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Curcumol inhibits colorectal cancer proliferation by targeting miR-21 and modulated PTEN/PI3K/Akt pathways

Haowei Liu[a,1], Juan Wang[b,c,1], Yexing Tao[b], Xumei Li[a], Jianli Qin[a], Zhun Bai[d], Bixia Chi[e], Wei Yan[f], Xu Chen[a,*]

[a] College of Pharmacy, Guilin Medical University, 541004 Guilin, China
[b] Research Center for Science, Guilin Medical University, Guilin 541004, China
[c] Xiangya Hospital, Central South University, 410008 Changsha, China
[d] Intensive Care Unit, Zhuzhou Central Hospital, 412007 Zhuzhou, China
[e] Digestive System Department, The Frist People's Hospital of Yueyang, 414000 Yueyang, China
[f] The Frist hospital of Fuzhou, 350000 Fuzhou, China

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ABSTRACT

Aims: The purpose of this study was to demonstrate how curcumol affected the expression of miR-21 and whether its effects on miR-21 was associated with the activation of PTEN/PI3K/Akt pathways in CRC cells.
Main methods: MTT and xenograft assay were used to examine how curcumol inhibits colorectal cancer (CRC) cells' growth. Q-PCR and western blot analysis were employed to test the role of miR-21 in the inhibition of curcumol on proliferation and PTEN/PI3K/Akt pathways of CRC cells.
Key findings: We found that curcumol effectively inhibited CRC cells from proliferating via the PTEN/PI3K/Akt pathways and reduced expression of miR-21 both in vitro and in vivo. miR-21 mimics were found to decrease the protein level of PTEN and increase the expression of PI3K, phospho-Akt (p-Akt) and NF-κB, while miR-21 sponge (miR-21-SP) enhanced the expression of PTEN and reduced the activity of PI3K, Akt and NF-κB. Furthermore, miR-21-SP strengthened the role of curcumol in up-regulating PTEN and inhibiting PI3K/Akt pathways, but miR-21 reversed the effect of curcumol on the PTEN/PI3K/Akt pathways.
Significance: Our research demonstrated that curcumol reduced the proliferation of CRC cells through PTEN/PI3K/Akt by targeting miR-21 and miR-21 could be a target molecule of curcumol for CRC treatment.

1. Introduction

Colorectal cancer (CRC) has became the top three deadly cancer in the US [1], it has also been one of the five most commonly diagnosed cancer in China [2], Its incidence rate has gradually increased year by year [3]. Although a small amount of targeted inhibitors of CRC have been used in clinically, due to the complex pathogenic mechanism of malignant tumors, those targeted chemotherapeutic drugs are only effective for a small number of people and have certain side effects. Complex pathogenic mechanism of malignant tumors refers to the mechanism of occurrence, development of tumor and the target of treatment as complicated and multi-faceted, with reference to cancer. Accordingly, it is necessary to explore new molecular targets and more effective but less toxic medicines, in order to address long-term prognosis and high chance of recurrence of CRC, as its main problem, and to delay CRC's progression.

MicroRNA (miRNA) is an endogenous none-coding RNA, inextricably linked with the occurrence and development of malignant tumors. miR-21, one of the most common miRNAs associated with cancer patient's diagnoses, has emerged as a novel molecular target for cancer therapy [4–6]. Since mature miR-21 is abundant in most cancer cell lines, and according to the literature [7], it is the only highly expressed miRNA in 540 human tumor from different races [8], which attracts our attention. It has been confirmed that over-expression or knockdown of miR-21 in breast, liver and glioma cells has an effect on cell growth and development [9–11]. In CRC, miR-21 is also significantly increased and associated with patients' poor survival, and high miR-21 has been reported as an emerging biomarker in colon cancer [8]. However, it has not yet been fully clarified which signaling pathways is involved with miR-21's inhibition in CRC cells' proliferation

* Corresponding author.
E-mail address: chenxu@glmc.edu.cn (X. Chen).
[1] Haowei Liu and Juan Wang contributed equally.

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yet. PI3K/Akt signaling pathways are associated with growth and proliferation of malignant cells, which is positively regulated by miR-21 [12,13]. Meanwhile, the phosphatase and tension homolog, which is missed on chromosome ten (PTEN), works as a tumor suppressor gene and participates in tumors' growth, apoptosis, adhesion, invasion and migration partially by inhibiting phosphorylation of Akt (p-Akt), which is the active form of Akt [14,15]. In addition, PTEN antagonizes PI3K-mediated cell growth and proliferation by converting PIP3 to PIP2 (phoSphatidylinositol-2-phosphate) [16]. As we all known, PTEN is one of miR-21's target genes; increasing evidence indicates thatmiR-21 promotes cell proliferation via PTEN-dependent PI3K/Akt activation in cancer cells [17,18]. Since miR-21 and PTEN/PI3K/Akt pathways are closely related to the occurrence of cancer and miR-21 has been reported to target and inhibit the activity of the PTEN/PI3K/Akt pathways in lung cancer, gastric cancer and esophageal cancer [19–21]. We hypothesized that miR-21 could target the PTEN/PI3K/Akt pathways to inhibit CRC cells' proliferation.

