Unbalanced YAP-autophagy Circuit Promotes the Malignant Progression of Pancreatic Cancer

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Abstract

Background: Pancreatic ductal adenocarcinoma (PDAC) is the most lethal malignancy in humans, and new therapeutic targets are urgently needed. Yes-associated protein (YAP) plays a significant role in tissue homeostasis and cancer progression. Autophagy is also closely associated with various human cancers. However, the interplay between YAP and autophagy in pancreatic cancer remains poorly understood.

Methods: The expression of YAP in PDAC was evaluated by immunohistochemistry (IHC). The effects of YAP on pancreatic cancer cells were evaluated by CCK-8, EdU, wound healing and Transwell invasion assays. Subsequent mechanistic studies were performed in PDAC cell lines by western blotting, qRT-PCR, chromatin immunoprecipitation (ChIP) assay, luciferase reporter assay and immunofluorescence assay. The consequence of the dual inhibition of YAP and autophagy on tumor growth was evaluated in AsPC-1 xenograft mice.

Results: YAP was upregulated and activated in PDAC tissues. Functional assays showed that YAP promoted PDAC cell proliferation, migration and invasion. Further analysis revealed a YAP-autophagy feedback loop in pancreatic cancer. Mechanistically, YAP activated autophagy by promoting Atg5 transcription via TEAD1-mediated binding, while autophagy negatively regulated YAP by controlling its degradation. The hyperactivation of YAP in PDAC unbalanced the YAP-autophagy circuit and promoted cancer progression. The dual inhibition of YAP and autophagy suppressed the malignant progression of pancreatic cancer.

Conclusions: Our study elucidates a novel mechanism involving a YAP-autophagy feedback loop and suggests that the YAP-autophagy circuit may represent a potential therapeutic target for PDAC.

Background

Pancreatic cancer, mostly pancreatic ductal adenocarcinoma (PDAC), is the most lethal malignancy in humans, with a 5-year survival rate of approximately 9% and a median survival of approximately 6 months [1–3]. The high mortality of pancreatic cancer is mainly due to the difficulty in early stage diagnosis, aggressive local invasion and easy metastasis [4]. Currently, gemcitabine, the first-line chemotherapeutic drug for pancreatic cancer, provides a limited survival advantage in treated patients and the therapeutic efficacy is unsatisfactory [5]. Activating mutations in KRAS are the most frequent genetic events and are present in the majority of pancreatic cancer patients [6]. Genetically engineered mouse models have also substantiated the critical role of KRAS mutations in pancreatic cancer initiation and progression [7–10]. Unfortunately, KRAS has been proven to be difficult to inhibit and the therapeutic strategy of directly blocking KRAS activity with small-molecule inhibitors has proven challenging [11]. Therefore, it is of great significance to elucidate the underlying mechanism of PDAC malignant progression and to develop new therapeutic strategies.
Studies have shown that Yes-associated protein (YAP), the major nuclear effector in the Hippo signaling pathway, enables the bypass of oncogenic KRAS addiction in pancreatic cancer [12]. In mammals, the Hippo pathway plays a critical role in organ size control, tissue homeostasis and stemness by controlling cell proliferation and death [13–16]. When the Hippo pathway is activated, MST1 and MST2 kinases phosphorylate and activate LATS1/2 kinases, which in turn phosphorylate YAP and TAZ, leading to their cytoplasmic retention or degradation [17]. When Hippo signaling is inhibited, hypophosphorylated YAP/TAZ is translocated into the nucleus where it mainly interacts with TEAD transcription factors acting as coactivators of the transcription of target genes [18, 19]. Accumulating evidence has suggested that dysregulation of the Hippo pathway plays a crucial role in cancer development [20]. YAP has been confirmed to be involved in a variety of human cancers [16], including breast cancer [21], lung cancer [22], ovarian cancer [23] and liver cancer [24]. In addition, YAP contributes to the proliferation and invasion of pancreatic cancer and inhibiting YAP expression suppresses pancreatic cancer progression [25]. Despite these research advances, the precise mechanisms underlying YAP dysregulation and the possibility of YAP-targeted therapy for pancreatic cancer remain to be explored.

