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ORIGINAL RESEARCH

POU2F2 aggravates cell proliferation and autophagy in lung adenocarcinoma

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Abstract

Lung adenocarcinoma (LUAD) is one deadly tumor with high incidence. POU class 2 homeobox 2 (*POU2F2*) has been notarized to exhibit roles in the regulation of various cancers by playing as a facilitator. Our previous study demonstrated that *POU2F2* is highly expressed and accelerates cell proliferation and motility in lung cancer. However, the additional regulatory functions of *POU2F2* in LUAD progression require further investigations. In this study, we first confirmed through the UALCAN (A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses) online database that *POU2F2* expression is elevated in LUAD tissues. Furthermore, we found that *POU2F2* expression is up-regulated in LUAD cell lines. Functional experiments revealed that *POU2F2* accelerates LUAD cell proliferation and enhances autophagy through increasing the LC3-II/LC3-I level and fluorescence intensity. Additionally, we discovered that *POU2F2* activates the phosphoinositide dependent protein kinase 1 (PDPK1)-mediated phosphatidylinositol-3 kinase (PI3K)/protein kinase B (AKT) pathway. In conclusion, our discovery firstly disclose that *POU2F2* exacerbates cell proliferation and autophagy in LUAD by modulating the PI3K/AKT pathway. This work suggests that *POU2F2* may be a hopeful bio-marker for treating LUAD.

Keywords

POU2F2; Lung adenocarcinoma; Autophagy; PI3K/AKT pathway

1. Introduction

Lung cancer is one major reason for cancer-associated death worldwide, ranks for about 28% of all cancers cases [1]. The rapidly increased incidence and mortality of lung cancer have been discovered in many countries [2, 3]. Lung adenocarcinoma (LUAD) is one kind of usual types in lung cancer, occupying 30–35% of primary lung cancers [4]. It is pressed for illustrating the key molecular mechanisms in LUAD to seek potential targets for early diagnosis and treatment of LUAD.

POU class 2 homeobox 2 (*POU2F2*, or *OCT2*) is a B cell-restricted transcription factor belonging to the POU domain family, which binds DNA through its POU domain [5, 6]. *POU2F2* has been shown to bind to the octamer DNA motif (5′-ATGCAAAT-3′) [7]. Importantly, the pivotal roles of *POU2F2* have been extensively studied in the progression of multiple cancers. For example, *POU2F2* activates the PDPK1-dependent PI3K/AKT/mammalian target of rapamycin (mTOR) pathway to regulate glycolytic reprogramming in glioblastoma [8]. Additionally, *POU2F2* is induced by nuclear factor-kappa B (NF-κB) activation to modulate roundabout guidance receptor 1 (ROBO1) transcription, promoting metastasis in gastric cancer [9]. The chromobox protein homologue 7 (CBX7)/*POU2F2*/Programmed Death Ligand 1 (PD-L1) axis modulates the immune response to ameliorate

bladder cancer [10]. Moreover, in breast cancer, *POU2F2*-mediated lncRNA Protein tyrosine phosphatase receptor type G-antisense RNA 1 (PTPRG-AS1) affects miR-376c-3p to target solute carrier family 7 member 11 (SLC7A11), thereby suppressing ferroptosis [11]. Interestingly, our previous research has uncovered that *POU2F2* exhibited highly expression in lung cancer, and its increased expression activates Argonaute 1 (AGO1) to enhance cell proliferation and motility [12].

Autophagy is one cellular catabolic process that provides some macromolecular precursors and energy [13, 14]. Autophagy has been found to support the growth and survival of various cancers by regulating tumor metabolism and eliminating damaged organelles [15, 16]. However, the regulatory effects of *POU2F2* on autophagy in LUAD progression remain unclear and warrant further explorations.

In conclusion, results reveal that accelerates cell proliferation and autophagy in LUAD by modulating the PI3K/AKT pathway. This discovery may offer novel opinions for the cure of LUAD, leading to the identification of potential therapeutic targets to inhibit the progression of the disease.

2. Materials and methods

2.1 Tissue samples

Twenty pairs of tumor tissues and adjacent normal tissues were gained from patients with LUAD at the First Affiliated Hospital, Fujian Medical University.

