associated proteins (MMP2 and MMP9). Results are the mean ± SD. ***P<0.001. PLOD2, procollagen-lysine 2-oxoglutarate 5-dioxygenase 2; YAP1, Yes-associated protein 1; Ov-NC, overexpression vector-negative control; Ov-YAP1, overexpression vector-YAP1; siRNA-NC, small interfering RNA-negative control; siRNA-PLOD2, small interfering RNA-PLOD2.

samples (logFC≥1 or logFC≤-1; P<0.05) as indicated by differential analysis of the CESC-related dataset GSE64217. The UALCAN database further confirmed that PLOD2 was significantly higher expressed in samples from patients with CESC compared with healthy samples, and the lower the expression, the better the prognosis. Additionally, when PLOD2 was knocked down, the migration of SiHa cells was diminished while the apoptosis and senescence were accelerated.

To explore the mechanism of PLOD2 in CESC, the molecules that PLOD2 may bind to were predicted using the BioGrid website. YAP1 was obtained and further simulations were performed to validate the binding of PLOD2 to YAP1 through H

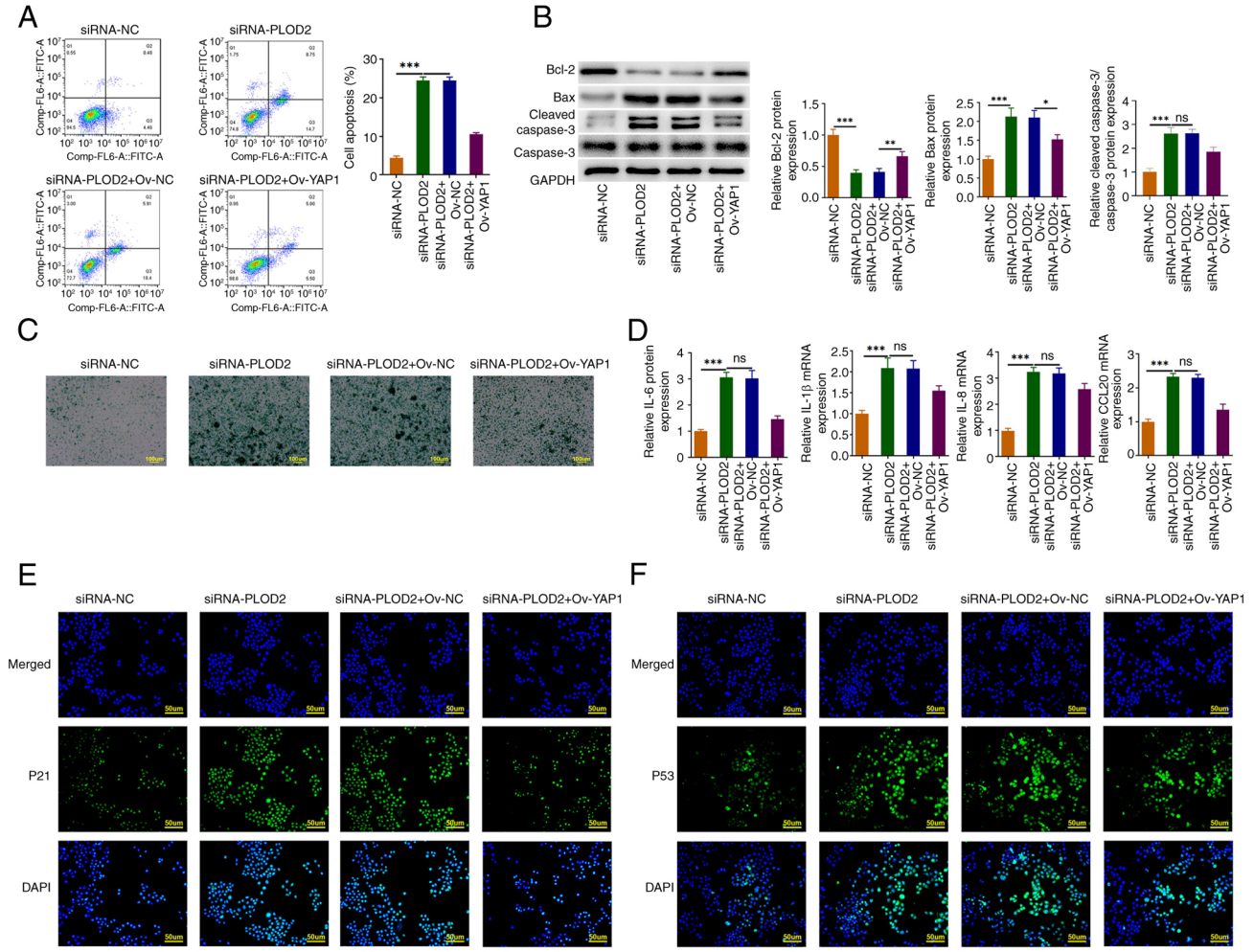

Figure 5. PLOD2 silencing promoted apoptosis and senescence in SiHa cells by binding to YAP1 and regulated the p53 pathway. (A) Cell apoptosis was examined by flow cytometric analysis. (B) Western blotting was used to assess apoptotic-related proteins (Bcl-2, Bax, cleaved caspase 3 and caspase 3) levels in SiHa cells. (C) The expression level of SA-β-Gal was detected by SA-β-Gal staining. (D) The levels of senescence-associated secretory phenotype genes (IL-6, IL-1β, IL-8 and CCL20) were identified by RT-qPCR. Immunofluorescence staining was carried out to test the levels of (E) p21 and (F) p53 in SiHa cells. Results are the mean ± SD. *P<0.05, **P<0.01, ***P<0.001. SA-β-Gal, β-galactosidase; PLOD2, procollagen-lysine 2-oxoglutarate 5-dioxygenase 2; YAP1, Yes-associated protein 1; siRNA-NC, small interfering RNA-negative control; siRNA-PLOD2, small interfering RNA-PLOD2.

Dock. YAP1, also termed YAP, is a member of the FOX family of transcription factors (43). The hyperactivation of YAP1 can drive the onset and progression of cervical cancer, including CESC (44). Activated YAP upregulates TGF-α, amphiregulin and EGFR, thus forming a positive signaling loop to drive cervical cancer cell proliferation (45). Liu *et al* (44) showed that YAP expression in the cytoplasm of samples from patients with CESC was significantly higher than that in normal cervical tissues. He *et al* (46) demonstrated that YAP1 hyperactivation in cervical epithelial cells increased HPV receptors, disrupted innate immunity of the host cells, and promoted HPV infection, which promotes cervical cancer development and progression. Deng *et al* (47) demonstrated that large tumor suppressor kinase 1 (LATS1) inhibited cervical cancer cell proliferation and invasion by regulating YAP1, and LATS1 overexpression decreased the protein level of YAP1 and increased YAP1 phosphorylation. In the present study, the binding of PLOD2 to YAP1 was confirmed by co-IP. After YAP1 was overexpressed, the migration, apoptosis and senescence of SiHa cells regulated by PLOD2 silencing were all reversed, indicating the role of YAP1 in PLOD2-silenced SiHa cells.