Macroautophagy (hereafter autophagy) is an evolutionarily conserved lysosome-dependent cellular catabolic degradation pathway [26]. Autophagy has a dual role in cancer. During tumor initiation, autophagy removes damaged organelles and reactive oxygen species to maintain genomic stability, thereby inhibiting malignant transformation. However, during tumor development and metastasis, autophagy may contribute to cell survival under low-nutrient conditions and other forms of cellular stress [27]. Recent studies have indicated that the Hippo pathway is involved in the regulation of autophagy. Maejima et al. showed that MST1 inhibits autophagy by promoting the interaction between Beclin1 and Bcl2, suggesting a role for the Hippo pathway in integrating autophagy and apoptosis during cellular stress [28]. Another study found that the Hippo kinases STK3/STK4 promote autophagy via direct phosphorylation of LC3 [29]. Moreover, a recent study showed that YAP is an autophagy substrate and YAP dysregulation is associated with hepatocarcinogenesis in autophagy-deficient livers [30]. Collectively, these studies suggest that YAP and autophagy may be mutually regulated, but how they are involved in pancreatic cancer progression remains unclear.

In the present study, we found that YAP was overexpressed and activated in PDAC, and was critical for promoting cancer progression. More importantly, YAP activated autophagy by promoting Atg5 transcription, while autophagy negatively regulated YAP through autophagic degradation. Aberrant hyperactivation of YAP in PDAC led to an imbalanced YAP-autophagy circuit. The dual inhibition of YAP and autophagy suppressed the malignant progression of pancreatic cancer, suggesting that the YAP-autophagy circuit is a potential therapeutic target.

Materials And Methods

Cell lines, culture conditions and reagents
Pancreatic cancer cell lines (AsPC-1, BxPC-3, PANC-1, CFPAC-1 and SW1990) were obtained from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China) and maintained in Dulbecco's modified Eagle media (DMEM) supplemented with 10% fetal bovine serum (Gibco, NY, USA). The cells were incubated at 37°C in a humidified atmosphere containing 5% CO₂. Antibodies against YAP (#14074), ATG5 (#2630) were purchased from Cell Signaling Technology. Anti-active YAP (ab205270) antibody was from Abcam. Antibodies against p62 (sc-28359) and GAPDH (sc-32233) were purchased from Santa Cruz Biotechnology. Anti-LC3 (NB100–2220) antibody was obtained from NOVUS. Anti-Flag antibody (F3165), cycloheximide (CHX, 01810), verteporfin (SML0534) and chloroquine (CQ, C6628) were obtained from Sigma-Aldrich.

RNA isolation and qRT-PCR

Total RNA was isolated using Total RNA Kit I (OMEGA) according to the manufacturer's instruction. For qRT-PCR, RNA was reverse transcribed to cDNA by using a PrimeScript™ RT reagent kit (Takara, Dalian, China). The cDNA was amplified on a 7500 Real-Time PCR System (Applied Biosystems) using SYBR Green Master Mix (Roche). All samples were normalized to GAPDH. The primer sequences were listed in supplementary Table 1.

Cell proliferation assays

Cell viability was detected by CCK8 and EdU assays. Cells were seeded in 96-well plates (4,000 cells/well) and incubated overnight for attachment, and were then treated with indicated agents for different times. The medium was replaced with CCK8 at 37°C for 2 hours and absorbance at 450 nm was measured in a microplate reader (BIO-RAD xMark). EdU assays were performed using the EdU Cell Proliferation Assay Kit (Ruibo, China) according to the manufacturer's instructions. All experiments were performed in triplicate.

Wound-healing assay

AsPC-1 and BxPC-3 cells were seeded in 12-well plates and grown to 90% confluence. Then, scratch wounds were generated by using a plastic pipette tip, which was recorded as 0 h. Then, the scratch was imaged at 24 h or 48 h. Cell migration was assessed by measuring the movement of cells into the scratch wounds.

