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Gossypol overcomes EGFR-TKIs resistance in non-small cell lung cancer cells by targeting YAP/TAZ and EGFR$^{L858R/T790M}$

Jian Xu[a,1], Guo-Yuan Zhu[b,1], Dai Cao[a], Hao Pan[c], Ying-Wei Li[c,*]

[a] Institute of Tropical Medicine, Guangzhou University of Chinese Medicine, Guangzhou, Guangdong, PR China
[b] State Key Laboratory of Quality Research in Chinese Medicine, Macau Institute for Applied Research in Medicine and Health, Macau University of Science and Technology, Macau, PR China
[c] Research Center of Integrative Medicine, School of Basic Medical Sciences, Guangzhou University of Chinese Medicine, Guangzhou, Guangdong, PR China

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ABSTRACT

EGFR tyrosine kinase inhibitors (EGFR-TKIs) improve the progression-free survival of patients with non-small cell lung cancer (NSCLC). However, most patients inevitably developed drug resistance. EGFR T790 M mutation is the major mechanism for resistance to EGFR-TKIs and becomes an obstacle for the treatment of NSCLC patients with EGFR activating mutations. Besides, YAP/TAZ also confers resistance to EGFR-TKIs. Our previous study identified gossypol as a YAP/TAZ inhibitor. In the current study, we found that gossypol inhibited cell growth and induced apoptosis in H1975 cells harboring EGFR$^{L858R/T790M}$. Also, gossypol treatment sensitized H1975 cells to EGFR-TKIs. Our mechanism studies showed that gossypol decreased the protein level of YAP/TAZ, which was abrogated by the proteasome inhibition. Moreover, over-expression of YAP/TAZ reversed the effects of gossypol on H1975 cells, and YAP/TAZ knockdown sensitized H1975 cells to gossypol treatment. Furthermore, gossypol reduced the protein level of EGFR$^{L858R/T790M}$ and inhibited the downstream ERK1/2 pathway in H1975 cells. Our findings suggested that gossypol might serve a promise drug candidate for overcoming EGFR-TKIs resistance by targeting both YAP/TAZ and EGFR$^{L858R/T790M}$.

1. Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide. It is the second most common cancer in the United States. The American Cancer Society estimated about 234,030 new cases of lung cancer and 154,050 deaths from lung cancer in 2018 [1,2]. Non-small cell lung cancer (NSCLC) accounts for approximately 80% of lung cancers [3]. In recent years, the discovery of oncogenic drivers leads to the great achievement of targeted therapy in NSCLC. The most commonly altered oncogenes are epidermal growth factor receptor (EGFR), KRAS, and anaplastic lymphoma receptor tyrosine kinase (ALK) rearrangements [4–7].

Mutant EGFR is found in around 15% of NSCLC patients in Caucasians, and about 40% of East Asian patients [8]. The two major activating mutations are the deletion of exon 19 and the L858R point mutation, which predict for the response to EGFR-tyrosine kinase inhibitors (EGFR-TKIs) [9]. Since 2015, the FDA (Food and Drug Administration) has successively approved several EGFR TKIs (e.g., gefitinib, erlotinib, and afatinib) for the first-line treatment of NSCLC patients with activating EGFR mutation [10,11]. However, despite the initial response to EGFR-TKIs, NSCLC patients eventually develop disease progression with acquired resistance to EGFR TKIs. EGFR T790 M mutation is shown in about 50–60% of resistant cases and becomes an obstacle for the treatment of NSCLC patients with EGFR activating mutations [12,13]. Therefore, it is valuable to develop therapeutic agents for overcoming EGFR-TKIs resistance.

Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ) are key transcriptional co-activators of the Hippo pathway [14]. The hyperactivation of YAP/TAZ is found in many solid tumors [15–17]. Aberrant activation of YAP/TAZ induces proliferation, epithelial-mesenchymal transition (EMT), migration, cancer stem cells features and chemoresistance [18–21]. The most recent

Abbreviations: EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; MEK, mitogen-activated protein kinase kinase; EMT, epithelial–mesenchymal transition; SHP2, SH2 domain-containing phosphatase-2; ALK, anaplastic lymphoma receptor tyrosine kinase; EGFR-TKIs, EGFR-tyrosine kinase inhibitors; FDA, Food and Drug Administration; YAP, yes-associated protein; TAZ, transcriptional co-activator with PDZ-binding motif

* Corresponding author at: Research Center of Integrative Medicine, Guangzhou University of Chinese Medicine, 510006, Guangzhou, PR China.
E-mail address: liyingwei@gzucm.edu.cn (Y.-W. Li).

[1] Contributed equally.

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studies show that YAP/TAZ plays a key role in the tumorigenesis and metastasis of NSCLC [22–24]. Moreover, growing evidence reveals that the activation of YAP/TAZ confers resistance to EGFR-TKIs [25,26]. Therefore, targeting YAP/TAZ might be a promising strategy for the treatment of NSCLC patients with primary or acquired resistance to EGFR-TKIs.

Natural products have always been valuable sources for drug discovery. In our previous study, we screened a natural compound library for YAP/TAZ modulators and identified gossypol as a YAP/TAZ inhibitor. In the present study, we used H1975 cell lines harboring EGFR L858R/T790 M mutation to investigate the effect and underlying mechanisms of gossypol on overcoming EGFR-TKIs resistance. We provided evidence that gossypol overcame EGFR-TKIs resistance by targeting both YAP/TAZ and $EGFR^{L858R/T790M}$. Our findings suggested that gossypol was a potential candidate to overcome EGFR-TKIs resistance in NSCLC patients.

