Curcumin inhibits prostate cancer progression by regulating the miR-30a-5p/PCLAF axis

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Abstract. Curcumin has been shown to inhibit the growth of a variety of tumor cells. However, the biological functions of curcumin in prostate cancer (PCa) have not yet fully elucidated. The objective of the present study was to investigate the role of curcumin on the proliferation, migration, invasion and apoptosis of PCa cells and the underlying mechanism. Cell Counting Kit-8 and flow cytometry were used to detect the effects of curcumin at different concentrations on the proliferation and apoptosis of PCa cell lines, PC-3 and DU145. BrdU and Transwell assays, western blotting and reverse transcription-quantitative PCR were used to determine the effect of curcumin on cell proliferation, migration and invasion, apoptosis-related protein expression, and microRNA (miR)-30a-5p and PCNA clamp associated factor (PCLAF) expression, respectively. In addition, bioinformatics analysis and Pearson's correlation test were used to verify the relationship between miR-30a-5p and PCLAF. Curcumin was observed to impede the proliferation, migration and invasion of PCa cells, and promote their apoptosis in a time- and dose-dependent manner. Curcumin enhanced miR-30a-5p expression and inhibited PCLAF expression; furthermore, there was a negative correlation between miR-30a-5p and PCLAF expression in PCa tissues. In addition, transfection of miR-30a-5p inhibitors partially reversed the function of curcumin on cell proliferation, migration, invasion and apoptosis. Overall, curcumin suppressed the malignant biological behaviors of PCa cells by regulating the miR-30a-5p/PCLAF axis.

Introduction

In 2019, prostate cancer (PCa) was one of the most common malignancies among males worldwide (1). Patients with early stages of PCa can be cured by radical surgery or radiotherapy, but the treatment of advanced prostate cancer is still dependent on androgen deprivation therapy (ADT) (2). Although ADT can effectively inhibit the progression of PCa, resistance of PCa cells to ADT can lead to tumor metastasis (3). Therefore, novel therapy strategies are urgently needed in the treatment of PCa.

Curcumin is a polyphenolic compound derived from *Curcuma longa* L., which is used as perfume in India and herbal medicine in China (4,5). Previous studies indicate that curcumin has anti-inflammatory, anti-oxidative, anti-infective, anti-fibrotic and anti-atherosclerotic effects, and that it exhibits low toxicity (5,6). The anticancer properties of curcumin have attracted increasing attention. Accumulating studies have indicated that curcumin inhibits the occurrence, development, chemoresistance and radioresistance of various malignancies (7-13). For example, curcumin inhibits the proliferation of colon cancer cells by inhibiting the Wnt/β-catenin signaling pathway, and can increase the radiosensitivity of PCa cells by inhibiting autophagy (11,12). In addition, curcumin suppresses the activity of JNK signaling in PCa cells, thereby impeding cell proliferation and promoting apoptosis (13). Nevertheless, the mechanism of curcumin in blocking PCa progression has not been clearly clarified.

Certain studies have reported that curcumin can inhibit cancer progression by regulating microRNA (miRNA/miR) expression (7,14-15). For example, in PCa, curcumin inhibits cancer cell proliferation and migration by regulating the miR-143/phosphoglycerate kinase 1 axis (14); curcumin induces the upregulation of miR-145, which in turn inhibits PCa cell proliferation by repressing octamer-binding transcription factor 4 expression (15). Therefore, the present study was designed to uncover the biological functions and specific mechanisms of curcumin/miR-30a-5p/PCNA clamp associated factor (PCLAF) axis in PCa via *in vitro* assays.

Materials and methods

Clinical specimens. PCa tissues and the adjacent benign tissues (at 5-cm distance) of 35 patients with PCa who visited Zhongshan Hospital Affiliated to Fudan University (Shanghai, China) from March 2017 to March 2019 were collected. All of the patients were diagnosed via biopsy and received radical prostatectomy. There were 21 male and 14 female patients.

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Their age range was 49-71 years (mean, 60.9±5.1). Among the patients enrolled, the Gleason score was ≥8 points in 11 cases and <8 points in 24 cases, and the serum prostate-specific antigen levels were ≤10 ng/ml in 19 cases and >10 ng/ml in 16 cases. All tissue specimens were surgically removed and quickly frozen in liquid nitrogen. The study was approved by The Ethics Review Board of Zhongshan Hospital Affiliated to Fudan University, and all participants provided written informed consent.

Cell culture, drug treatment and transfection. Both PC-3 and DU145 cells were purchased from the China Center for Type Culture Collection. The miR-30a-5p inhibitor (5'-CUUCCAGUCGAGGAUGUUUACA-3') and non-targeting control (5'-ACAUAGGGCCCAUGCUAACUGC-3') were procured from Guangzhou RiboBio Co., Ltd. PC-3 and DU145 cells were cultured in RPMI-1640 medium (Thermo Fisher Scientific, Inc.) containing 10% FBS (Thermo Fisher Scientific, Inc), 100 μg/ml penicillin and 100 μg/ml streptomycin (Sigma-Aldrich; Merck KGaA) at 37°C in 5% CO_2. When the cells had reached 70-80% confluence, the miR-30a-5p inhibitor and control were transfected into PC-3 and DU145 cells at 37°C for 6 h at a final concentration of 50 μM according to the manufacturer's instructions of Lipofectamine® 2000 (Thermo Fisher Science, Inc.). After 24 h, cells were treated with curcumin (cat. no. 239802; Sigma-Aldrich; Merck KGaA) at different concentrations (10, 20, 30, 40 or 50 μmol/l) at 37°C for 12, 24 or 48 h. After that, cells were harvested for subsequent experiments.