Transwell invasion assay

Matrigel-coated invasion assay was performed using a 24-well Transwell chamber system (Corning, USA) according to our previous work [31]. Briefly, 5×10⁴ cells in 400 μL serum-free culture medium were placed into the upper chamber, which was coated with Matrigel (BD, New Jersey, USA). A total of 600 μL medium supplemented with 20% FBS was added into the lower chamber. After incubation for 24 h, cells were fixed in 4% paraformaldehyde and stained with 0.1% crystal violet (Sigma-Aldrich, USA) for 30 min. The stained cells were analyzed.

ChIP analysis
ChIP assays were carried out using the EZ-ChIP kit (Millipore) according to the manufacturer's instructions, using the antibody against YAP. Briefly, cells were lysed and then sonicated to obtain DNA fragments (500-800 bp). Next, samples were immunoprecipitated overnight at 4°C with YAP antibody, supplemented with magna ChIP™ protein A/G beads. After washing, elution and de-cross-linking, the amount of immunoprecipitated DNA was analyzed by qRT-PCR using the indicated primers listed in supplementary Table 2.

**Luciferase reporter assay**

293T cells were seeded in 24-well plates and transfected with the pGL3-Atg5 (wild type or mutated TEAD1-binding site) promoter reporter plasmid, pcDNA3.1/YAP or an empty vector, and a Renilla luciferase vector for normalization. Relative luciferase activity was measured with the Dual-luciferase Reporter Assay System (Promega).

**Plasmid constructs and transfection**

Plasmid encoding the human YAP was cloned into pcDNA3.1 vector with the Flag-tag. For transient transfection, plasmids were pretransfected with lipofetamine 2000 (Invitrogen) for 24 hours and then processed with the indicated treatment as described. siRNAs against human YAP, Atg5 and scramble control RNA oligos were produced by GenePharma (Suzhou, China) and transfected using Lipofectamine RNAiMAX Transfection Reagent (Invitrogen). For stable YAP knockdown, the following Addgene plasmids were used, pLKO1-shYAP#1 (27368) and pLKO1-shYAP#2 (27369). Stable cell lines with YAP knockdown established as previously described [31].

**Tissue microarray slides and immunohistochemistry (IHC)**

In vivo active-YAP expression was detected by IHC using tissue microarrays (PA2081a, AlenaBio, Xi’an, China). The tissues were incubated with primary anti-active-YAP antibody (1:100, ab205270, Abcam) and biotin conjugated secondary antibody. Hematoxylin was used as the counterstain. Immunostaining degree of each sample was evaluated independently by two pathologists based on nuclear staining intensity (0, negative; 1, weak; 2, moderate; 3, strong) and percentage of positive cells (0, <5% positive cancer cells; 1, 6-25% positive cancer cells; 2, 26-50% positive cancer cells; 3, 51-75% positive cancer cells; 4, ≥76% positive cancer cells). The final immunoreactivity score is the product of the intensity score and the extent score.

**Cycloheximide (CHX) chase assay**

AsPC-1 cells were incubated at 90% confluency in complete growth media containing the protein synthesis inhibitor CHX (50 μM) or vehicle. Cells were collected in RIPA buffer containing proteinase inhibitors at 0, 4, 8, 16 and 24 h after incubation. The lysate was sonicated, centrifuged for 10 min at 12,000 × g and the resulting supernatants analyzed by immunoblotting.

**Immunofluorescence analysis**
For immunofluorescence analysis, cells were plated in chamber slides then fixed in methanol for 10 min at room temperature, permeabilized with 5% bovine serum albumin (BSA) in PBST. Cells were then exposed to primary antibodies (anti-YAP 1:200) diluted in PBST containing 5% BSA overnight at 4°C. After washing three times with PBS, secondary antibody (Alexa Fluor 488 goat anti-rabbit 1:200) diluted in PBST was added and incubated for 1h at room temperature. Cells were then washed in PBS and mounted using 4,6-diamidino-2-phenylindole (DAPI) to counterstain DNA. Images were collected using a confocal microscope (Olympus FV-1000).