2. Materials and methods

2.1. Cell lines and reagents

The human NSCLC cell lines H1975 (EGFR L858R/T790 M), H441, and A549 were purchased from the American Type Culture Collection (ATCC) (Manassas, VA, United States). H1975 and H441 cells were cultured in RPMI1640 medium supplemented with 10% fetal bovine serum (FBS, Thermo Fisher Scientific, Carlsbad, CA, USA). A549 cells were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% FBS. We maintained the cells in a humidified atmosphere of 95% air and 5% CO2 at 37 °C. Gossypol (S2303, purity > 99%) was obtained from Selleckchem (Houston, TX, USA). MG132 was purchased from Calbiochem (San Diego, CA, USA). Dimethyl sulfoxide (DMSO) was purchased from MP Biomedicals (Santa Ana, CA, USA). All compounds were dissolved in DMSO for in vitro studies. Sulforhodamine B (SRB) was purchased from MilliporeSigma (Burlington, MA, USA).

2.2. Plasmids construction and transfection

The human YAP1 and TAZS89 A plasmids were cloned into the BamHI and EcoRI restriction site of the pcDNA3 vector as previously described [27]. Plasmid was transfected into cells for 24 h using Lipofectamine 3000 DNA Transfection Reagent (Thermo Fisher Scientific, Waltham, MA, USA), according to the manufacturer's instruction. The increased level of YAP and TAZ was confirmed by Western blotting.

We constructed the sgRNAs targeting exon 1 of the human WWTR1 (TAZ, NM_001168278) and the Yes-associated protein 1 (YAP1, NM_001195044). The target sequences were listed in Table 1. We verified the constructs by sequencing. Cells were transfected with 2.5 μg of each of the sgRNAs by using Lipofectamine 3000 DNA Transfection Reagent (Thermo Fisher Scientific). Gene-edited cells were selected with 1.5 μg/ml puromycin (Thermo Fisher Scientific) for 48 h. The knockdown of YAP and TAZ was confirmed by Western blotting.

Table 1
Target sequence of sgRNA plasmids.

Plasmid	Target sequence (5'-3')
sgTAZ-1	CACCGCGCGAGTGCGAGCCCGAATC
	AAACGATTCGGGCTCGCACTCGCGC
sgTAZ-2	CACCGGCAAGTGATCCACGTCACGC
	AAACGCGTGACGTGGATCACTTGCC
sgYAP-1	CACCGGCACGATCTGATGCCCGGCG
	AAACCGCCGGGCATCAGATCGTGCC
sgYAP-2	CACCGGTGCACGATCTGATGCCCGG
	AAACCCGGGCATCAGATCGTGCACC

Table 2
The primers used in RT-qPCR.

Genes	Forward primer (5'-3')	Reverse primer (5'-3')
CTGF	GCAGAGCCGCCTGTGCATGG	GGTATGTCTTCATGCTGG
CYR61	CACACCAAGGGGCTGGAATG	CCCGTTTTGGTAGATTCTGG
YAP	TCCTGATGGATGGGAACAAG	ATGGCAAAACGAGGGTCA
TAZ	CAGCAATGTGGATGAGATGG	TCAAGGAAATCAGGGAAACG
β-Actin	GGTGAAGGTCGGAGTCAACGG	GAGGTCAATGAAGGGGTCATTG

2.3. Sulforhodamine B (SRB) cell growth assay

In this study, we used the SRB protein assay to analyze cell proliferation. In brief, the NSCLC cells were seeded at a density of 6000 cells per well on 96-well plates. Gossypol (0–80 μM) was added to the indicated wells and plates were incubated for 24, 48, or 72 h. At the end of incubation, SRB assay and calculation of IC50 were performed as previously described [28].

2.4. Colony formation assay

H1975 cells were seeded on 24-well plates (100 cells / per well) and cultured overnight. Cells were treated with indicated concentration of gossypol for 72 h and replaced with fresh medium. Seven days later, cells were stained with 0.1% crystal violet solutions. Visible colonies were photographed and counted.

2.5. Flow cytometry analysis of apoptosis

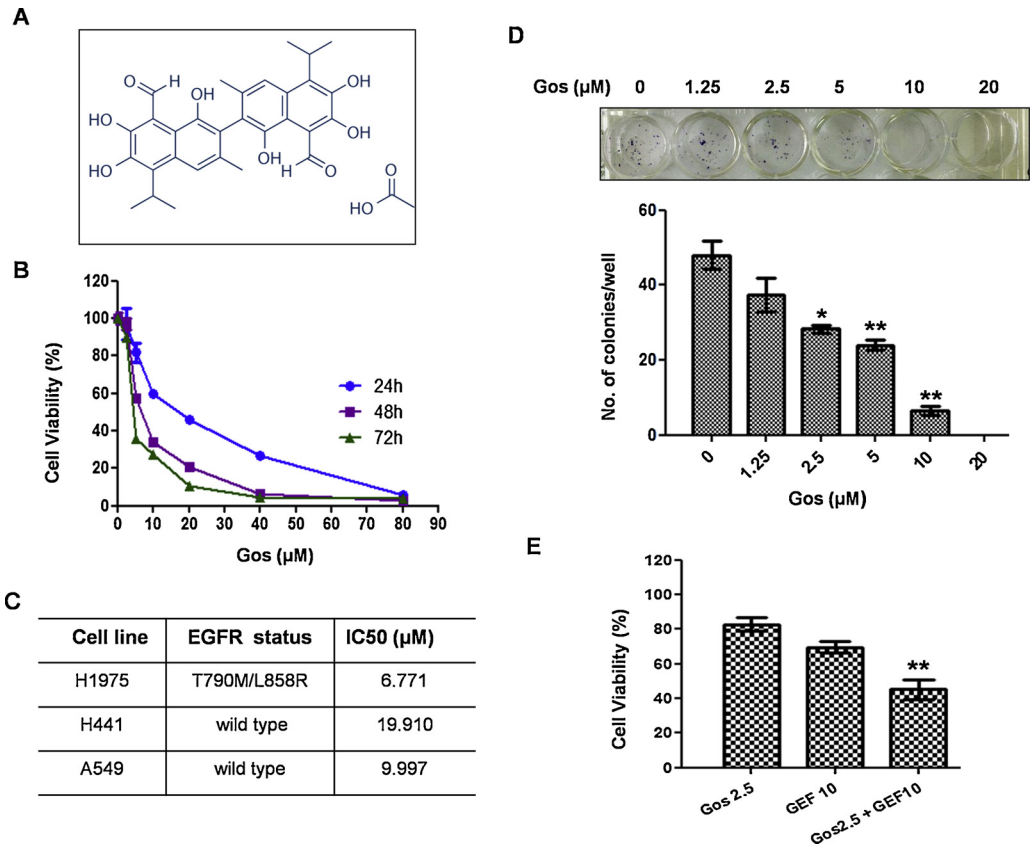
We used the annexin-V-FITC/PI double staining assay to examine the apoptotic cell death. After cells were treated with different concentration of gossypol for 48 h, cells were collected and washed with phosphate-buffered saline (PBS) three times. Annexin V-FITC/PI assay was performed according to the manufacturers' instruction (BBI LIFE SCIENCES, Shanghai, China). Apoptotic cells were detected by using the BD Accuri C5 (BD Biosciences, Franklin Lakes, NJ, USA).