Cell Counting Kit-8 (CCK-8) assay. PC-3 and DU145 cells in the logarithmic growth phase were seeded in a 96-well cell plate ($2x10^3$ cells/well). Subsequently, the cells were randomly divided into six groups with different concentrations of curcumin: i) Control group (0 μmol/l); ii) 10 μmol/l curcumin group; iii) 20 μmol/l curcumin group; iv) 30 μmol/l curcumin group; v) 40 μmol/l curcumin group; and vi) 50 μmol/l curcumin group. CCK-8 solution (10 μl; Beyotime Institute of Biotechnology) was supplemented to each well at 12, 24 and 48 h, and then the cells were incubated at 37°C for a further 4 h. After that, the absorbance value of each well was detected by a microplate reader (GENios FL Fluorescence Microplate Reader FI TRF; Tecan Group, Ltd.) at a wavelength of 450 nm, and the average absorbance value of three wells were used to evaluate the viability of cells.

Flow cytometry analysis. The Annexin V-FITC/propidium iodide (PI) double staining method was used to analyze the apoptosis of PCa cells in this study. A total of $1x10^6$ PC-3 or DU145 cells from each group were harvested and resuspended using 100 μl 1X binding buffer (BSL Bioservice), followed by the addition of 5 μl Annexin V-FITC solution and 5 μl PI solution (both from Beyotime Institute of Biotechnology), mixed thoroughly and incubated in the dark at room temperature for 15 min. Subsequently, the apoptosis rate was detected using a flow cytometer (BD Biosciences) within 1 h and the data were analyzed using FlowJo v10.07 software (FlowJo, LLC).

Reverse transcription-quantitative PCR (RT-qPCR). PC-3 and DU145 cells treated with 30 μmol/l curcumin for 24 h were collected. Total cellular RNA was extracted using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.). RNA concentration and purity were measured on the NanoDrop 2000 (NanoDrop Technologies; Thermo Fisher Scientific, Inc.) using 1 μl of RNA. After the concentration and purity of RNA were determined, total RNA was reverse transcribed into cDNA using a RevertAid First Strand cDNA Synthesis Kit (1 h at 37°C; Thermo Fisher Scientific, Inc.). RT-qPCR was conducted using a CFX96 quantitative PCR system (Bio-Rad Laboratories, Inc.) with an SYBR® Green Premix Ex Taq II kit (Takara Biotechnology Co., Ltd.) in accordance with the manufacturer's instructions. The following temperature protocol was used for reverse transcription: 37°C For 2 min, 23°C for 10 min, 55°C for 10 min and 85°C for 10 min. U6 was identified as the internal reference for miR-30a-5p, and β-actin was used as the internal reference for PCLAF. The levels of miR-30a-5p and PCLAF mRNA expression were calculated using the $2^{-\Delta\Delta Cq}$ method (16). The primer sequences were shown in Table I.

BrdU experiment. PC-3 and DU145 cells were prepared into a single-cell suspension and inoculated in a 96-well plate ($1x10^4$ cells/well). The cells were then divided into control- and curcumin-treated groups. After being treated with 30 μmol/l curcumin for 24 h, BrdU solution (Beyotime Institute of Biotechnology) was added at a final concentration of 30 μmol/l and then the cells were incubated at 37°C for 8 h. The cells were washed with PBS three times. Subsequently, the cells were fixed with 4% paraformaldehyde at room temperature for 30 min and rinsed with PBS for three times again. A total of 2 mol/l HCl was added to denature the DNA for 30 min at 37°C and then cells were immersed in PBS containing 0.1% Tween-20 for 30 min. After that, non-specific antigens were blocked with 3% BSA (Sigma-Aldrich; Merck KGaA) for 1 h at room temperature. Following which, mouse anti-BrdU antibody (1:1400; cat. no. 5292S; Cell Signaling Technology, Inc.) was incubated with the cells for 2 h at room temperature. The cells were rinsed for three times with PBS, and the secondary antibody incubated with the cells for 1 h at room temperature. Subsequently, DAPI staining solution was used to stain the nuclei at 37°C for 30 min. The cells were observed and images captured under a fluorescence microscope (magnification, x400; Olympus Corporation). Overall, five visual fields were observed under fluorescence microscope for each sample. Cell proliferation rate = the number of BrdU staining positive cells/total DAPI staining positive cells x100%.