Curcumol, a natural monomeric sesquiterpenoid product extracted from *Rhizoma Curcumae*, could inhibit cancer cells' proliferation in several cancers, including lung carcinoma, breast carcinoma, gastric adenocarcinoma, nasopharyngeal carcinoma and colorectal cancer [22–26]. We found that curcumol could inhibit CRC from growing through the PI3K/Akt pathways. However, it has rarely been reported whether curcumol could inhibit the proliferation of CRC cells via miR-21 and there is the correlation between miR-21 and PTEN/PI3K/Akt pathways in CRC. Considering the gaps, we considered whether miR-21 is a target of curcumol on CRC. Therefore, the purpose of this experiment was to demonstrate how curcumol inhibits the proliferation of CRC and verify whether miR-21 is a potential target of curcumol and affects the PTEN/PI3K/Akt pathways in a cascade on CRC cells.

2. Materials and methods

2.1. Reagents and cell lines

RPMI 1640 was purchased from Gibco BRL (Grand Island, NY, USA), fetal bovine serum (FBS) was purchased from Gibco (Auckland, New Zealand), penicillin-streptomycin was purchased from Solarbio Biotechnology Company (Shanghai, China), curcumol was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO) were purchased from Amresco Chemical Company (Solon, Ohio, USA), miR-21 mimics was purchased from TSINGKE Biological Technology Company (Beijing, China), miR-21 sponge was purchased from ViGene Biosciences Company (Shandong, China), lipofectamine 2000 was purchased from Thermo Fisher Scientific (Waltham, MA, USA), BALB/c nude mice was purchased from Slac Laboratory Animal Company (Shanghai, China), Antibodies Akt, p-Akt, p85, p-p85, PTEN, NF-κB, were purchased from Wanlei Biotechnology Company (Shenyang, China), antibody β-actin was purchased from Santa Cruz Biotechnology Company (Santa Cruz, CA, USA), peroxidase-conjugated goat anti rabbit or mouse IgG second antibodies were purchased from Emarbio Science Technology Company (Beijing, China), All-in-One miRNA Detection Kit was purchased from GeneCopoeia Company (Rockville, MD, USA), Trizol reagent (TIANGEN, Beijing, China), SYBR premix Ex Taq (TAKARA, Dalian, China).

2.2. Cell culture and treatment

Human colorectal cancer cells lines LoVo, HCT-116, SW480 were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 U/mL streptomycin. The cells were grown on 700 mm culture dishes at 37 °C in a humidified atmosphere of 5% CO_2, and were routinely passaged 3–4 times a week. Curcumol is a sesquiterpene compound, it is hardly soluble in water, but soluble in organic solvents such as ethanol and DMSO. Therefore, we consulted the literature and pre-experiment and chose to dissolve the curcumol with absolute ethanol as a 10 mg/mL stock solution, then dilute to the desired concentration with cell culture medium before using.

2.3. MTT assay

The cells were seeded in 96-well plates at 1000 cells per well, and treated with different concentration of curcumol (0, 12.5, 25, 50,100 μg/mL), After 24, 48, 72, 96, 120 h later, each well was supplemented with 20 μL MTT(5 mg/mL) and incubated for 4 h at 37 °C, then removed the medium, added 150 μL DMSO to each well and incubated the plates at 37 °C for 30 min without light, the optical density was read at 490 nm on TECAN M200 plate reader. According to the difference in absorbance, the value of cells' proliferation under different concentrations of drugs was measured. All experiments were repeated at least 3 times.

2.4. Cell treatment and transfection

Cells were seeded into 700 mm dishes, and treated with different concentrations of curcumol (0, 12.5, 25, 50, 100 μg/mL), after treated for 48 h, the cells were collected for RNA extraction and western blot analysis. 1×10^5 cells were plated into 6-well plates, following 12 h of incubation with non-antibodies and FBS-free medium, the cells were transfected with miR-21 mimics or sponge (5 ng/well) by lipofectamine 2000 for 24 h according to the manufacturer's protocol, then removed the supernatant, after that, cells were treated with curcumol (50 μg/mL) for 2 days. The cells were collected for miRNA and RNA extraction and western blot analysis.

2.5. Xenograft assays in nude mice and tissue preparation

LoVo cells (2.5×10^7/mL) were suspended in 200 μL of sterile PBS were injected subcutaneously into the right flanks of five week old BALB/c nude mice. Curcumol was dissolved in 90% propylene glycol, and the mice were randomly divided into four groups: control (200 μL 90% propylene glycol), low (20 mg/kg per day), medium (40 mg/kg per day), high (80 mg/kg per day), these doses of curcumol selected were based on accepted previous research, and mode of administration is by intragastric. The tumor size and mice body measured by calipers every three days and the tumor volume were calculated according to the formula: $V = (\text{length} \times \text{width}^2 / 2)$. With being treated for three weeks, sodium pentobarbital was used to anaesthetize mice before they were killed to minimize the suffering, the tumor issues were collected in EP tube and store at −80 °C for future use.