2.2 Immunohistochemistry (IHC) assay

The tumor tissues were cut into 4 μm paraffin-embedded sections, and then sections were subjected to dewaxing and re-hydration. After blocking, sections were co-cultured with POU2F2 antibody (2 μg/mL; ab243153; Abcam, Shanghai, China) (4 °C, overnight). Next, secondary antibody (1:1000, ab7090, Abcam, Shanghai, China) was mixed. The staining with diaminobenzidine and counterstaining with hematoxylin were made for sections. Images were gained from a microscope (E200, Nikon, Tokyo, Japan).

2.3 Cell line and cell culture

The LUAD cell lines (H1299, PC-9, A549, SPC-A1) and normal lung epithelial cell line (BEAS-2B) were bought from American Tissue Culture Collection (ATCC, USA). In a humidified incubator (37 °C, 5% CO_2), RPMI-1640 medium (31800022, Gibco Company, Grand Island, NY, USA) was employed for cultivation.

2.4 Cell transfection

The pcDNA3.1 vectors targeting *POU2F2* (POU2F2) with negative control (Control) and short-hairpin RNA (sh-RNA) targeting *POU2F2* (shPOU2F2) with negative control (shNC) were acquired from GenePharma (Shanghai, China). The transfection for these constructed plasmids into LUAD cells was done by Lipofectamine 2000 (11668019, Invitrogen, Carlsbad, CA, USA).

2.5 Western blot

The extraction of proteins from LUAD cells was made using ice-cold radio immunoprecipitation assay (P0013B, Beyotime, Shanghai, China). The separation of proteins was executed by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), next moving proteins to polyvinylidene fluoride (PVDF) membranes (Beyotime, Shanghai, China). After blocking, primary antibodies were put to the membranes, next adding secondary antibodies (1:2000; ab7090, Abcam, Shanghai, China). The protein bands were visualized using a chemiluminescence detection kit (89880, Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Primary antibodies used were POU2F2 (1:5000; ab179808; Abcam, Shanghai, China), LC3-I (1 μg/mL; ab48394), LC3-II (1 μg/mL; ab48394), Beclin-1 (1:2000; ab207612), P62 (1:1000; ab207305), PDPK1 (1:1000; ab52893), p-PI3K (1:500; ab182651), PI3K (1:1000; ab86714), p-AKT (1:1000; ab8933), AKT (1:500; ab8805) and β-actin (1 μg/mL; ab8226).

2.6 Cell counting kit-8 (CCK-8) assay

In one 96-well plate, LUAD cells (1000 cells/well) were plated. CCK-8 solution (CK04, 10 μL, Dojindo Laboratories, Kumamoto, Japan) filled into each well was made. After 2 hours, cell viability was measured using a spectrophotometer (ND-ONE-W, Thermo Fisher Scientific, Waltham, MA, USA).

2.7 5-ethynyl-2′-deoxyuridine (EDU) assay

The Click-iT®EDU Imaging Kit (C10339, Invitrogen, Carlsbad, CA, USA) was used. The EdU (50 μM) was treated to LUAD cells for 2 hours. After being fixed (4% paraformaldehyde) and permeabilized, cells were treated to dyeing. 4′,6-diamidi-diamidino-2-phenylindole (DAPI) was utilized for nucleus visualization. EDU-positive cells were examined using fluorescence microscopy (DM2000, Leica, Hilden, Germany).

2.8 Immunofluorescence (IF) assay

In the 24 well plates, LUAD cells (1×10^5) were subjected to fixation (4% paraformaldehyde), blocking (5% bovine serum albumin), and adding primary antibody against LC3 (ab192890, Abcam, Shanghai, China). The secondary antibody (RGAR002, Proteintech Group, Inc., Wuhan, China) was applied. DAPI was for nucleus staining, and images were gained from the Olympus BX53 fluorescence microscope (BX53, Olympus Optical Co. Ltd., Tokyo, Japan).