Scratch assay. Wound healing was used to evaluate PCa cell migration. PC-3 and DU145 cells were seeded into six-well plates at a density of $5x10^5$ cells/well. After the cells were cultured to 100% confluence and serum starved for 24 h, a sterile 200-μl pipette tip was used to scratch the cells at the center of the well. The cells were washed with PBS to remove the cell debris, and the cells were cultured in serum-free medium. Closure of the wound was observed under an inverted fluorescence microscope (magnification, x400; Olympus Corporation) at 0 and 24 h after scratching.

Transwell assay. Cells in each group were trypsinized, harvested and resuspended in serum-free medium to adjust the

Table I. Primer sequences used for reverse transcription-quantitative PCR.

Name	Primer sequences, 5'-3'
miR-30a-5p forward	GGGCCTGTAAACATCCTCG
miR-30a-5p reverse	GAATACCTCGGACCCTGC
PCLAF forward	ATGGTGCGGACTAAAGCAGAC
PCLAF reverse	CCTCGATGAAACTGATGTCGAAT
U6 forward	GCTTCGGCAGCACATATACTAAAAT
U6 reverse	CGCTTCACGAATTTGCGTGTCAT
β-actin forward	TCCCTCAAGATTGCTAGCAA
β-actin reverse	AGATCCACAACGGATACATT

miR, microRNA; PCLAF, PCNA clamp associated factor.

cell concentration to $5x10^5$ cells/ml. To evaluate the migration of cells, a Transwell system (8-μm pore-size; Corning, Inc.) was used. A total of 200 μl cell suspension was added to the upper chamber, 600 μl RPMI-1640 medium containing 10% FBS (both from Thermo Fisher Scientific, Inc.) was added to the lower chamber and the cells were cultured in an incubator for 24 h. Subsequently, the cells in the upper chamber were carefully wiped off. The migrated cells were fixed with formaldehyde at 37˚C for 15 min, and then stained with crystal violet solution at 37˚C for 30 min. After being rinsed with PBS, the cells were dried and observed under an inverted fluorescence microscope (magnification, x400). Overall, five visual fields were observed under fluorescence microscope for each sample. To test cell invasion, the membrane of the Transwell system was coated with Matrigel for 1 h at 37˚C (BD Biosciences) before the experiment, and the other steps were as the same as the migration assay.

Western blotting. PC-3 and DU145 cells were treated with 30 μmol/l curcumin for 24 h, and then the protease inhibitor-containing RIPA buffer (Beyotime Institute of Biotechnology) was added to lyse the cells on ice for 40 min. Then, the lysate was centrifuged at 1,500 x g for 15 min to obtain the supernatant. Protein concentration determination was performed using the BCA protein determination assay and 30 μg of protein was loaded per lane. Thereafter, the protein samples were loaded, 10% SDS-PAGE was performed and then the proteins were electrically transferred onto the PVDF membrane (EMD Millipore). After that, 5% skim-milk was used to block the non-specific antigens at room temperature. Next, primary antibodies were added to incubate the membrane at 4˚C overnight, and then the membrane was subsequently incubated with horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. Then, ECL chemiluminescent solution (Beyotime Institute of Biotechnology) was used to visualize the bands and the bands were quantified using ImageJ software (v1.52; National Institutes of Health). The antibodies used in this study were obtained from Abcam: Anti-PCLAF Antibody (cat. no. ab226255; 1:1,000), anti-Bax antibody (cat. no. ab32503; 1:1,000), anti-Bcl-2 antibody (cat. no. ab185002; 1:1,000), anti-caspase-3 antibody (cat. no. ab13847, 1:1,000), anti-cleaved caspase-3 antibody (cat.

no. ab2302; 1:500), anti-β-actin antibody (cat. no. ab8227; 1:500) and horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (cat. no. ab205718; 1:1,000).

Bioinformatics analysis. The Gene Expression Profiling Interactive Analysis (GEPIA) database (http://gepia.cancer-pku.cn/) was used to analyze PCLAF expression in PCa samples. The StarBase v3.0 database (http://starbase.sysu.edu.cn/) was used to analyze the correlation between miR-30a-5p expression and PCLAF expression in PCa samples. Cut-off values were as follows: |Log2FC| Cut-off =1, P-value cut-off =0.01.

Statistical analysis. All data are expressed as mean ± standard deviation (unless otherwise shown). SPSS v22.0 (IBM Corp.) and GraphPad Prism 8.0 (Version X; GraphPad Software, Inc.) were applied for statistical analysis. All experiments were performed in triplicate. Whether the data were normally distributed was examined using a one-sample Kolmogorov-Smirnov test. For normally distributed data, an independent sample t-test was used to make the comparison between two groups. One-way ANOVA was used to compare three or more groups. If there was a significant difference, Student-Neuman-Keuls or Tukey's tests were used to determine significant differences between two groups. Correlation was analyzed using Pearson correlation coefficient. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Function of curcumin on the viability and apoptosis of PCa cells. PCa cell lines PC-3 and DU145 cells were treated with different concentrations of curcumin (0, 10, 20, 30, 40 and 50 μmol/l), and a CCK-8 assay was employed to detect cell viability at 12, 24 and 48 h after treatment. As shown in Fig. 1A, compared with the untreated group, curcumin inhibited the viability of PCa in concentration- and time-dependent manners. As seen in Figs. 1B and S1, after being treated with curcumin for 24 h, the apoptosis rate of PCa cells gradually increased with increasing concentrations of curcumin. Moreover, when PCa cells were treated with 30 μmol/l curcumin, the apoptotic rate gradually increased with the treatment time (Figs. 1C and S1). These observations suggested that curcumin can inhibit cell viability and promote apoptosis.