**Autophagy analysis**

Autophagy was measured by quantitation of GFP-LC3 puncta using fluorescence microscopy according to our previous work [26]. Cells were infected with appropriate amounts of lentivirus carrying GFP-LC3 to express the close-to-endogenous level of GFP-LC3. After treatment, cells were fixed with 4% paraformaldehyde for 20 min and rinsed with PBS twice. Total number of cells on images was determined by nuclei staining with 4,6-diamidino-2-phenylindole. Cells were mounted and visualized under a confocal microscope (Olympus FV-1000).

**Xenograft tumor-formation assay and therapeutic treatment**

Female BALB/C nude mice at 4-5 weeks of age were obtained from Beijing Vital River Laboratory Animal Technology Co.,China. 5×10^6 AsPC1 cells were subcutaneously inoculated into the right flank of mice to establish pancreatic cancer xenografts. Approximately 8 days after subcutaneous implantation, the mice were randomly divided into four groups and delivered verteporfin (50 mg/kg; intraperitoneally), CQ (60 mg/kg; intraperitoneally), verteporfin plus CQ, or PBS as a control every 2 days. During the treatment, tumour volume was measured every 4 days and calculated using the formula: length × width^2 × π/6. Mice with tumor implants were euthanized 32 days after drug treatment, and the tumor xenografts were excised and weighed.

**Statistical analysis**

GraphPad Prism software (Version 5.0) was used for experimental data analysis. All experiments were independently repeated at least three times with triplicate samples. The Student's t test was used to detect significance between groups and statistical significance was determined when p < 0.05 (two-tailed). Values are expressed as the mean ± SEM.

**Results**

**YAP is upregulated and hyperactivated in PDAC**

YAP has been reported to be overexpressed in pancreatic cancer [25, 32]. To validate the mRNA expression of YAP in PDAC, we analyzed the RNA-sequencing data from the TCGA and GTEx databases using the web-based tool GEPIA (Gene Expression Profiling Interactive Analysis, http://gepia.cancer-
The analysis showed that YAP mRNA levels were significantly upregulated in PDAC tissues compared with normal pancreatic tissues (Fig. 1a). Moreover, patients with high YAP expression had significantly worse overall survival than those with low YAP expression (Fig. 1b). Using qRT-PCR, we evaluated the expression of YAP in tumor and corresponding peritumor tissues from 20 PDAC patients. Our results showed a significant upregulation of YAP in PDAC tissues compared with peritumoral tissues (Fig. 1c). Consistently, increased YAP expression was also detected in five PDAC cell lines (AsPC-1, PANC-1, BxPC-3, CFPAC-1 and SW1990) compared with that in the immortalized human normal pancreatic duct epithelial cell line HPDE6-C7 (Fig. 1d-e). In total YAP protein, only dephosphorylated YAP can enter the cell nucleus to activate gene transcription, thus playing an oncogenic role. To accurately evaluate the level of activated YAP in PDAC tissues, we used an antibody specifically recognizing the active (unphosphorylated) form of YAP. We then carried out immunohistochemical (IHC) staining for activated YAP in a human pancreatic cancer tissue microarray containing 40 PDAC and 20 normal pancreatic tissues (Fig. 1f). The results showed that the active YAP expression level was significantly increased in PDAC tissues, compared to normal tissues (Fig. 1g). Moreover, we found that active YAP expression in PDAC correlated with histological grade, and the active YAP score in grade 1, grade 2 and grade 3 PDAC was gradually increased (Fig. 1h). These data suggested the overexpression and hyperactivation of YAP in PDAC.