2.9 Statistical analysis

All data were analyzed by GraphPad Prism Software 9 (GraphPad Software, La Jolla, CA, USA) and exhibited as mean $\pm$ standard deviation (SD). Three times were done in each experiment. Student's *t*-test for two groups or one-way one-way analysis of variance (ANOVA) for multiple groups was utilized for statistical comparisons. The $p < 0.05$ was judged as statistically significant.

3. Results

3.1 *POU2F2* owned higher expression in LUAD

Using the UALCAN online database, *POU2F2* showed higher expression in LUAD tissues ($p < 0.01$) (Fig. 1A). IHC assay also revealed enhanced POU2F2 protein expression in LUAD tissues (Fig. 1B). Moreover, POU2F2 protein expression was indicated to be elevated in LUAD cell lines (Fig. 1C). H1299 and PC-9 cell lines were elected for next experiments, confirming the increased expression of *POU2F2* in LUAD.

3.2 *POU2F2* accelerated cell proliferation

The impacts of *POU2F2* in modulating cell proliferation in LUAD progression was investigated. Transfection of pcDNA3.1/*POU2F2* (or shPOU2F2) plasmids confirmed the overexpression (or knockdown) of *POU2F2* (Fig. 2A). Cell viability was enhanced with *POU2F2* overexpression and reduced with *POU2F2* inhibition (Fig. 2B). Furthermore, EDU assay demonstrated increased cell proliferation with *POU2F2* overexpression and decreased proliferation with *POU2F2* suppression (Fig. 2C). These data revealed that *POU2F2* accelerated cell proliferation.

3.3 *POU2F2* heightened autophagy in LUAD

Further experiments were executed to ascertain the regulatory effects of *POU2F2* on autophagy in LUAD progression. Overexpression of *POU2F2* led to the elevated levels of LC3-II/LC3-I and Beclin-1, and the declined levels of P62 protein, while *POU2F2* knockdown had the opposite effect (Fig. 3A). The fluorescence intensity of LC3 was also increased with *POU2F2* overexpression and decreased with *POU2F2* inhibition (Fig. 3B). Overall, *POU2F2* was found to enhance autophagy in LUAD.
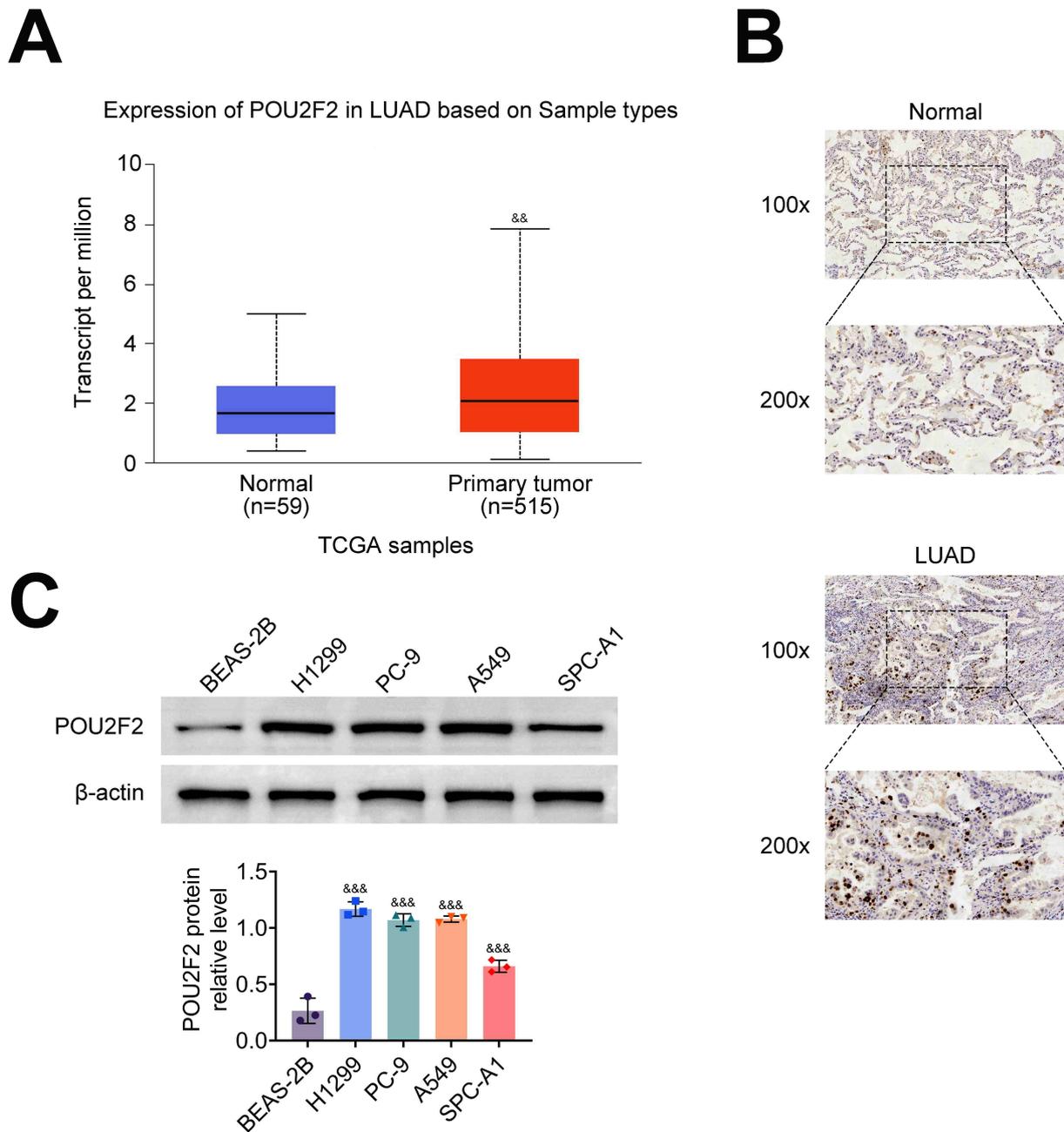