Curcumin inhibits the proliferation, migration and invasion of PCa cells and promotes apoptosis. To further examine the role of curcumin on PCa cell proliferation, migration and invasion, BrdU, wound healing and Transwell assays were performed. As shown in Fig. 2A-C, after PC-3 and DU145 cells were treated with 30 μmol/l curcumin for 24 h, cell proliferation, migration and invasion were significantly reduced compared with the control untreated group. Western blotting was used to detect the expression of apoptosis-related proteins. As shown in Fig. 2D, after treatment with curcumin, the expression levels of Bax and cleaved caspase-3 were increased in both PC-3 cells and DU145 cells, while the expression levels of Bcl-2 and caspase-3 were decreased. These results further indicated that curcumin exhibited inhibitory effects on PCa cells.
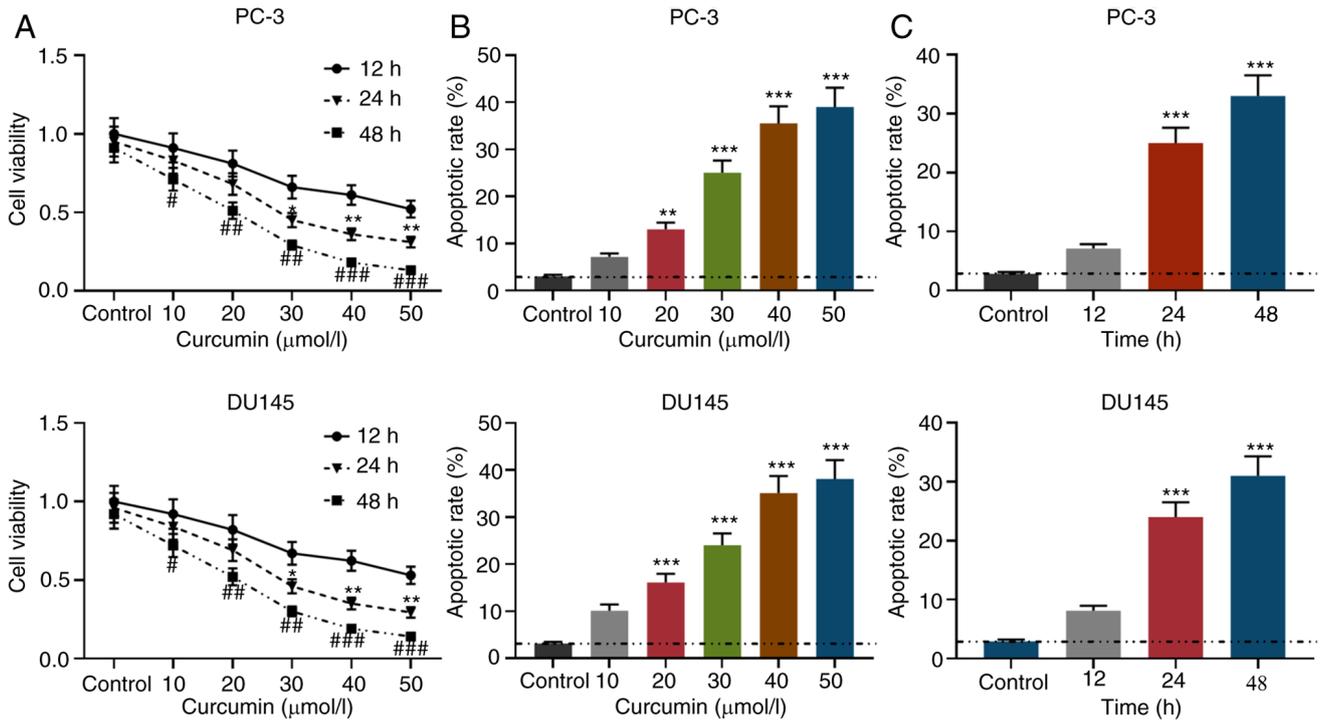
Figure 1. Curcumin inhibits PCa cell viability and promotes apoptosis. (A) Cell Counting Kit-8 was used to detect the viability of PCa cells treated with different concentrations of curcumin for different times. Flow cytometry was used to detect the apoptosis rates of PCa cells (B) treated with 0-50 μmol/l of curcumin for 24 h and (C) treated with 30 μmol/l curcumin for different times. *P<0.05, **P<0.01, ***P<0.001 vs. 12 h or Control; $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 vs. 24 h. PCa, prostate cancer.