2.6. Western blot

Tissues were grinded with liquid nitrogen and the collected cells were washed by PBS. They were lysed using RIPA lysis buffer with 1 mM PMSF, 14 mM DTT, 2 μg/mL Aprotinin, 5 μg/mL Leupeptin and 1 μg/mL Pepstain. The lysates were broke by ultrasonic crusher, then centrifuged at 12,000 rpm for 20 min at 4 °C. The proteins concentration of the supernatant was quantified with BCA Protein Assay Kit (Beyotime, China). Equal amount of lysate (30 μg) were separated by 10% SDS-PAGE and transferred onto polyvinylidene difluoride (PVDF) membranes. The membranes were blocked with 5% (w/v) non-fat milk in PBST(with 0.1% Tween 20) at room temperature for 1.5 h, then those membranes were incubated with antibodies overnight at 4 °C, followed by incubation with horseradish peroxidase-conjugated goat anti rabbit or mouse IgG second antibodies for 1.5 h at room temperature. The proteins were detected by enhanced chemiluminescence reagent, and the bands were exposed to medical X-ray film. The results were measured using Gel-pro software.

Table 1
Sequences of forward and reverse primers used in Q-PCR.

RNA species	Primer pairs
miR-21	UAGCUUAUCAGACUGAUGUUGA
U6	Forward: 5′-GCTTCGGCAGCACATATACTAAAAT-3′ Reverse: 5′-CGCTTCACGAATTTGCGTGTCAT-3′

2.7. miRNA and RNA extraction and Quantitative Real-Time PCR analysis

Tissue grinding with liquid nitrogen and the collected cells washed by PBS. The total RNA was extracted using Trizol reagent. For miRNA, the total RNA extracted from cells or tissue was reversely transcripted to cDNA by All-in-One miRNA Detection Kit, according to the manufacturer's proposal. Quantitative Real-Time PCR analysis (Q-PCR) was performed using All-in-One miRNA Detection Kit too. The reactions were performed in 8-tube strip on 7500 fast Real-Time PCR Detection System (ABI, Foster, CA, USA), and U6 was as the inner control. The primers used for Q-PCR are shown in Table 1.

2.8. Statistical analysis

Statistical analysis was performed using SPSS software, all experiments repeated at least three times and the results were expressed as mean ± SD. One-way analysis of variance (ANOVA) was used for statistical analysis of data, and the comparison between different treatment groups were analyzed by using student's *t*-test. The data was considered as statistically significant when $P < 0.05$.

3. Result

3.1. Differential expression of miR-21 in CRC cells

We detected the expression of miR-21 in HCT-116, LoVo and SW480 three different CRC cell lines. According to the results of Q-PCR assay, the expression of miR-21 in HCT-116 cells was highest, and in SW480 cells its expression is the lowest (Fig. 1a). Curcumol can inhibits SW480, HCT-116, LoVo cells' proliferation, we treated three CRC cell lines (HCT-116, LoVo, SW480) with 0, 12.5, 25, 50,100 μg/mL curcumol for five different times. According to the results of MTT assay, all three cell lines showed a significantly decreased survival rate with the increased concentration of curcumol (Fig. 1b, c, and d). The IC50 were 76.15 μg/mL, 93.59 μg/mL, 209.09 μg/mL forHCT-116, LoVo and SW480 cell lines at 72 h, which means curcumol might exert a stronger inhibition on the higher expression miR-21 CRC cells. Therefore, combining these data, we hypothesized that miR-21 may be a potential target of curcumol on CRC cells.

3.2. Curcumol inhibits the proliferation of CRC cells by depressing the expression of miR-21 both in vitro and in vivo

The next, we want to confirm that whether curcumol can reduce the expression of miR-21 in CRC cells. LoVo cells were treated with curcumol (0, 12.5, 25, 50, 100 μg/mL) for 48 h (Fig. 2a), and then the