2.6. Caspase activity assay

We measured the caspase3/7 activity by using the Caspase-Glo® 3/7 assay kit according to the manufacturer's protocol (Promega, Fitchburg, WI, USA). Cells were seeded on 96 well plates and incubated overnight. Gossypol (1.25–5 μM) was added to the indicated wells. After 48 h, the activity of caspase-3/-7 was measured according to the manufacturer's guidelines. We measured the luminescence by using a microplate reader (PerkinElmer, Waltham, MA, USA). The data was presented as the ratio of the DMSO-treated cells (control).

2.7. Western blotting analysis

The following antibodies were purchased from Cell Signaling Technology (Danvers, MA, United States): YAP/TAZ, YAP, TAZ, Gapdh, CTGF, and CYR61. HRP-conjugated anti-mouse and HRP-conjugated anti-rabbit were purchased from Bio-Rad (Bio-Rad, Hercules, CA, USA). EGFR, Phospho-EGFR (Y1068), ERK1/2, and Phospho-ERK antibodies were purchased from ABclonal (Wuhan, China). Cells were lysed with RIPA buffer containing protease inhibitors (Thermo Fisher Scientific) and cleared by centrifugation. Protein concentration was determined with BCA Protein Assay Kit (Thermo Fischer Scientific). Western blotting was performed according to the standard protocol. ECL detection reagents (Thermo Fischer Scientific) were used to detect protein bands by using the chemiluminescent imaging system (Tanon, Shanghai, China).



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Cell line	EGFR status	IC50 (μM)
H1975	T790M/L858R	6.771
H441	wild type	19.910
A549	wild type	9.997

Fig. 1. Gossypol (Gos) inhibited cell growth in NSCLC cells. (A) Chemical structure of Gos. (B) H1975 cells were treated with Gos (0–80 μM) for 24, 48 and 72 h, and cell viability was measured via the SRB assay. Cell viability is expressed as the percentage of the DMSO control cells. (C) H1975, H441, and A549 cells were treated with Gos (0–80 μM) for 48 h and the IC50 values were calculated with GraphPad Prism 5 software. (D) Representative images and summary of the number of colonies. H1975 cells were plated in 24-well plates and treated with indicated concentration of Gos for 72 h, and then replaced with fresh medium. After 7 days, the plates were stained with 0.1% crystal violet solution. The results shown are representative of at least three independent experiments. Data are shown as the means ± S.D. (*$P < 0.05$ and **$P < 0.01$ compared with the control group). (E) Co-treatments are indicated at the bottom of the graph, and cell viability is expressed as the percentage of the DMSO-treated cells (**P < 0.01 compared with the gefitinib alone group).