Curcumin suppresses PCLAF expression by upregulating miR-30a-5p in PCa cells. miR-30a-5p exerts a tumor-suppressive effect in colorectal cancer and melanoma by inhibiting cell proliferation, metastasis, differentiation and the cell cycle (17-19). A recent study reported that miR-30a-5p has low expression levels in PCa, and downregulation of miR-30a-5p promotes the progression of PCa by targeting PCLAF (20). After PC-3 and DU145 cells were treated with 30 μmol/l curcumin for 24 h, the cells were collected and the levels of PCLAF and miR-30a-5p expression in PC-3 and DU145 cells were detected via RT-qPCR and western blotting. The results showed that in comparison with the untreated group, miR-30a-5p expression was significantly increased in PCa cells, while PCLAF expression was decreased (Fig. 3A and B).

Through analyzing the data from GEPIA, it was demonstrated that PCLAF expression was upregulated in PCa samples compared with normal tissues adjacent to PCa cancer tissues (Fig. 3C). Further analysis of the data from the StarBase database found that in prostate adenocarcinoma samples, miR-30a-5p expression and PCLAF expression were significant (r=-0.215; Fig. 3D). Levels of miR-30a-5p and PCLAF mRNA expression were also detected in the 35 pairs of PCa tissues/adjacent tissues collected from patients; miR-30a-5p was downregulated in PCa tissues, while PCLAF was upregulated (Fig. S2A and B). Additionally, the expression levels of miR-30a-5p and PCLAF were negatively correlated in PCa tissues (Fig. 3E). Considering that PCLAF is a target gene of miR-30a-5p (20), it was concluded that curcumin could inhibit the expression of PCLAF by promoting the expression of miR-30a-5p.

Curcumin inhibits the malignant biological behaviors of PCa cells partly by regulating miR-30a-5p. To investigate whether curcumin affected PCa cells through regulating miR-30a-5p and PCLAF, before PCa cells were treated with 30 μmol/l curcumin for 24 h, miR-30a-5p inhibitors were transfected into PCa cells (Fig. S2C). As shown in Fig. 4A, the transfection of miR-30a-5p inhibitors partially reversed the inhibitory effect of curcumin on the expression of PCLAF in both PCa cell lines. Subsequently, through the BrdU, wound healing and Transwell assays, it was found that curcumin significantly suppressed PCa cell proliferation, migration and invasion, while miR-30a-5p inhibitors partially reversed these effects (Figs. 4B-D and S1). Additionally, transfection of miR-30a-5p inhibitors partially reversed the effects of curcumin on the expression of Bax, Bcl2, cleaved caspase-3 and caspase-3 (Fig. 4E). These results further validated that the miR-30a-5p/PCLAF axis was a significant downstream effector of curcumin.

Discussion

A number of studies have shown that curcumin exerts significant anticancer effects in diverse malignancies, inhibiting cancer cell proliferation and metastasis, promoting apoptosis, and increasing chemosensitivity and radiosensitivity (21-24). Curcumin suppresses the proliferation, migration and invasion of PCa cells by regulating multiple signaling pathways. For example, it inhibits the expression of membrane type 1-MMP and MMP2 in PCa cells, and suppresses the viability and metastasis of cancer cells via the Notch-1 signaling pathway (21). A similar study reports that curcumin induces PCa apoptosis and
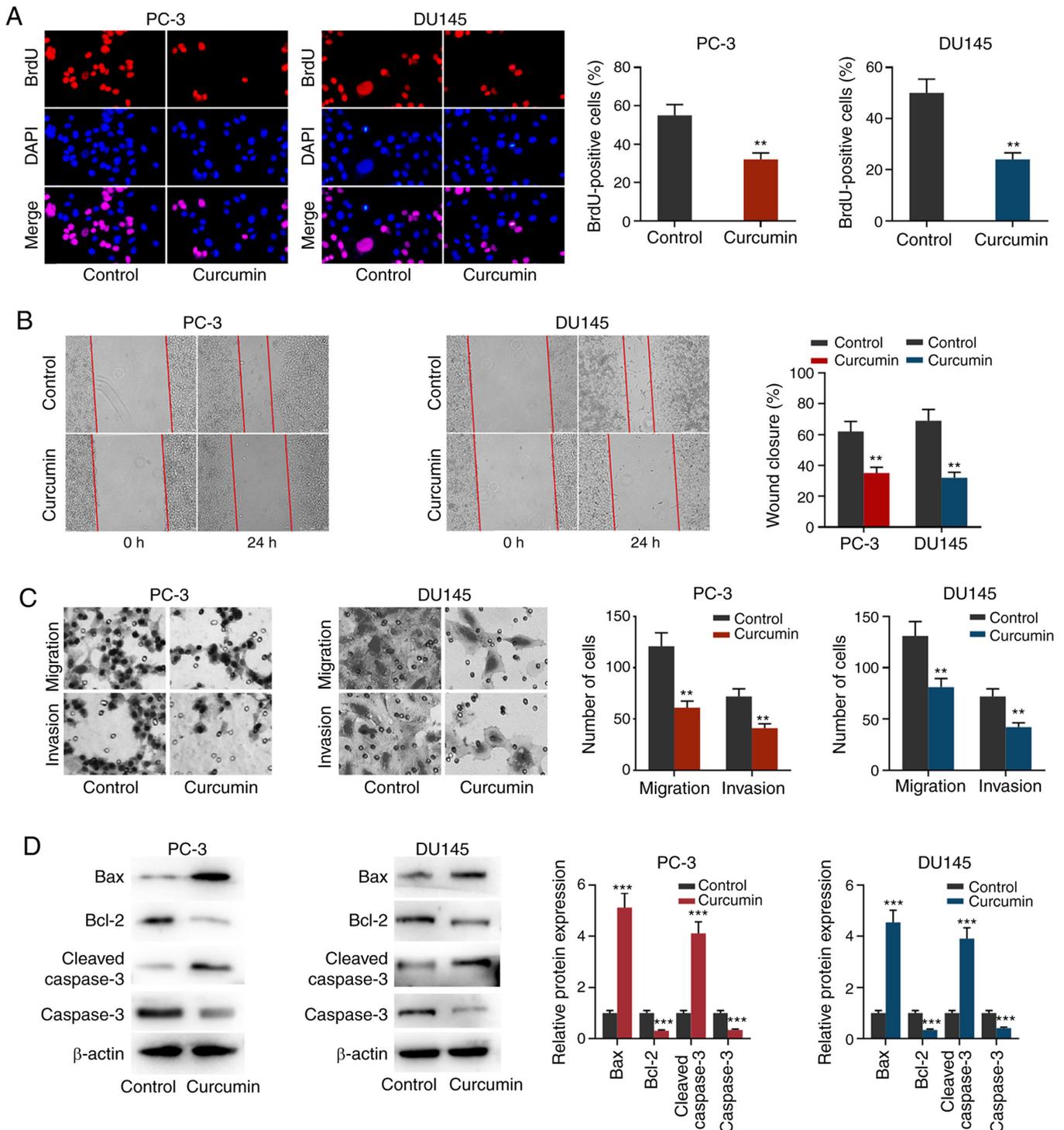
Figure 2. Curcumin inhibits PCa cell proliferation, migration and invasion, and regulates apoptosis-related proteins. (A) BrdU assay was used to detect the proliferation of PCa cells treated with 30 μmol/l curcumin for 24 h (magnification, x400). (B) Wound healing (magnification, x400) and (C) Transwell assays were used to detect the of migration and invasion of PCa cells treated with 30 μmol/l curcumin for 24 h (magnification, x400). (D) Western blotting was used to detect the effect of curcumin on the expression of apoptosis-related proteins. **P<0.01, ***P<0.001 vs. Control. PCa, prostate cancer.