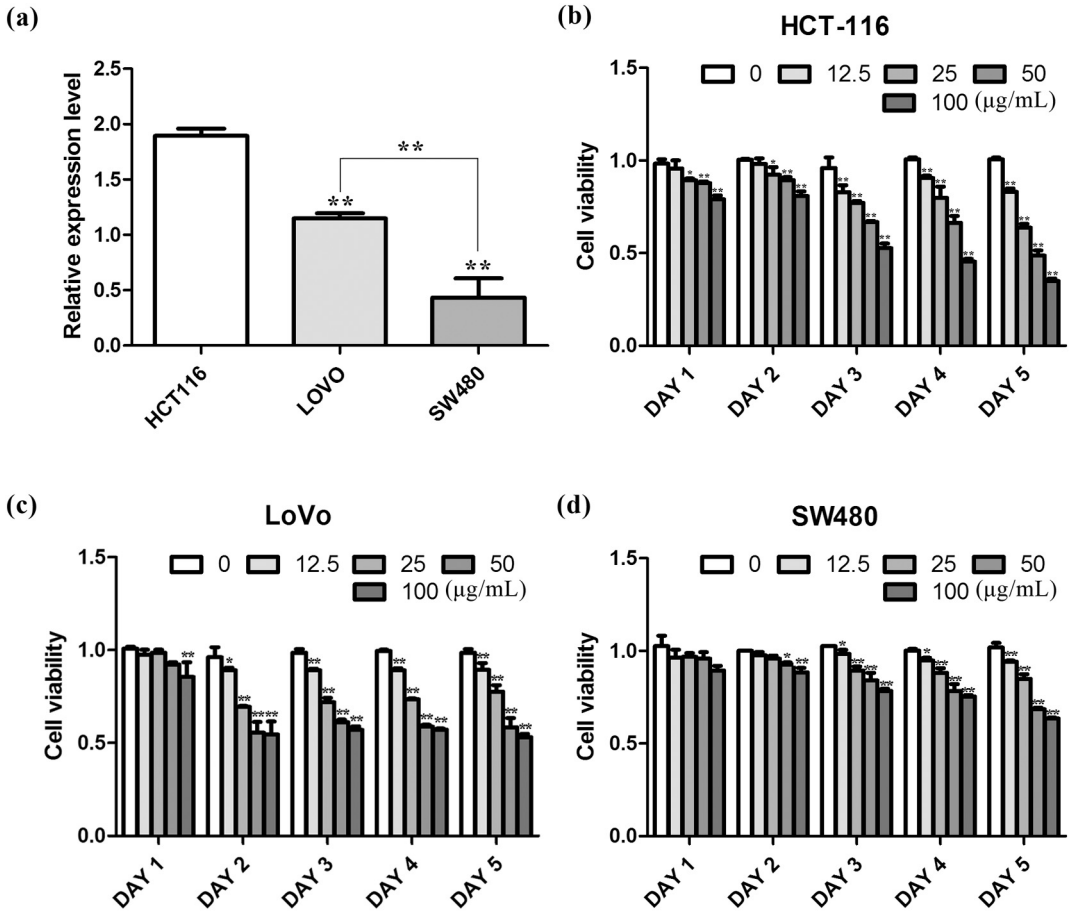

Fig. 1. Differential expression of miR-21 in CRC cells. (a) The expression of miR-21 in HCT-116, LoVo and SW480 were detected by Q-PCR. (b) HCT-116 cells were treated by the indicated concentrations of curcumol by MTT assay. (c) LoVo cells were treated by the indicated concentrations of curcumol by MTT assay. (d) SW480 cells were treated by the indicated concentrations of curcumol by MTT assay. All data were representative of at least three independent experiments. *P < 0.05, **P < 0.01, significantly different from the control group.