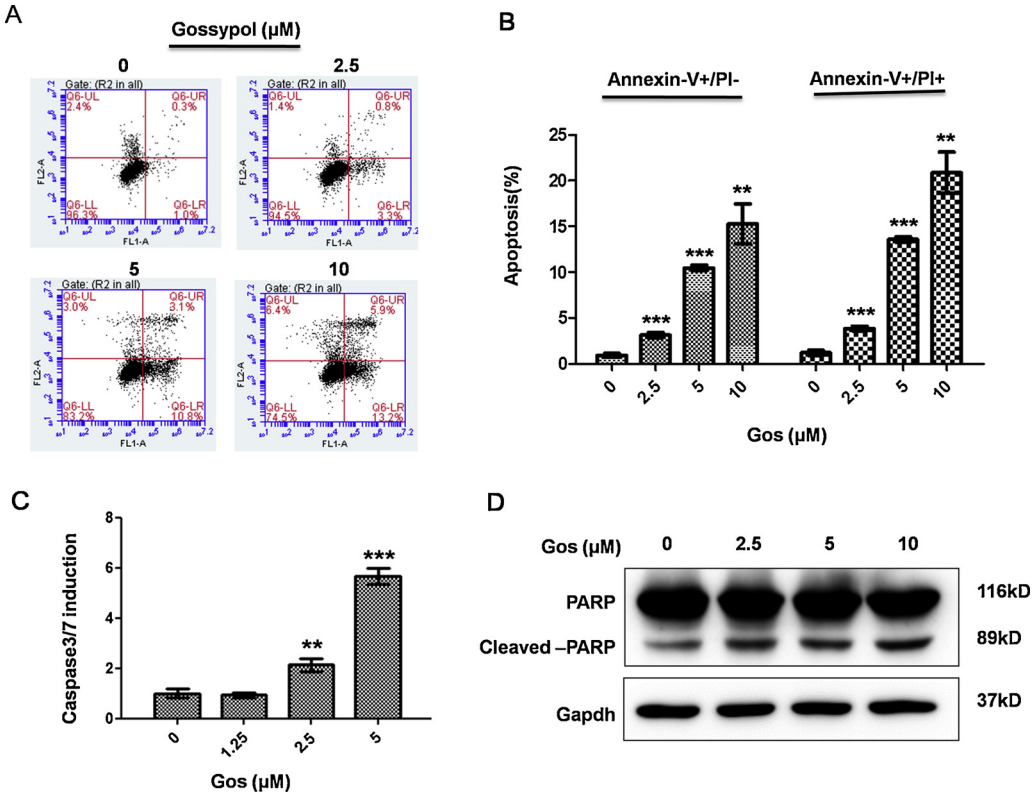

Fig. 2. Gossypol induced apoptosis in H1975 cells. (A) Representative images of annexin-V-FITC/PI assay. (B) Statistical analysis of Gos-induced early and late apoptosis. (C) Activation of the caspase3/7 after treatment with Gos for 48 h. The activity of caspase3/7 activity is expressed as fold change to the DMSO-treated cells. Each experiment was performed in triplicate. (**P < 0.01 and ***p < 0.001 compared with the control group). (D) Western blot analysis of PARP in Gos-treated and DMSO-treated cells. Gapdh was used as loading control. The results shown are representative of at least three independent experiments.

2.8. Real-time PCR

We applied the TRIzol reagent (Thermo Fisher Scientific) for total RNA extraction. Reverse transcriptase reaction was performed by using a cDNA Synthesis Kit following the manufacturer's instructions (Thermo Fischer Scientific). Real-time polymerase chain reaction (PCR) analysis was conducted by using the Power SYBR Green PCR Master Mix (Roche, Germany) on a Bio-Rad CFX96 detection system. The mRNA levels of

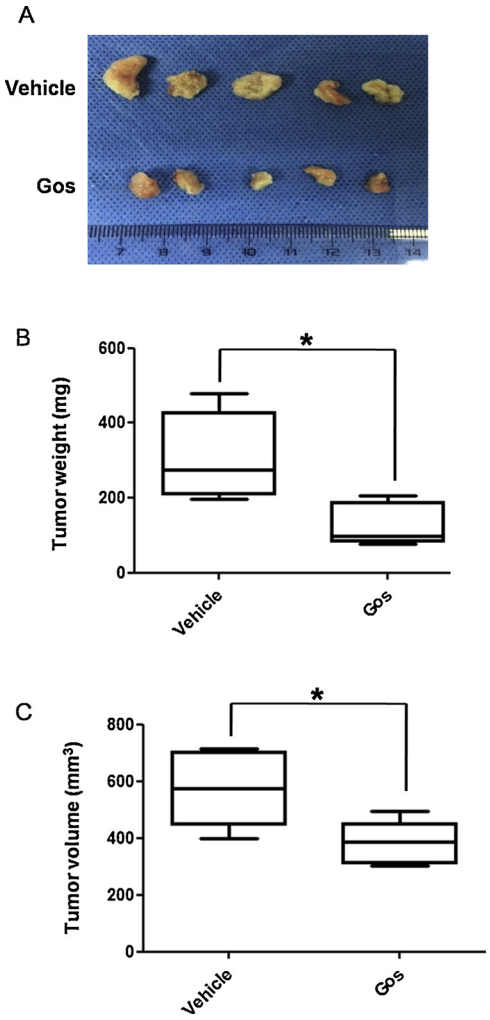

Fig. 3. Gossypol inhibited H1975 xenograft tumor growth. H1975 cells were subcutaneously injected into the flanks of Balb/c nude mice. Drug treatments began after one week of injection (day 0). Mice were treated with vehicle and gossypol (20 mg/kg/d) by oral gavage, and tumor size was measured by calipers. (A) The tumors harvested at the end of the experiment. (B) The tumor volumes at the end of the experiment. Tumor volumes were calculated according to the formula $LW^2/2$. (C) Tumor weights at the end of the experiment. The statistical significance was determined by Student's t-test. The difference between Gos and vehicle control is significant (*p < 0.05).

target genes were standardized to the level of β-Actin. Primer sequences (in the 5′-3′direction) are listed in Table 2.

2.9. Tumorigenic evaluation assays

We purchased female BALB/c nude mice (5–6 weeks old) from Charles River (Beijing, China). All procedures involving animals were approved by the Institutional Animal Care and Use Committee (Guangzhou University of Chinese Medicine, Guangzhou, China). Mice were injected subcutaneously with 1×10^6 H1975 cells. After one week, we randomly assigned mice to two groups (five mice per group). For the following 21 days, the vehicle control group was given 1% carboxymethylcellulose sodium, and the drug-treated group was given 20 mg/kg gossypol via daily oral gavage. The weight and tumor sizes of mice were recorded once weekly. Tumor volumes were calculated as previously described [28].

2.10. Statistical analysis

The data are presented as the mean ± SD. Prism version 5.0 (GraphPad, La Jolla, CA) was used for statistical analyses. $P < 0.05$ was considered significant (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$).