FIGURE 1. *POU2F2* owned higher expression in LUAD. (A) The expression of *POU2F2* in LUAD tissues and normal tissues was confirmed through the UALCAN online database (http://ualcan.path.uab.edu/index.html). (B) The POU2F2 protein expression was verified in Normal tissues and LUAD tissues through IHC assay. (C) The protein expression of POU2F2 was assessed in the LUAD cell lines (H1299, PC-9, A549, SPC-A1) and normal lung epithelial cell line (BEAS-2B) through western blot. $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$. LUAD: Lung adenocarcinoma; *POU2F2*: POU class 2 homeobox 2; UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses; IHC: Immunohistochemistry.
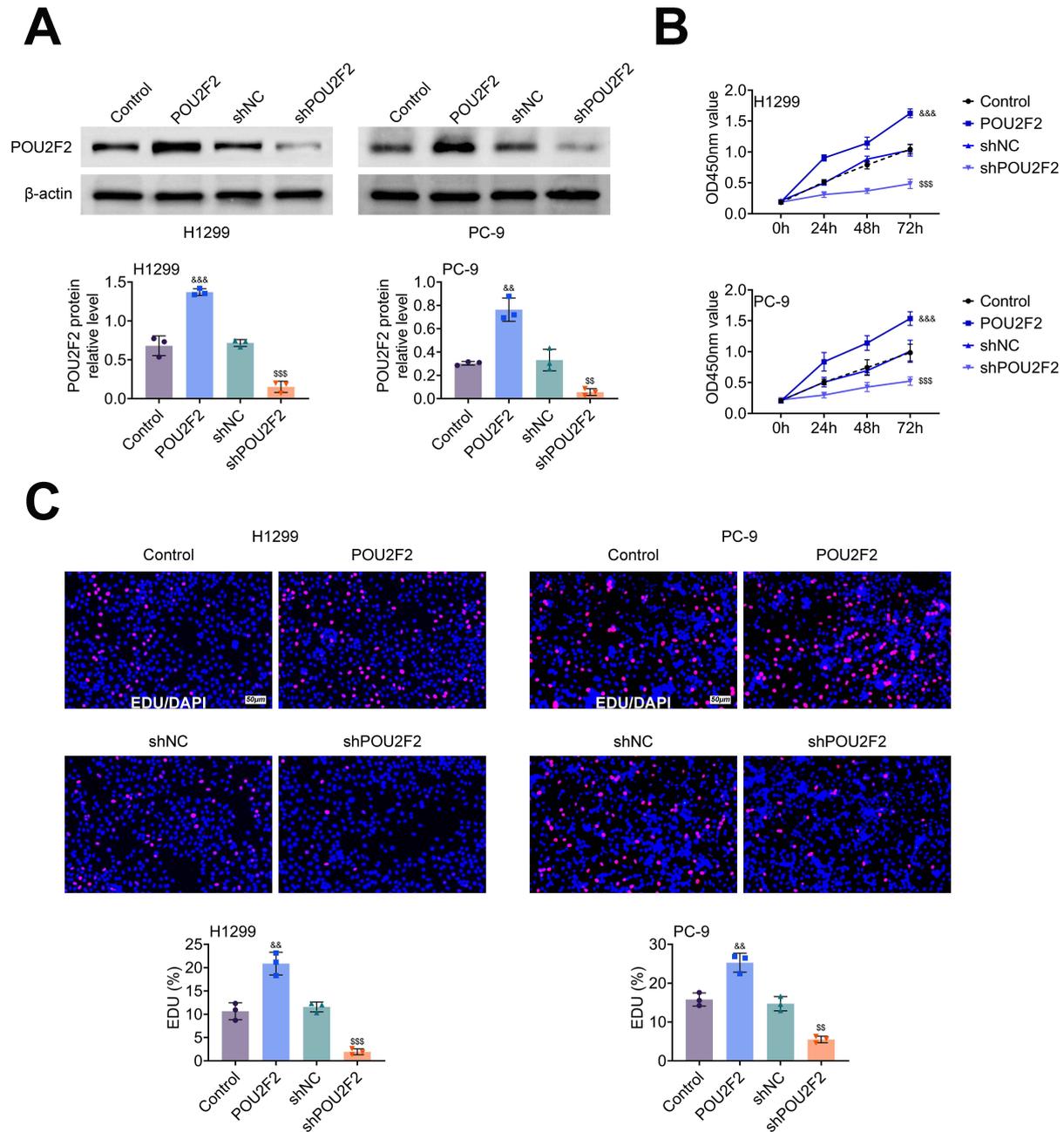
FIGURE 2. *POU2F2* accelerated cell proliferation. (A) The protein expression of POU2F2 was determined in the Control, POU2F2, shNC and shPOU2F2 groups by western blot. (B) The cell viability was examined in the Control, POU2F2, shNC and shPOU2F2 groups by CCK-8 assay. (C) The cell proliferation ability was determined in the Control, POU2F2, shNC and shPOU2F2 groups by EDU assay. $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$ *vs.* the Control group; $^{\$\$}p < 0.01$, $^{\$\$\$}p < 0.001$ *vs.* the shNC group. *POU2F2*: POU class 2 homeobox 2; CCK-8: Cell counting kit-8; EDU: 5-ethynyl-2′-deoxyuridine.