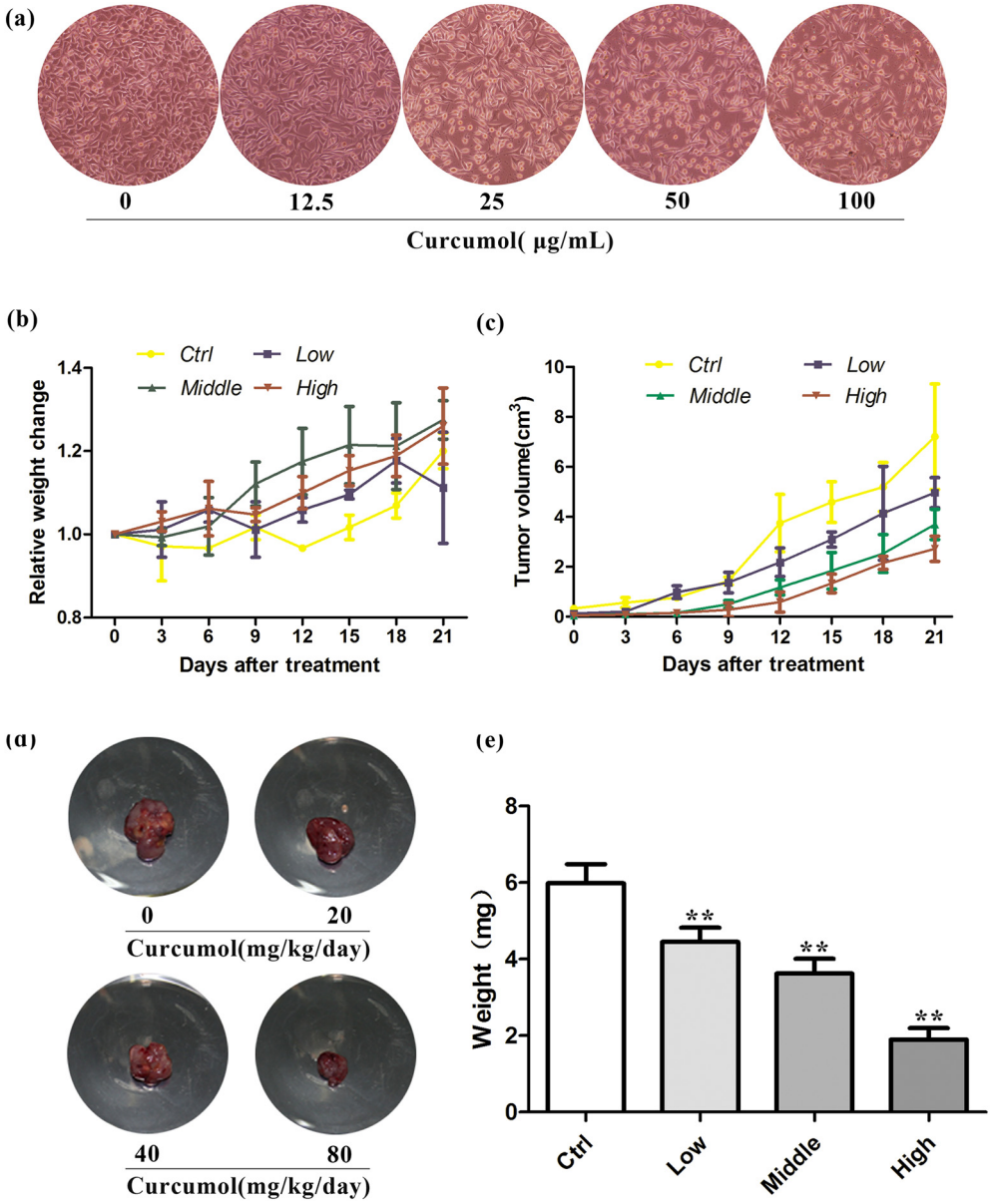

Fig. 2. Curcumol inhibits the proliferation of CRC cells by depressing the expression of miR-21 both in vitro and in vivo. (a) LoVo cells were treated with different concentrations of curcumol. (b) LoVo cells were inoculated in BALB/c nude mice, after giving different doses of curcumol, the changes of body weight in mice. (c) The changes of subcutaneous tumor volume in nude mice. (d, e) The tumor tissue was removed and weighed. *P < 0.05, **P < 0.01, significantly different from the control group.

expression of miR-21 was analyzed by the real-time Q-PCR, the data are normalized with house-keeping gene U6. The Q-PCR data suggested that miR-21 expression was down-regulated with the increased concentration of curcumol (Fig. 3a). Furthermore, we investigate the effect of curcumol on miR-21 in vivo. LoVo cells were subcutaneously transplanted into BALB/c nude mice to establish xenografts model, and then the mice were given intragastric administration of curcumol at doses of 20, 40 or 80 mg/kg/day, the control group was treated with vehicle in the same administration method. During the course of treatment, the changes in body weight and the volume of subcutaneous tumor tissue in each dose group were shown in Fig. 2b and c. The results showed that compared with the control group, the mice in the drug intervention group had normal living conditions and no weight loss, but the tumor volume was significantly reduced. After three weeks later, the mice were sacrificed and the tumor tissues were weighed, tumors weight lost as drug dose increased (Fig. 2d and e), and the expression of miR-21 in tumor tissues was also decreased with the increased concentration of curcumol (Fig. 3b).

3.3. The effect of miR-21 on the PI3K/Akt pathway

From the above, we found that curcumol can decrease CRC cells' miR-21 expression in vitro and in vivo, and previous study demonstrated that miR-21 regulated cancer cells proliferation through the PI3K/Akt pathway. So we want to verify that whether miR-21 was involved in the PI3K/Akt pathway in CRC cells. We used miR-21 mimics and miR-2-SP (miR-21 inhibitors) to determine the effect of miR-21 in PI3K/AKT pathways (Fig. 3c). As indicated in Fig. 3d and e, the expressions of p85, p-p85,p-Akt, NF-κB were significantly increased in SW480 cells after transfected with miR-21 mimics and there was no evident effect on the expression of total Akt, while the expression of PTEN were decreased. On the contrary, the expression of p85, p-p85, p-Akt and NF-κB were down-regulated and the expression of PTEN was increased in SW480 cells after transfected with miR-21 sponge. Based