G0/G1 arrest by inhibiting Notch signaling (22). In addition, curcumin represses the monoamine oxidase A/mTOR/hypoxia inducible factor-1α signaling pathway, impedes the production of reactive oxygen species, inhibits the expression levels of C-X-C chemokine receptor 4 and IL-6 receptors, and suppresses the epithelial-mesenchymal transition of PCa cells mediated by cancer-associated fibroblasts (23). Moreover, curcumin inhibits nuclear β-catenin transcription activity

in PCa cells by activating polycystin-1, thereby suppressing the proliferation, colony formation and motility of cancer cells (24). In the present study, it was observed that curcumin could inhibit PCa cell proliferation, migration and invasion, and promote apoptosis in a concentration- and time- dependent manner. These experiments indicated that curcumin exhibited tumor-suppressive properties in PCa, which is consistent with previous reports.
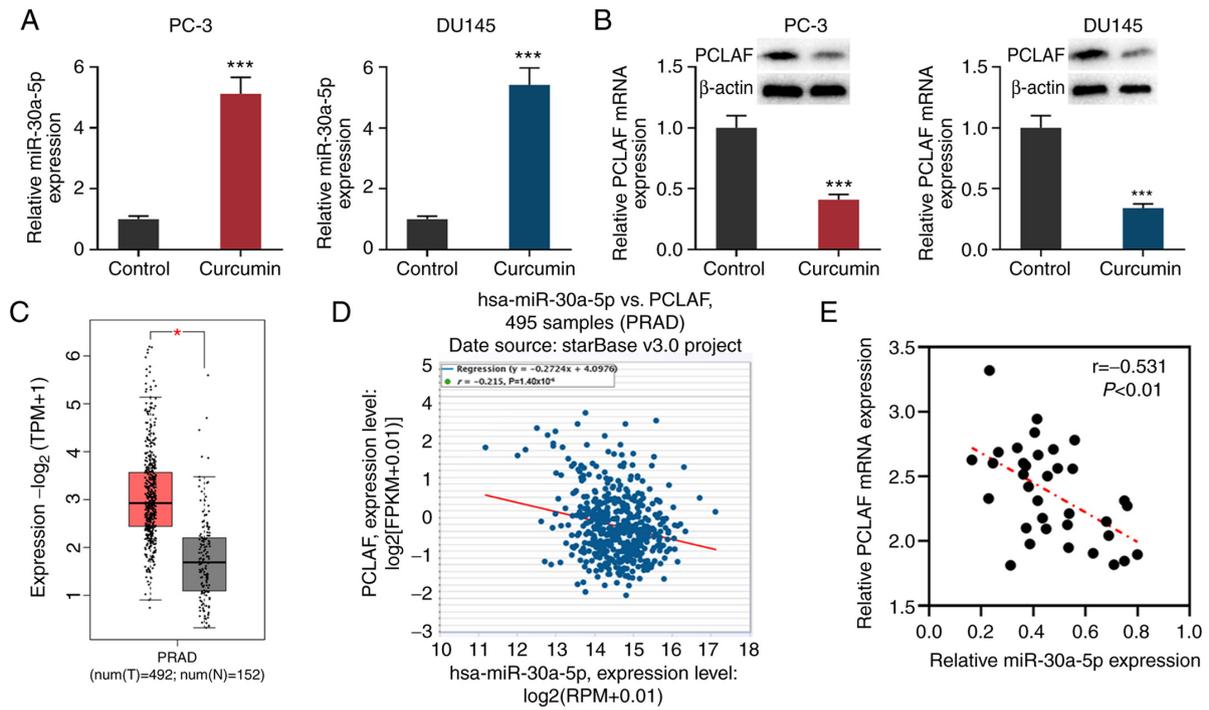