3.4 *POU2F2* triggered PDPK1-mediated PI3K/AKT pathway

The PI3K/AKT pathway exhibits pivotal roles in LUAD progression. *POU2F2* has been shown to activate the transcription of PDPK1, triggering the PI3K/AKT/mTOR pathway to glioblastoma progression. Therefore, the concertation between *POU2F2* and the PDPK1-mediated PI3K/AKT pathway in LUAD progression need deeply explorations. Results

from Fig. 4 demonstrated that levels of PDPK1, p-PI3K/PI3K and p-AKT/AKT were increased with *POU2F2* up-regulation and decreased with *POU2F2* down-regulation, suggesting that *POU2F2* triggered PDPK1-mediated PI3K/AKT pathway.

4. Discussion

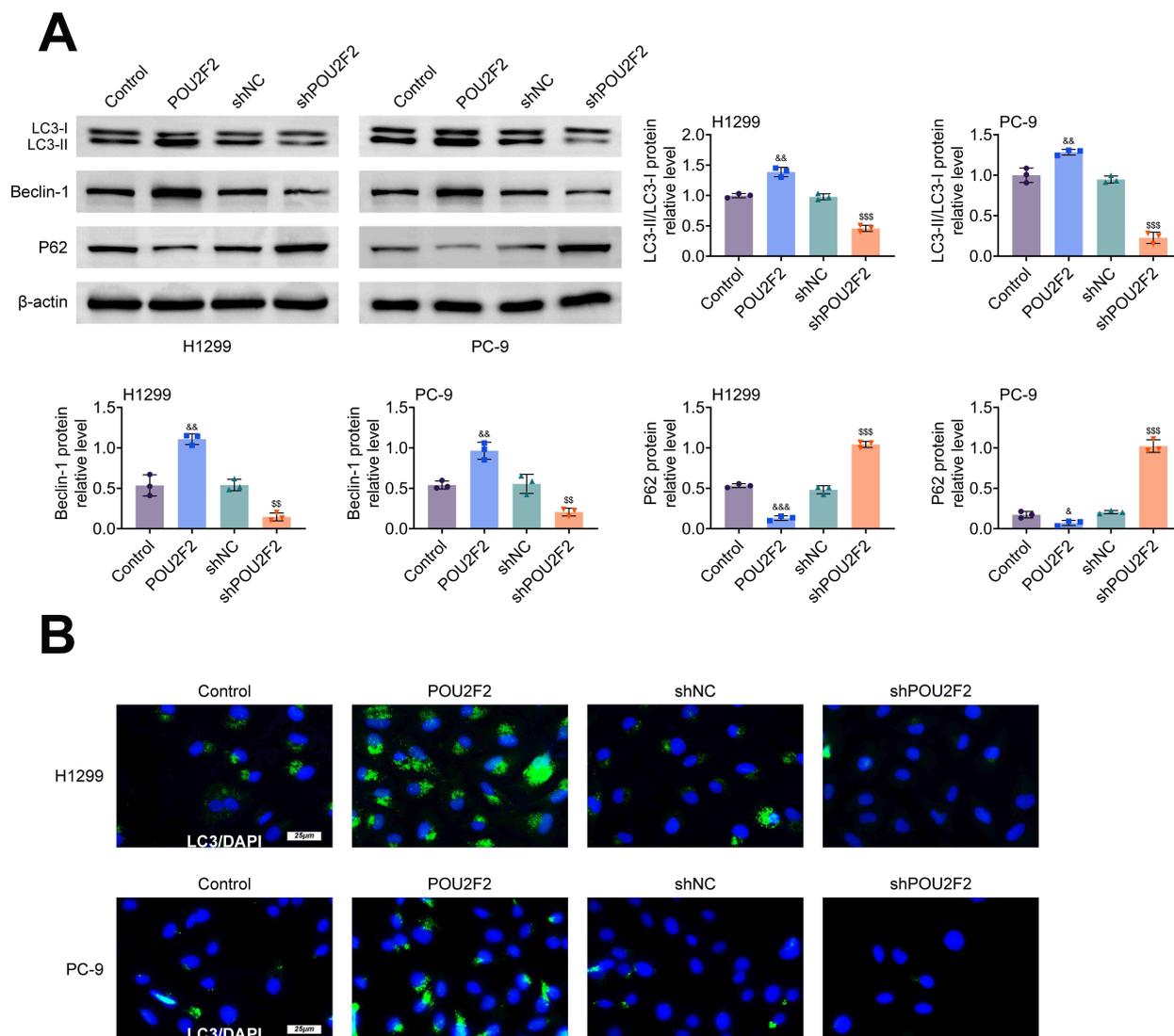The additional regulatory impacts of *POU2F2* in LUAD progression require further investigations. In this study, it was

FIGURE 3. _POU2F2_ heightened autophagy in LUAD. (A) The protein expressions of LC3-I, LC3-II, Beclin-1 and P62 were measured in the Control, POU2F2, shNC and shPOU2F2 groups through western blot. (B) The fluorescence intensity of LC3 was verified in the Control, POU2F2, shNC and shPOU2F2 groups through IF assay. $^{\&}p < 0.05$, $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$ _vs._ the Control group; $^{\$\$}p < 0.01$, $^{\$\$\$}p < 0.001$ _vs._ the shNC group. _POU2F2_: POU class 2 homeobox 2; LUAD: Lung adenocarcinoma; IF: Immunofluorescence.

initially confirmed through UALCAN online database that _POU2F2_ has higher expression in LUAD tissues. Furthermore, the up-regulation of _POU2F2_ expression in LUAD cell lines was also validated. Functional experiments revealed that _POU2F2_ accelerated LUAD cell proliferation.