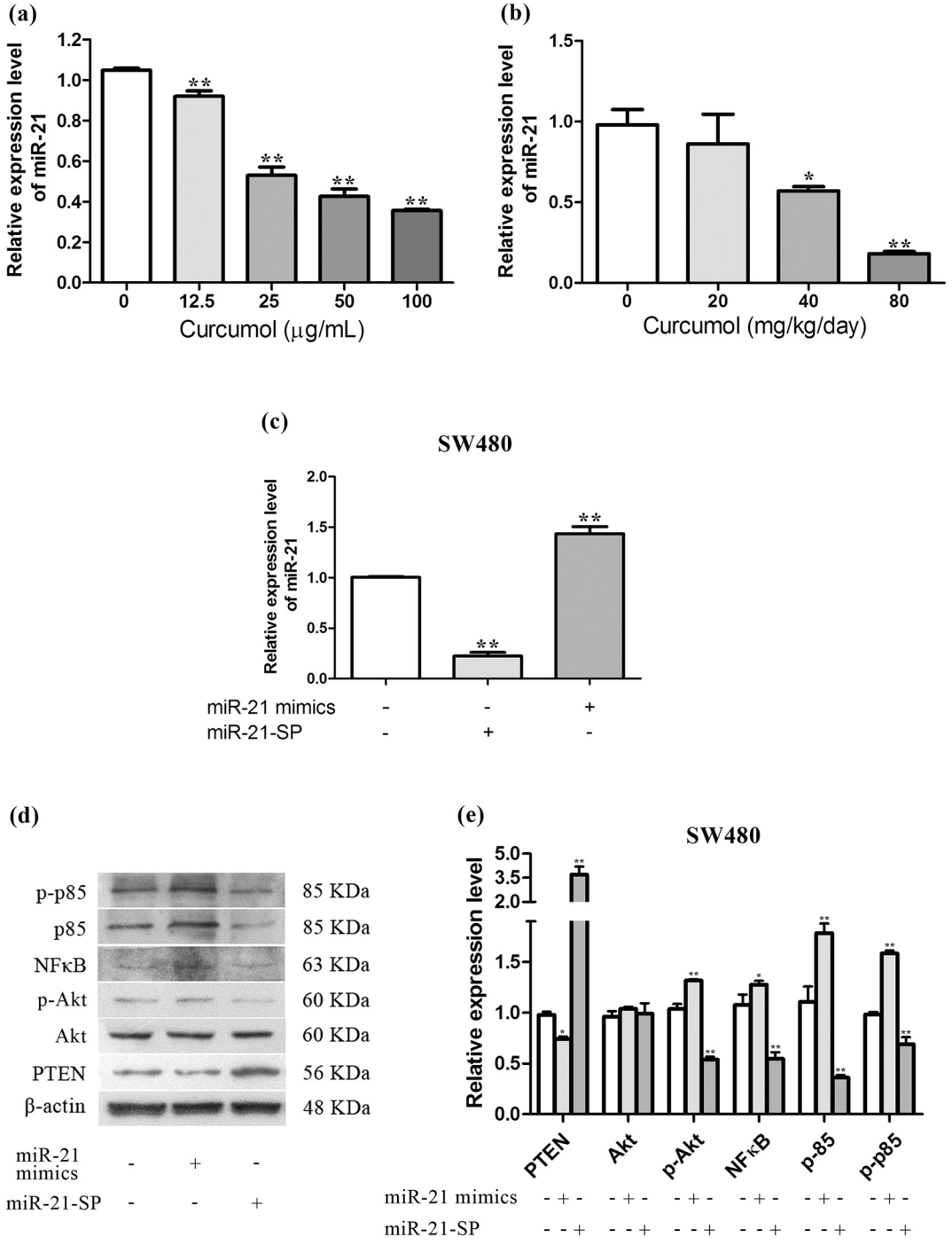

Fig. 3. The effect of miR-21 in the PI3K/AKT pathways. (a) The expression of miR-21 decreases with increasing concentration of curcumol in LoVo was detected by Q-PCR. (d) The expression of miR-21 decreases with increasing concentration of curcumol in tumor tissues was detected by Q-PCR. (c) The expression of miR-21 after transfection of miR-21-SP and mimics. (d, e) The protein expression of p85, p-p85, Akt, p-Akt, NFκB, PTEN in SW480 cells after transfection of miR-21 inhibitors and mimics by western blot. The data of miR-21 are normalized with house-keeping gene U6.All data were representative of at least three independent experiments (*P < 0.05, **P < 0.01).

on these findings, we concluded that miR-21 may modulate PI3K/Akt pathway by target PTEN to regulate a variety of physiological functions in CRC cells.

3.4. Curcumol inhibited the proliferation of CRC cells through PTEN/PI3K/Akt pathway by targeting miR-21

To further investigate the relationship between the effect of curcumol on PTEN and the effect of curcumol on miR-21, we first detected the proliferation inhibition of curcumol on the miR-21 mimics and miR-21 sponge CRC cells. The results showed that miR-21 can promote the CRC cells growth, while miR-21 expression inhibited can suppress CRC cells' viability. Curcumol (50 μg/mL) significantly inhibited the growth of SW480 cells in the miR-21-SP transfected group, but curcumol showed slight inhibition effects on miR-21 mimics transfected CRC cells. Compared with the curcumol (50 μg/mL) group, the inhibition of curcumol was slightly increased by transfection of miR-21-mimics, but there was no significant difference, this may be related to the