3. Results

3.1. Gossypol inhibited cell proliferation in NSCLC cells

In this study, we performed the SRB protein assay to investigate the effect of gossypol on cell proliferation in three NSCLC cell lines. A549 and H441 cells carried wild-type EGFR, and H1975 cells carried L858R/T790 M mutation. Our results demonstrated that gossypol inhibited cell growth in a dose- and time-dependent manner in all three cell lines. H1975 cells harboring EGFR $^{L858R/\ T790M}$ mutation were more sensitive to gossypol treatment than the other two cell lines (Fig. 1B and Supplementary Fig. 1). The IC50 values of gossypol in H1975, H441 and A549 cells were approximately 6.771 μM, 19.91 μM, and 9.997 μM respectively, at 48 h (Fig. 1C). We next investigated if gossypol affected the ability of colony formation in H1975 cells. Results of our studies showed that gossypol reduced the number of colony formation in H1975 cells (Fig. 1D). Moreover, treatment with a fixed combination of gossypol and gefitinib significantly decreased cell growth in H1975 cells, as compared with gefitinib alone (Fig. 1E).

3.2. Gossypol induced apoptosis in NSCLC cells

We next performed annexin-V-FITC/PI double staining assay to investigate whether gossypol inhibited cell growth by inducing apoptosis in NSCLC cells. Our data showed that gossypol increased the percentage of early and late apoptotic cells in H1975 cells. (Fig. 2A, B). Gossypol also induced the activation of caspase-3/-7 in H1975 cells (Fig. 2C). Consistent results from western blot analysis showed that gossypol increased cleaved PARP (poly ADP ribose polymerase) in H1975 cells (Fig. 2D). Gossypol also induced apoptosis in A549 cells (Supplementary Fig. 2).

3.3. Gossypol inhibited H1975 xenograft tumor growth

We confirmed that gossypol suppressed tumor growth in vivo. BALB/c nude mice were injected subcutaneously with 1×10^6 H1975 cells. After 7 days, mice were given 20 mg/kg gossypol or vehicle by daily oral gavage for 21 days. As shown in Fig. 3, gossypol treatment significantly decreased the tumor weight and volume at the end of the assay ($P < 0.05$, $n = 5$). The body weights of the mice were not significantly altered by gossypol treatment (Supplementary Fig.3).

3.4. Knockdown of YAP/TAZ suppressed cell growth in H1975 cells

CRISPR/Cas9-mediated genome editing was used in this study to ablate the expression of YAP/TAZ in H1975 cells. As shown in Fig. 4A and B, the level of YAP and TAZ were decreased in H1975 cells. Either YAP or TAZ knockdown significantly decreased the number of colony formation H1975 cells (Fig. 4C, D).

3.5. Knockdown of YAP/TAZ sensitized NSCLC cells to EGFR-TKIs

We proceeded to examine whether the level of YAP/TAZ was related to EGFR-TKIs sensitivity in NSCLC cells with EGFR L858R/T790 M mutation. H1975 scram, H1975 sgYAP, and H1975 sgTAZ cells were treated with the indicated concentration of gefitinib for 72 h. The SRB assay was performed to evaluate cell viability. Our results showed that H1975 cells with YAP/TAZ knockdown became more sensitive to gefitinib treatment (Fig. 5A, B). Knockdown of TAZ also sensitized A549