**YAP promotes PDAC cell proliferation, migration and invasion**

To further analyze the potential role of YAP as an oncogene in PDAC, YAP expression was knocked down in AsPC-1 and BxPC-3 cells, which have relatively high endogenous YAP expression. Then, shRNA-resistant YAP S127A or YAP S127D, representing the active and inactive states of YAP respectively, was transfected into YAP-knockdown cells (Fig. 2a-b). YAP knockdown significantly suppressed the proliferation of the PDAC cells, as determined by CCK-8 and EdU cell proliferation assays. Moreover, YAP knockdown-induced suppression of cell proliferation was rescued by YAP S127A but not by YAP S127D (Fig. 2c-f). Wound healing (Fig. 2g-h) and Transwell invasion assays (Fig. 2i-j) indicated that YAP knockdown attenuated the migration and invasion of PDAC cells, whereas YAP S127A, but not YAP S127D, reversed the effects of YAP knockdown in the assays. These data showed that activated YAP drives PDAC cell proliferation, migration and invasion.

**YAP activates autophagy by promoting Atg5 transcription**

It has been reported that the Hippo pathway is involved in autophagy regulation [28, 29]. We wondered whether YAP plays a role in regulating autophagy in PDAC. As shown in Fig. 3a, GFP-LC3, a highly specific fluorescent marker of autophagosomes, was significantly increased as puncta in YAP overexpressing AsPC-1 cells. To investigate whether the increased GFP-LC3 puncta observed upon YAP overexpression reflect increased autophagic flux or blocked autophagosome turnover, the effects of YAP were analyzed in the presence of chloroquine (CQ), an inhibitor of autophagosome degradation. The conversion of the soluble form of LC3 (LC3I) to the lipiddated form (LC3II) is a sign of autophagy activation, and p62 is recognized as a substrate of autophagic degradation. We found that YAP
overexpression plus CQ treatment had a synergistic effect in inducing the accumulation of LC3II, and CQ blocked YAP-induced p62 degradation (Fig. 3b). These results suggest that YAP overexpression leads to an increase in autophagic flux. To complement these conclusions, we applied Earle’s balanced salt solution (EBSS) to induce nutrient starvation and trigger autophagosome formation, which is the initial stage of autophagy. Indeed, YAP knockdown inhibited autophagy initiation. Even under nutrient starvation conditions, YAP depletion significantly inhibited autophagy activation (Fig. 3c). While our findings indicated that YAP activates autophagy by targeting autophagosome formation, YAP has been reported to interfere with autophagic flux by enhancing autolysosome degradation in breast cancer cells [34], suggesting diverse, context-specific regulatory roles of YAP in autophagy.

Since YAP exerts its transcriptional coactivator function predominantly via interaction with the transcription factor TEAD1 [35], we used the JASPAR database [36] to analyze the promoter regions of several crucial autophagy-related genes, such as ULK1, Beclin1, Atg5 and Atg7. Notably, multiple TEAD1-binding sites were identified in the promoter region of Atg5 (Supplementary Table 3). The binding site with the highest prediction score in the promoter region of Atg5 was pursued as a candidate for detailed study (Fig. 3d). To investigate whether Atg5 is a direct target gene of YAP-TEAD1, we performed a chromatin immunoprecipitation (ChIP) assay, and CTGF was used as a positive control. YAP was recruited to the promoters of Atg5 and CTGF, but not to the negative control GAPDH gene (Fig. 3e). The competency of TEAD1 binding was further examined using a luciferase reporter assay. Verteporfin, an antagonist of the YAP-TEAD association, was applied to inhibit YAP transcriptional activity [37]. YAP overexpression and verteporfin treatment enhanced and reduced Atg5 promoter activity, respectively, whereas mutation of the TEAD1-binding site abrogated the effects of YAP overexpression and verteporfin treatment (Fig. 3f-g). In addition, we analyzed the correlation of YAP and Atg5 expression in the TCGA PAAD (pancreatic adenocarcinoma) database and found a positive correlation between YAP and Atg5 mRNA expression levels (R = 0.63, p < 0.001) (Fig. 3h). As expected, ATG5 protein expression was increased in YAP overexpressing PDAC cells and decreased in verteporfin-treated PDAC cells (Fig. 3i-j). In addition, we validated the effect of Atg5 on autophagy induction by YAP and found that Atg5 knockdown inhibit YAP-induced autophagy (Fig. 3k). Collectively, these findings strongly suggest that YAP promotes Atg5 transcription through the TEAD1-binding site.