Figure 3. Curcumin promotes miR-30a-5p expression and inhibits PCLAF expression. (A) RT-qPCR was employed to detect the effect of curcumin on miR-30a-5p expression. (B) RT-qPCR (left) and western blotting (right) were used to detect the effect of curcumin on PCLAF mRNA and protein expression. (C) PCLAF expression in PCa samples was analyzed using the Gene Expression Profiling Interactive Analysis database. (D) StarBase database was used to analyze the correlation between miR-30a-5p expression and PCLAF mRNA expression in PCa samples. (E) miR-30a-5p expression and PCLAF mRNA expression in 35 patients with PCa were detected using RT-qPCR and their correlation was analyzed. *P<0.05 vs. normal tissue. ***P<0.001 vs. Control. miR, microRNA; RT-qPCR, reverse transcription-quantitative PCR; PCLAF, PCNA clamp associated factor; PCa, prostate cancer; PRAD, prostate adenocarcinoma; N, normal; T, tumor.
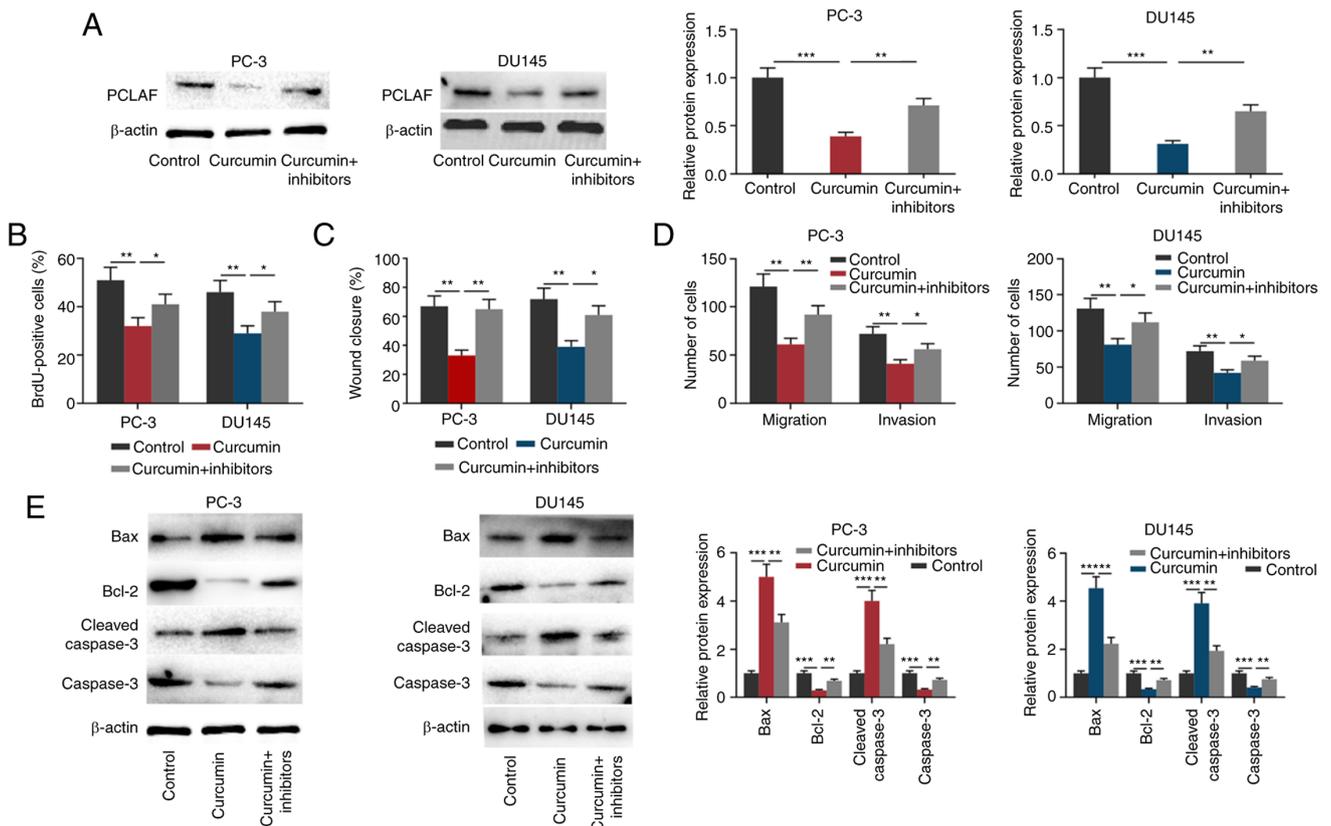