It was observed that cell senescence is partly induced by the activation of p53 (48). For example, DeFilippis *et al* (49) demonstrated that activation of p53 by inhibiting E6 protein expression triggered cervical cancer cell senescence and apoptosis, which in turn inhibited the pathological development of cervical cancer. In addition, activation of p53 can inhibit the pathological development of CESC, for example, up-regulation of p53 by LncRNA WT1-AS inhibits the proliferation of CESC cells (50). Thus, activation of p53 can mediate CESC cell senescence and inhibit the pathological development of CESC. In addition, CLP36 can promote the pathological development of p53-deficient tumors through upregulation of YAP1 (51). Xu *et al* (52) demonstrated that knockdown of YAP1 in glial cells significantly promotes premature senescence of glial cells, including reduced cell proliferation, morphological hypertrophy, increased SA-β-Gal activity and upregulation of several senescence-associated genes such as p16, p53 and NF-κB. It was also found that the levels of p53 and p21 were affected by PLOD2 silencing and YAP1 overexpression, which was in agreement with the aforementioned findings.

To conclude, the findings of the present study indicated that the expression of PLOD2 was elevated in CESC tissues and cell lines, and PLOD2 silencing caused the inhibition of CESC cell proliferation and migration, and promotion of apoptosis and senescence of CESC cells. PLOD2 was predicted to be bound to YAP1 and YAP1 overexpression reversed the effects of PLOD2 silencing on CESC cell proliferation, cell migration, apoptosis and senescence. In addition, PLOD2 facilitated CESC progression by regulating the p53 pathway through YAP1. These findings demonstrated the impacts of PLOD2 silencing on CESC cells and reported the role of the binding of PLOD2 and YAP1 in CESC cells, which suggests that PLOD2 could be a prospective therapeutic target for CESC.

However, there were some limitations to the present study. Firstly, generalizability and clinical translational potential of the present findings were not verified by animal models or clinical samples. In the current *in vitro* experiments, a single cervical cancer cell line was used, and the results of the aforementioned experiments require validation with multiple cervical cancer cell lines. Moreover, the overexpression or silencing of PLOD2 at different concentrations must be investigated to comprehensively evaluate the effects of PLOD2 on CESC cells at different expression levels for a more comprehensive understanding of the mechanism of action of PLOD2. Although the present study reveals the mechanism by which PLOD2 regulates the p53 signaling pathway through YAP1 to promote CESC progression, the specific molecular mechanisms, such as the mechanisms by which PLOD2 binds to YAP1, what their binding sites are and how YAP1 regulates p53, still need to be further investigated by bioinformatics and experimentally. Therefore, future endeavors will further investigate the aforementioned research elements.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

MY conceptualized the study and wrote the original draft of the manuscript; YW performed the investigation and the formal analysis; TQ was responsible for conceptualization, methodology, writing, review, editing and revising the manuscript critically for important intellectual content. All authors read and approved the final version of the manuscript. MY, YW and TQ confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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