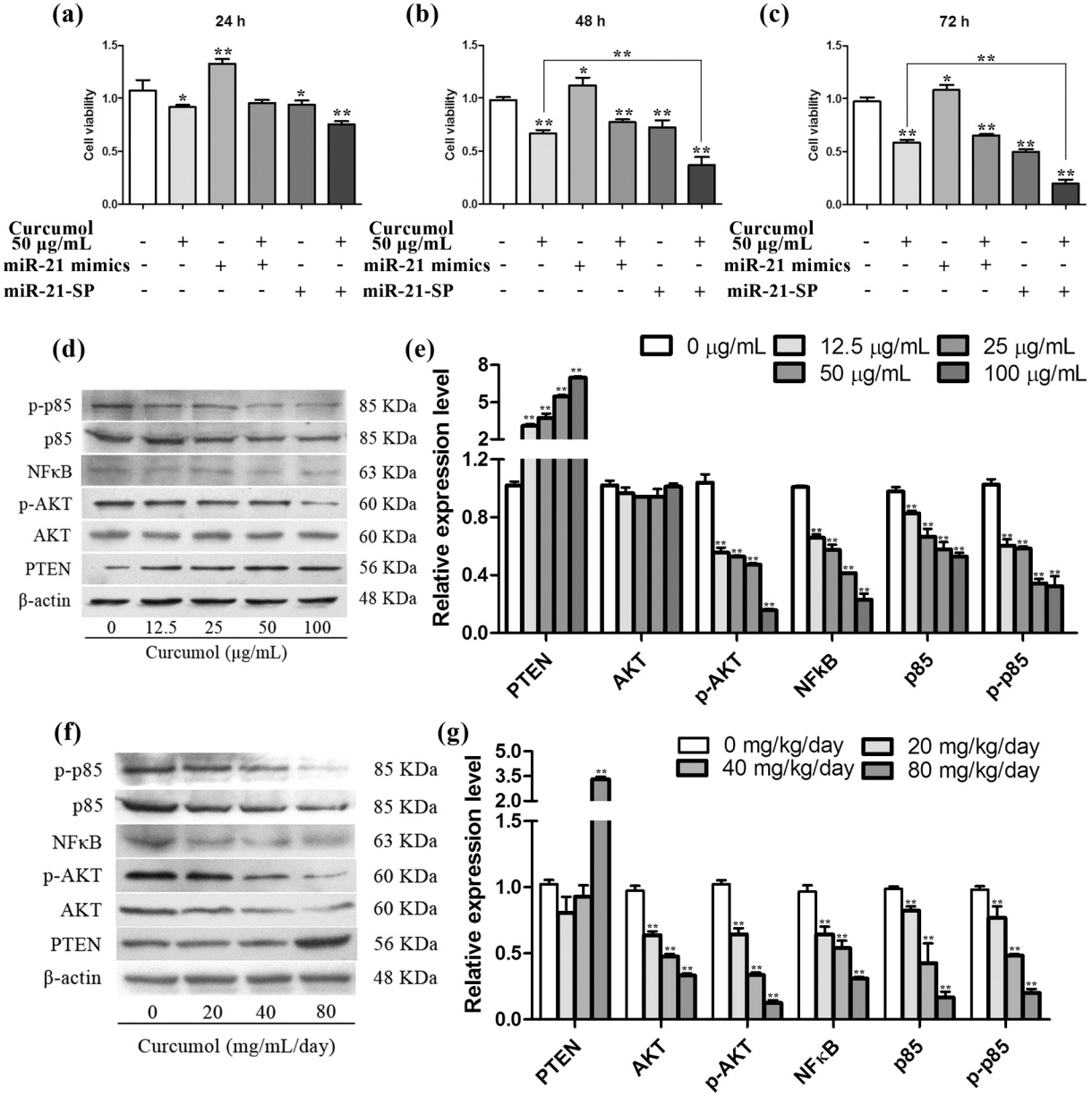

Fig. 4. Curcumol inhibited CRC cells proliferation by target miR-21. (a, b, c) The cell viability of SW480 after treating with curcumol and (or) miR-21 mimics and (or) miR-21-SP. (d, e) The protein expression of p85, p-p85, Akt, p-Akt, NF-κB, PTEN in LoVo cells were detected by western blot. (f, g) The protein expression of p85, p-p85, Akt, p-Akt, NF-κB, PTEN in extracted tumor tissues were detected by western blot (*P < 0.05, **P < 0.01).

interference of transfection reagents on cells (Fig. 4a, b and c). Then we verified the effect of curcumol on PTEN/PI3K/Akt pathway of CRC cells in vitro and in vivo. We detect the expression of PTEN and its downstream factor PI3K, Akt and NF-κB activity by western blot after the cells treating with curcumol. Our results showed that curcumol increased the expression of PTEN and decreased the expression of p85, p-p85, Akt, p-Akt and NF-κB in a dose-dependent manner (Fig. 4d, e, f and g). Thirdly, we identified the activation of PTEN /PI3K/Akt pathway in the up-regulated and down-regulated miR-21 CRC cells. As shown in Fig. 5a and b compared with control group, after 50 μg/mL curcumol treated, the expressions of PTEN was increased and the expressions of p-85, Akt, p-Akt were all decreased in the miR-21-SP group. Moreover, compared with the single curcumol treated group, miR-21 mimics could obviously reverse curcumol's effect on PTEN, p-Akt and p-p85 (Fig. 5c and d).

Collectively, they are further indicated that miR-21 may be a potential target of curcumol to inhibit the proliferation of CRC cells, and curcumol regulated the activity of PTEN/PI3K/Akt pathway partly through miR-21 in CRC cells.