**Autophagy plays a role in the malignant progression of PDAC**

Since autophagy plays a dual role in cancer, we further investigated the effect of autophagy on YAP-promoted malignant progression of PDAC cells. We first examined the transfection efficiency of shAtg5 and Flag-YAP in AsPC-1 and BxPC-3 cells (Fig. 4a-b). As expected, Atg5 depletion robustly attenuated YAP-enhanced cell proliferation, as determined by CCK-8 and EdU cell proliferation assays (Fig. 4c-f). Wound healing (Fig. 4g-h) and Transwell invasion assays (Fig. 4i-j) indicated that Atg5 knockdown significantly attenuated the promoting effect of YAP overexpression on the migration and invasion of PDAC cells. Although YAP may affect cancer progression through multiple downstream pathways, these data suggest that autophagy plays an important role in YAP-promoted malignant progression of PDAC.
Autophagy negatively regulates YAP through autophagic degradation

Generally, when the Hippo pathway is activated, YAP is phosphorylated and degraded through the βTrCP-mediated proteasomal pathway. However, a novel mechanism of YAP degradation has been recently identified. The study found that YAP is an autophagy substrate, and is an essential downstream mediator of hepatic differentiation and carcinogenesis in autophagy-deficient livers [30]. Therefore, we investigated whether autophagy has an effect on the degradation of YAP in PDAC cells. Atg5 knockdown increased YAP accumulation in AsPC-1 cells (Fig. 5a). To confirm that YAP colocalized with autophagosomes, AsPC-1 cells were transfected with GFP-LC3. EBSS induced the colocalization of YAP and GFP-LC3, indicating that YAP may be degraded in autophagosomes (Fig. 5b). To investigate whether ubiquitin is involved in autophagy-mediated YAP degradation, we performed a co-IP assay and found no significant change in the ubiquitination level of YAP after Atg5 knockdown (Fig. 5c). This result suggests that autophagy-mediated YAP degradation is ubiquitin-independent. Moreover, according to cycloheximide (CHX) chase assay, the YAP half-life was increased in Atg5-knockdown cells (Fig. 5d-e). Similarly, the YAP half-life was also increased in CQ-treated cells (Fig. 5f-g). In contrast to the effect of CQ, 3-Methyladenine (3-MA) inhibits autophagy by blocking autophagosome formation via the inhibition of class III PI3K. As expected, LC3 II levels were decreased by treatment with 3-MA, whereas CQ increased the accumulation of LC3 II (Fig. 5h). However, both 3-MA and CQ, inhibiting autophagosome formation and inhibiting autophagosome degradation respectively, led to increased YAP protein levels (Fig. 5h). Moreover, both CQ and 3-MA significantly enhanced CTGF and Atg5 transcription compared to the control (Fig. 5i).
Collectively, these results suggest that YAP is degraded by autophagy in PDAC cells and that autophagy plays a role in the negative feedback regulation of YAP.

Dual inhibition of YAP and autophagy suppressed the malignant progression of PDAC

We further examined the effects of the dual inhibition of YAP and autophagy in pancreatic cancer. As expected, dual inhibition of YAP and autophagy by verteporfin and CQ significantly inhibited the proliferation of AsPC-1 and BxPC-3 cells compared with either the verteporfin or CQ treatment alone (Fig. 6a-d). Similar effects on cell migration and invasion were also observed in PDAC cells (Fig. 6e-h). We also analyzed the combinational antitumor effect in vivo, by establishing a subcutaneous pancreatic cancer xenograft model in nude mice injected with AsPC-1 cells. We found that the combination of verteporfin and CQ treatment profoundly inhibited tumor growth in mice (Fig. 7a-c). These results imply that inhibiting YAP and autophagy may be a potential therapeutic strategy for pancreatic cancer.