Figure 4. Curcumin inhibits the malignant phenotypes of PCa cells via miR-30a-5p. (A) After transfection of miR-30a-5p inhibitors and curcumin treatment, PCLAF expression in PC-3 cells and DU145 cells was detected via western blotting. (B) BrdU assay was used to detect the proliferation of PCa cells. (C) Wound healing and (D) Transwell assays were used to detect the migration and invasion of PCa cells. (E) Effects of curcumin and miR-30a-5p inhibitors on the expression of apoptosis-related proteins were detected via western blotting. *P<0.05, **P<0.01, ***P<0.001. PCa, prostate cancer; miR, microRNA; PCLAF, PCNA clamp associated factor.

An increasing number of studies have shown that miRNAs have notable effects on cell proliferation, apoptosis and differentiation (25-28). A number of studies report that miRNAs are implicated in carcinogenesis and cancer progression. For example, miR-9-5p can suppress the proliferation of glioma cells by downregulating forkhead box P2 expression (28). In PCa, miR-381 suppresses the proliferation and invasion of PCa cells by downregulating androgen receptor expression (29). Curcumin can exert tumor-suppressive effects by regulating the expression of a diverse number of miRs (12,30-33). For example, in laryngeal squamous cell carcinoma, curcumin inhibits the activity of the PI3K/Akt/mTOR signaling pathway by upregulating miR-145 expression, thereby inhibiting the proliferation, cell cycle procession and metastasis of cancer cells (32). In nasopharyngeal carcinoma, curcumin suppresses cancer cell proliferation and metastasis by regulating the miR-7/Skp2/p21 axis (33). In PCa, curcumin represses autophagy through the miR-143/ATG2B axis, thereby increasing the radiosensitivity of cancer cells (12).

The present study observed that miR-30a-5p expression was induced by curcumin treatment in both PC-3 and DU-145 cells. Notably, after PCa cells were transfected with miR-30a-5p inhibitors, the antitumor activity of curcumin was attenuated. These results suggested that miR-30a-5p was a downstream effector of curcumin, and the function of curcumin in inhibiting PCa progression was partly dependent on miR-30a-5p.

miR-30a-5p is well known as a tumor suppressor (34-36). In breast cancer, miR-30a-5p targets the regulation of lactate dehydrogenase A to inhibit the Warburg effect, and impedes the proliferation and metastasis of cancer cells (37). In non-small cell lung cancer, miR-30a-5p downregulates CD73 expression and inhibits proliferation, metastasis and epithelial-mesenchymal transition by regulating the EGF signaling pathway (38). In PCa, miR-30a-5p is also significantly downregulated in cancer tissues, and it suppresses the proliferation of PCa cells by inhibiting PCLAF (20). PCLAF, also known as KIAA0101, is an upregulated oncogene in a variety of tumors (39). Previous studies have shown that PCLAF plays a vital role in cell proliferation, cell survival, DNA repair and tumorigenesis (40-42). For example, PCLAF-knockdown suppresses cancer cell proliferation and cell cycle progression by promoting the formation of p53/Sp1 complex in breast cancer (41). miR-429 inhibits the progression of epithelial ovarian cancer by targeting PCLAF (42). The present study demonstrated that PCLAF was highly expressed in PCa samples and that miR-30a-5p expression was negatively associated with PCLAF expression in PCa samples. These data further evidenced the regulatory effect of miR-30a-5p on PCLAF, which is consistent with previous reports (20). Moreover, curcumin treatment downregulated PCLAF expression in both PC-3 and DU145 cell lines. Collectively, these data showed that curcumin could inhibit PCLAF to block the progression of PCa by inducing the expression of miR-30a-5p. However, the weak correlation between miR-30a-5p and PCLAF predicted by bioinformatics in the present study is limitations the conclusions of this study.

In summary, curcumin inhibited the proliferation and metastasis, and promoted the apoptosis of PCa cells by regulating the miR-30a-5p/PCLAF axis. The present study provides novel insights into the mechanism of curcumin's tumor-suppressive functions. Notably, that curcumin may repress the malignant phenotypes of PCa cells by regulating other miRNAs and proteins, which remains to be clarified in future studies.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

LP, JS and JG conceived and designed the experiments. LP, WL and TB performed the experiments. YW and WL performed the statistical analysis. LP, JS and TB drafted the manuscript. LP, JS and JG confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by The Ethics Review Board of Zhongshan Hospital Affiliated to Fudan University and all participants provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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