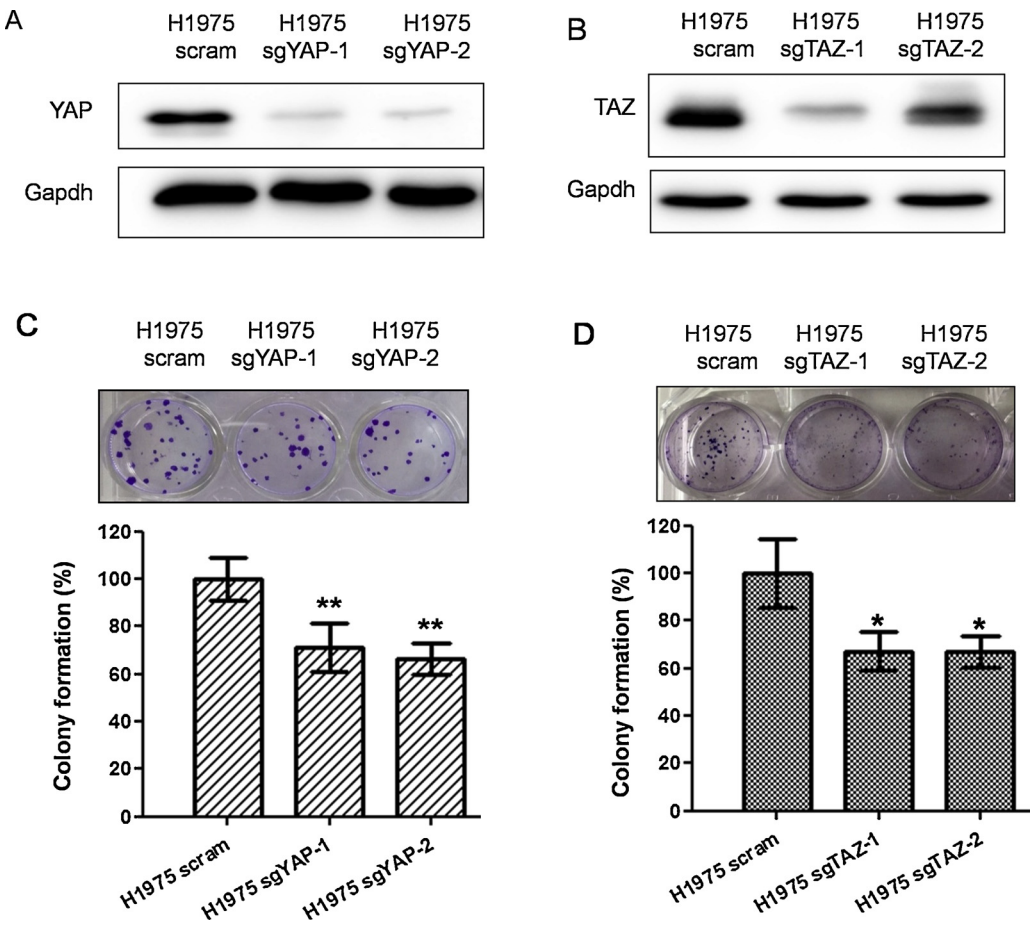

Fig. 4. Knockdown of YAP/TAZ suppressed cell growth in H1975 cells. (A, B) The decreased expression of YAP/TAZ was validated by western blot analysis. (C) Representative images and statistical analysis of colony formation in H1975 cells with or without knockdown of YAP. Both scramble and sgYAP cells were plated in 24-well plates and incubated for 10 days. (D) Representative images and statistical analysis of colony formation in H1975 cells with or without knockdown of TAZ. Both scramble and sgTAZ cells were plated in 24-well plates and incubated for 10 days. Cells were stained and counted as previously described (*p < 0.05 and **P < 0.01 compared with the scramble cells).

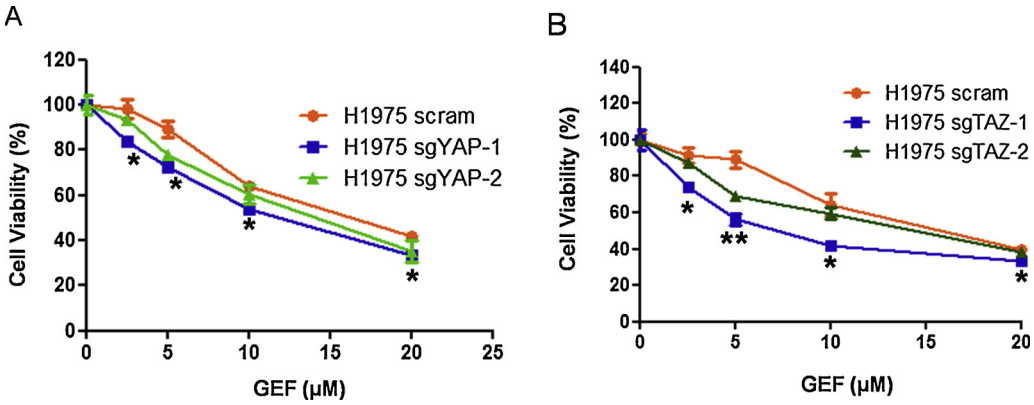

Fig. 5. Knockdown of YAP/TAZ sensitized H1975 cells to Gefitinib treatment. (A, B) The scramble, sgYAP, and sgTAZ cells were treated with gefitinib (0–20 μM) for 72 h, and cell viability was measured via the SRB assay. Cell viability is expressed as the percentage of the DMSO-treated cells. Data are shown as the means ± S.D. (*P < 0.05 and **P < 0.01 compared with the control group).

cells to gefitinib treatment (Supplementary Fig. 4A and B).

3.6. Gossypol targeted YAP and TAZ in NSCLC cells

In our previous study, we identified gossypol as a potential YAP/TAZ inhibitor. Therefore, we hypothesized that gossypol overcame EGFR-TKIs resistance by targeting YAP/TAZ. As expected, gossypol treatment down-regulated the protein level of YAP and TAZ in a dose-dependent manner (Fig. 6A). Consistently, gossypol decreased the mRNA and protein level of YAP/TAZ target genes, including CTGF and CYR61 (Fig. 6A, B). However, the mRNA levels of YAP and TAZ were not affected by gossypol treatment (Fig. 6C). We next examined whether gossypol induced the proteasomal degradation of YAP/TAZ. Our results demonstrated that treatment with MG132, a proteasome inhibitor, rescued the decrease of YAP/TAZ by gossypol treatment in H1975 cells (Fig. 6D). In A549 cells, gossypol treatment down-regulated the protein level of TAZ, and MG132 blocked the reduction of TAZ protein by gossypol treatment (Supplementary Fig. 4C and D).

3.7. YAP/TAZ mediated the anti-cancer activities of gossypol

We proceeded to detect whether the expression of YAP/TAZ affected the response of H1975 cells to gossypol treatment. Our results demonstrated that over-expression of YAP/TAZ reversed the effects of gossypol on H1975 cells (Fig. 7A, B), and knockdown of YAP/TAZ sensitized H1975 cells to gossypol treatment (Fig. 7C, D), which indicated that the anti-cancer activities of gossypol could be partly due to down-regulation of YAP/TAZ.