Autophagy plays as a crucial role in cancers progression and regulates key molecules that drive tumor growth [17]. This process has also been shown to be involved in the regulation of LUAD. For example, exosomal LOC85009 modulates autophagy-related gene 5 (ATG5)-triggered autophagy to repress docetaxel resistance in LUAD [18]. CWHM-1008 can modulate the AKT/mTOR pathway, thereby intensifying autophagy in LUAD [19]. Additionally, Cezanne modulates PIK3C2A transcription to aggravate autophagy in LUAD [20]. Furthermore, UBE2T affects the

p53/Adenosine 5′-monophosphate (AMP)-activated protein kinase (AMPK)/mTOR signaling pathway to strengthen autophagy in LUAD [21]. Besides, tripartite motif 13 (TRIM13) modulates the Kelch like ECH associated protein 1 (KEAP1)/nuclear factor erythroid 2-related factor 2 (NRF2) pathway to restrain cell proliferation and stimulate autophagy in LUAD [22]. In this study, it was demonstrated that _POU2F2_ enhances autophagy in LUAD by increasing LC3-II/LC3-I level and fluorescence intensity.

The PI3K/AKT pathway exhibits critical functions in cancer progression, including LUAD. For instance, Mex3a/laminin subunit alpha 2(LAMA2) axis accelerates tumor metastasis in LUAD by affecting the PI3K/AKT pathway [23]. Moreover, centromere protein N (CENPN) can evoke the PI3K/AKT pathway to aggravate LUAD progression [24]. ZNF687
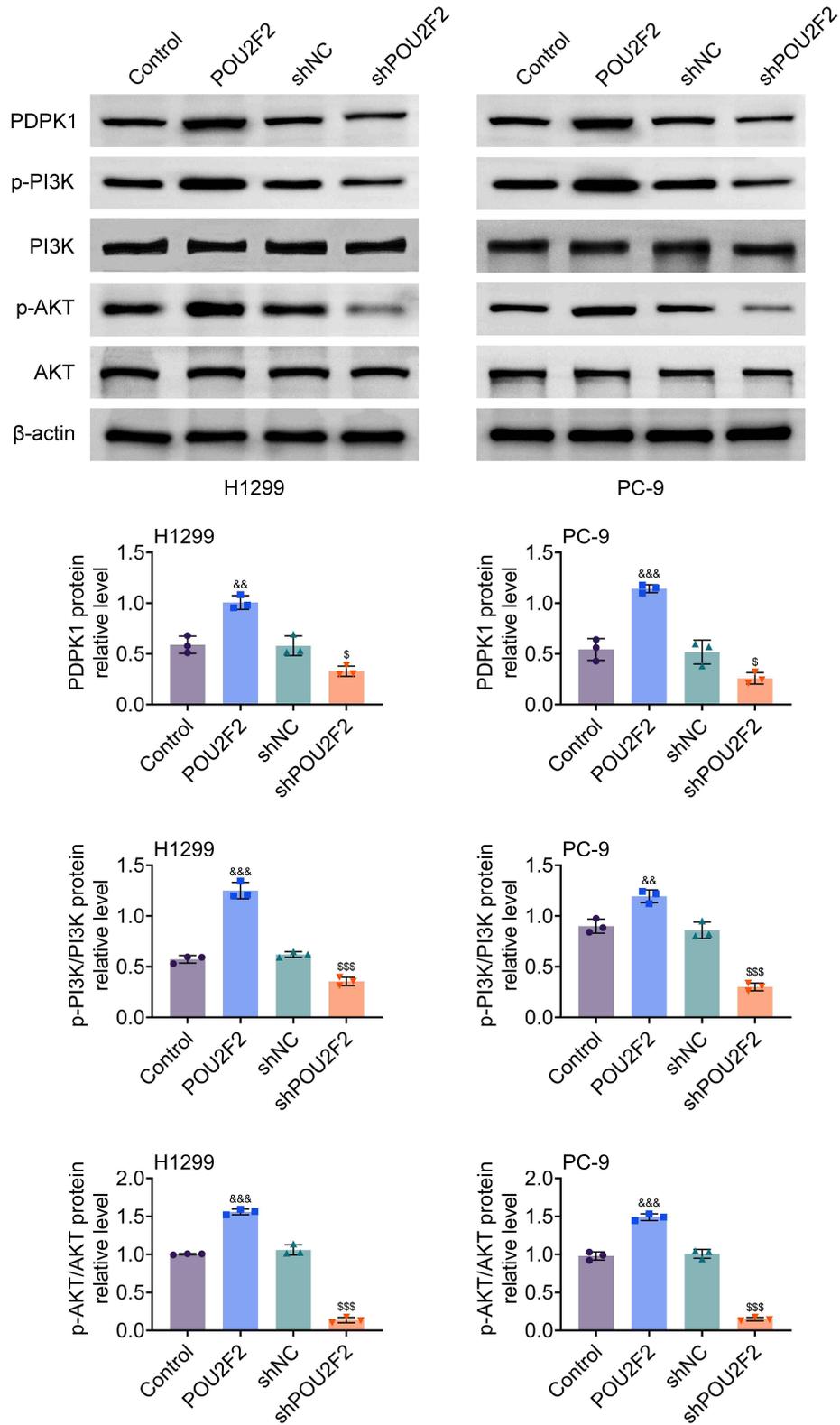
F I G U R E 4. *POU2F2* **triggered PDPK1-mediated PI3K/AKT pathway.** The protein expressions of PDPK1, p-PI3K, PI3K, p-AKT and AKT were inspected in the Control, POU2F2, shNC and shPOU2F2 groups by western blot. $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$ *vs.* the Control group; $^{\$}p < 0.01$: $^{\$\$\$}p < 0.001$: *vs.* the shNC group. *POU2F2*: POU class 2 homeobox 2; PDPK1: phosphoinositide dependent protein kinase 1; PI3K: phosphatidylinositol-3 kinase; AKT: protein kinase B.