Discussion

Cell-intrinsic negative feedback loops are important to ensure proper physiological regulation and homeostasis of the cells. The findings presented here support the existence of a feedback loop consisting of YAP and autophagy in the regulation of PDAC progression. YAP activated autophagy via TEAD1-mediated transcription of Atg5. Moreover, autophagy negatively regulated YAP through autophagic degradation. The complex regulatory circuit of YAP and autophagy provides feedback regulation of YAP
and thus ensures tissue homeostasis. However, aberrant hyperactivation of YAP disrupts this feedback loop and promotes the malignant progression of PDAC. The dual inhibition of YAP and autophagy suppressed PDAC progression (Fig. 7d).

YAP is essential for cancer initiation and progression but dispensable for normal homeostasis in adult organs, making it an attractive target for cancer therapy [16]. In our study, YAP was upregulated in PDAC and associated with poor prognosis. Hyperactivation of YAP promoted PDAC cell proliferation, migration and invasion. These findings suggest that YAP may serve as an oncogene in PDAC. Since YAP association with TEADs is essential for YAP transcriptional effects in most cellular contexts [38], blocking the YAP-TEAD interaction represents one of the most promising strategies for realizing anti-YAP therapy. Verteporfin can disrupt the interaction between YAP and TEAD, thus abrogating YAP-induced transcription [37]. As a clinical photosensitizer for the treatment of macular degeneration, verteporfin has also been shown to be safe in clinical trials [39].

Autophagy is a mechanism by which cellular material is delivered to lysosomes for degradation, leading to the basal turnover of cell components and providing energy and macromolecular precursors. Autophagy has opposing, context-dependent roles in cancer. Although sometimes controversial, targeting autophagy has been proposed as a potential therapeutic strategy for cancer [40]. Chloroquine (CQ) is an anti-malaria drug that inhibits autophagy through the inhibition of autophagosome-lysosome fusion [41]. The antitumor effect of CQ has been reported in different types of cancer [42, 43].

In our study, inhibition of YAP by verteporfin or inhibition of autophagy suppressed the malignant phenotype of PDAC to some extent both in vitro and in vivo. However, the combination of verteporfin and CQ was more robust in suppressing PDAC progression. This result suggests that targeting the YAP-autophagy circuit might yield better outcomes.

**Conclusions**

In summary, our findings reveal the existence of a YAP-autophagy feedback loop in regulating PDAC progression. In normal pancreas development and homeostasis, YAP transcriptionally activate Atg5 via interaction with TEAD1, which in turn initiates a negative feedback regulation through autophagic degradation of YAP. However, in PDAC, aberrant hyperactivation of YAP disrupts homeostasis, inducing YAP-autophagy circuit imbalance and subsequent cancer malignant progression. Moreover, our study indicates that targeting the YAP-autophagy signaling circuit represents a novel therapeutic strategy for pancreatic cancer.

**Abbreviations**

ChIP: chromatin immunoprecipitation; CQ: chloroquine; EGFR: epidermal growth factor receptor; EMT: epithelial-to-mesenchymal transition; IHC: immunohistochemical; PDAC: pancreatic
ductal adenocarcinoma; qRT-PCR: Quantitative real-time polymerase chain reaction; YAP: Yes-associated protein; 3-MA: 3-Methyladenine.

**Declarations**

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Not applicable.

**Authors’ contributions**

TS conceived and designed the study. WM and HP performed most of the experiments. JM and LJ critically revised the manuscript for important intellectual content. LM and HL provided technical and material support. TS drafted the manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

**Ethics approval and consent to participate**

The Ethics Committee of the The First Affiliated Hospital of Zhengzhou University provided ethical approval. Xenograft experiments in nude nice were approved by the Animal Experimentation Ethics Committee of Zhengzhou University.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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