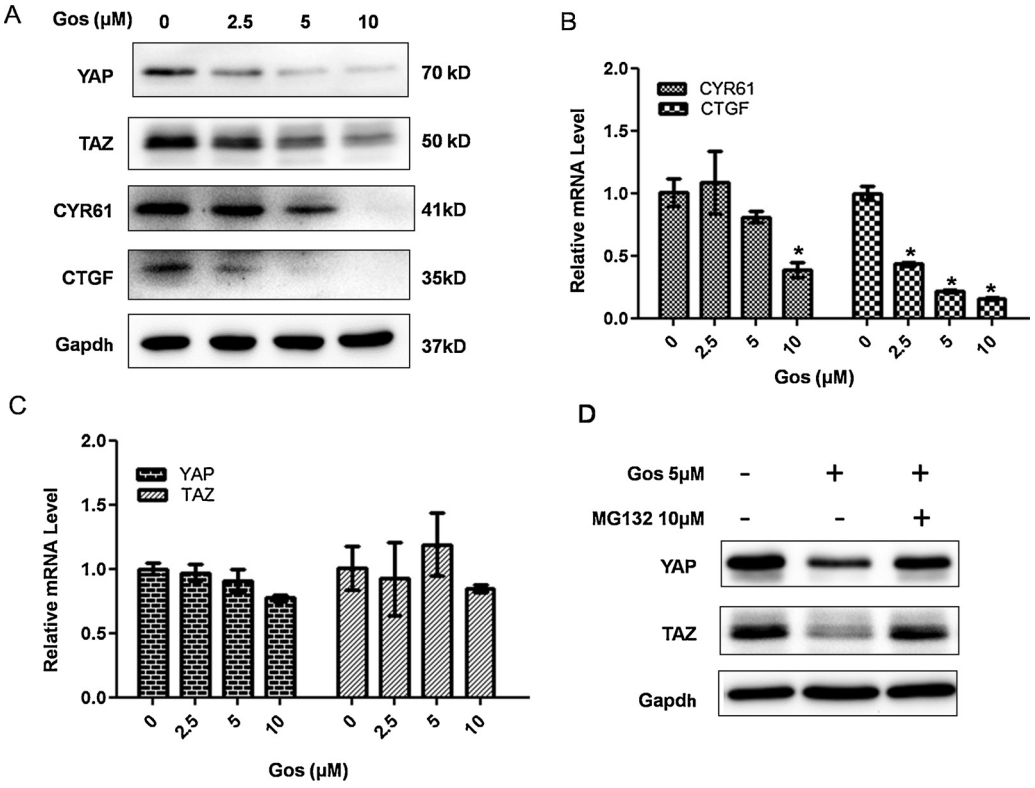

Fig. 6. Gossypol targeted YAP and TAZ in H1975 cells. (A) Cells were treated with indicated concentration of Gos for 48 h, YAP/TAZ, CTGF and CYR61 were determined by western blot analysis. (B, C) mRNA expression levels of CTGF, CYR61, YAP and TAZ were detected by real-time qPCR and calculated by the $2^{-\Delta\Delta Ct}$ method. Each experiment was performed in triplicate. The results shown are representative of at least three independent experiments. Data are shown as the means ± S.D. (*P < 0.05 compared with the control group). (D) Cells were treated with 5 μM Gos for 6 h, and 10 μM MG132 was added to the indicated wells for another 6 h. YAP/TAZ protein expression levels were detected by western blot analysis. The results shown are representative of three independent experiments.

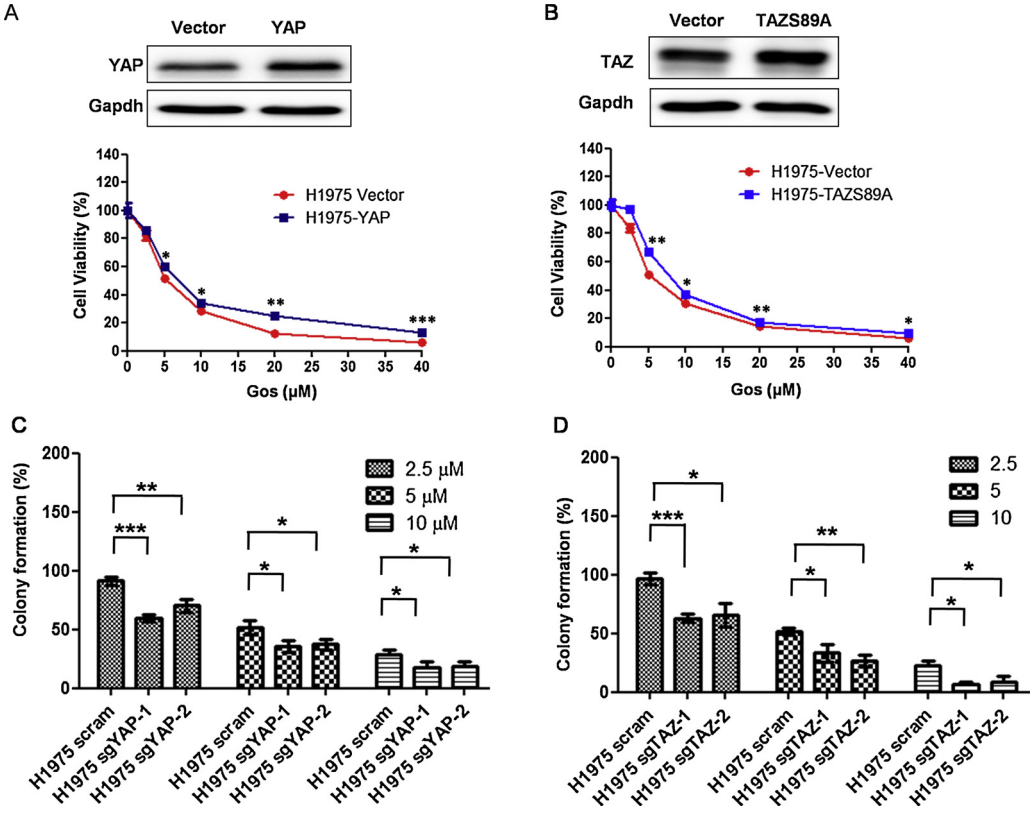

Fig. 7. YAP/TAZ mediated the anticancer activities of gossypol. (A) H1975 cells were transfected with YAP or Vector for 24 h, and the expression of YAP was detected by western blot analysis at 48 h post transfection. Both Vector and YAP cells were plated in 96-well plates and treated with gossypol (0–80 μM) for 48 h. Cell viability was measured via the SRB assay. (B) Increased expression of TAZ was validated by western blot analysis. Both Vector and TAZ cells were treated with gossypol (0–80 μM) for 48 h, and cell viability was measured via the SRB assay. (C) Both scramble and sgYAP cells were plated in 24-well plates and treated with indicated concentration of gossypol for 72 h, and then replaced with fresh medium. (D) Both scramble and sgTAZ cells were plated in 24-well plates and treated with indicated concentration of gossypol for 72 h, and then replaced with fresh medium. Cells were stained and counted as previously described (*P < 0.05, **P < 0.01 and ***p < 0.001 compared with the scramble group upon treatment with the same concentration of gossypol).