regulates the PI3K/AKT signaling pathway to facilitate tumor development in LUAD [25]. Additionally, insulin-like growth factor 2 mRNA-binding protein 3 (IGF2BP3) controls the PI3K/AKT pathway to exacerbate LUAD progression [26]. Interestingly, it has been disclosed that *POU2F2* can stimulate PDPK1 transcription, thereby triggering the PI3K/AKT/mTOR pathway to relieve glioblastoma progression [8]. Therefore, further explorations needed to illustrate the concertation between *POU2F2* and the PDPK1-mediated PI3K/AKT pathway in LUAD progression. Consistent with these findings, our study revealed that *POU2F2* triggers the PDPK1-mediated PI3K/AKT pathway in LUAD.

5. Conclusions

Results manifested that *POU2F2* accelerates cell proliferation and autophagy in LUAD by modulating the PI3K/AKT pathway. Nevertheless, limitations occur in this work, such as the lack of investigations in other cellular phenotypes, human samples and animal samples. Further experiments will be made to ascertain the additional regulatory functions of *POU2F2* in LUAD progression.

AVAILABILITY OF DATA AND MATERIALS

The authors declare that all data supporting the findings of this study are available within the paper and any raw data can be obtained from the corresponding author upon request.

AUTHOR CONTRIBUTIONS

RGL and YZ—designed the study and carried them out; RGL, YZ, QD and RDX—supervised the data collection, analyzed the data, interpreted the data; RGL and YZ—prepare the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval was obtained from the Ethics Committee of the First Affiliated Hospital, Fujian Medical University (Approval no. 2022-0830). Written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article.

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Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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