3.8. Gossypol decreased EGFR$^{L858R/T790M}$ expression and suppressed EGFR downstream signaling

We further investigated whether gossypol inhibited EGFR$^{L858R/T790M}$ signaling in NSCLC cells. Our results demonstrated that gossypol reduced the protein level of EGFR$^{L858R/T790M}$ and suppressed the downstream ERK signaling (Fig. 8). However, the protein level of EGFR wild type was not affected by gossypol treatment in A549 cells (Supplementary Fig. 5), indicating that gossypol specially targeted EGFR$^{L858R/T790M}$ mutation.

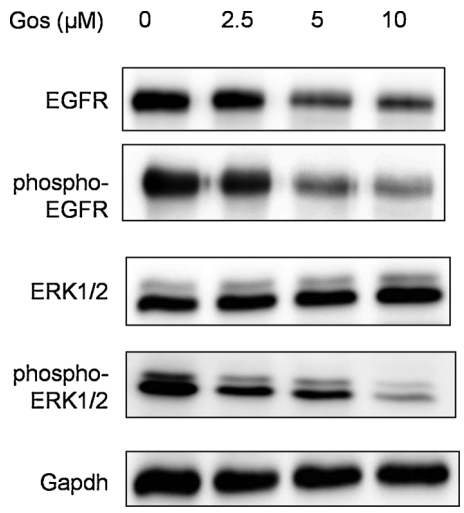

Fig. 8. Gossypol decreased EGFR$^{L858R/T790M}$ expression and suppressed EGFR downstream signaling. After cells were treated with indicated concentration of Gos for 48 h, the expression of EGFR, Phospho-EGFR, ERK, Phosphor-ERK, and Gapdh was detected by western blot analysis.

4. Discussion

Several natural compounds have been validated to overcome EGFR-TKIs resistance via different mechanisms in preclinical studies. Dioscin was reported to down-regulate the expression of SH2 domain-containing phosphatase-2 (SHP2) to overcome EGFR-TKIs resistance [29]. Butein was proved to overcome gefitinib-induced resistance by targeting both EGFR and MET in NSCLC patients [30]. Therefore, natural products might be valuable sources for the discovery of new agents overcoming EGFR-TKIs resistance.

Gossypol was a polyphenol derived from cotton seeds. Gossypol exerted anti-cancer effects in several types of cancer, including prostate cancer, malignant mesothelioma, leukemia, and breast cancer [31–34]. In a recent study, gossypol was proved to enhance gefitinib sensitivity, but the underlying mechanisms were not fully elucidated [35]. In this study, we used H1975 cell lines harboring EGFR$^{L858R/T790M}$ to investigate the effect of gossypol on overcoming EGFR-TKIs resistance. Our results showed that gossypol inhibited cell proliferation via inducing apoptosis in H1975 cells. Gossypol also inhibited H1975 xenograft tumor growth. Moreover, gossypol treatment sensitized H1975 cells to gefitinib.

YAP and TAZ were proved to be related to EGFR-TKIs resistance. The combination of YAP/TAZ inhibition with EGFR-TKIs overcame primary and acquired EGFR-TKIs resistance in lung adenocarcinoma [36,37]. Our study confirmed that knockdown of YAP/TAZ inhibited cell growth and sensitized H1975 cells to EGFR-TKIs. We previously identified gossypol as a potential YAP/TAZ inhibitor. Therefore, we further examined the effects of gossypol on the Hippo pathway in NSCLC cells. Our results demonstrated that gossypol decreased the protein level of YAP and TAZ without affecting their mRNA expression. We further investigated whether the protein stability of YAP/TAZ was decreased by gossypol. MG132, a known proteasome inhibitor, could reverse YAP/TAZ protein attenuation after gossypol treatment, which suggested that gossypol down-regulated the expression of YAP/TAZ in a proteasome-dependent manner. Moreover, over-expression of YAP/TAZ reversed the effects of gossypol on H1975 cells, and YAP/TAZ knockdown sensitized H1975 cells to gossypol treatment, indicating that gossypol exerted the anti-cancer effects, at least partially, by inhibiting YAP/TAZ activity.

Mutated EGFR exerts its oncogenic roles through activation of the MEK/ERK and PI3K/AKT signaling pathways [38]. In this study, we found that gossypol specially reduced the protein level of EGFR$^{L858R/T790M}$, and subsequently inhibited the phosphorylation of EGFR and downstream ERK1/2 in H1975 cells.

Thus far, our work is limited by the fact that we demonstrated the regulatory role of YAP/TAZ in EGFR-TKIs resistance through in vitro experiments. Results of our study may form a basis for further studies to investigate the role of YAP/TAZ in regulating human EGFR-TKIs resistance through clinical data analysis and patient-derived tumor xenograft.

5. Conclusion

In summary, targeting both YAP/TAZ and EGFR L858R/T790 M mutation might be a promising therapeutic approach to overcome EGFR-TKIs resistance. Our study demonstrated for the first time that gossypol targeted both YAP/TAZ and EGFR$^{L858R/T790M}$ in NSCLC cells, suggesting that gossypol was a potential candidate to overcome EGFR-TKIs resistance in NSCLC patients.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgements

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Appendix A. Supplementary data

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