4. Discussion

CRC is a common malignant tumor and its mortality rate increases year by year every year in China. Abnormal proliferation of CRC weakens the normal functioning of the body, eventually it causes cachexia, metabolic abnormalities, co-infection and other abnormal physiological conditions leading to death. Current chemotherapy medicines for CRC have evident side effects for its patient to tolerable and poorly help patients with overall survival and prognosis. miRNAs belong to the small non-coding RNA (19–22 nucleotides) family, which negatively regulate gene expression at the post-transcriptional level [27]. Previous studies have shown that miR-21 is involved in the cell division and proliferation of many cancer cells, and it is one of the most differentially expressed miRNAs in CRC compared to normal tissues [8]

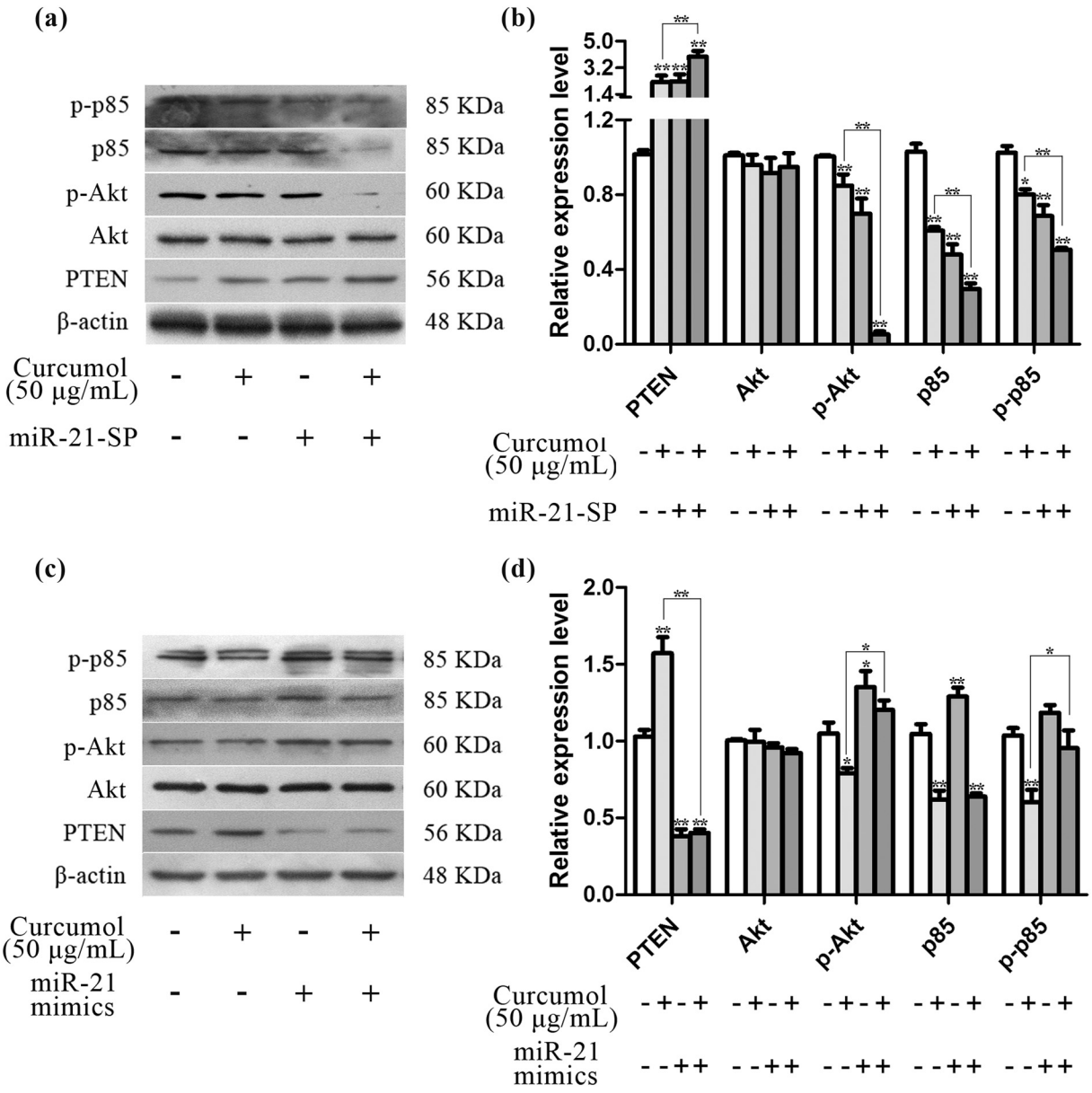

Fig. 5. Curcumol inhibited CRC cells proliferation by target miR-21. (a, b) The protein expression of p85, p-p85, Akt, p-Akt, PTEN in SW480 cells after treating with curcumol and (or) miR-21-SP by western blot. (c, d) The protein expression of p85, p-p85, Akt, p-Akt, PTEN in SW480 cells after treating with curcumol and (or) miR-21 mimics by western blot. All data were representative of at least three independent experiments (*P < 0.05, **P < 0.01).

Those are demonstrated that miR-21 has been to be a novel biomarker in CRC and is a good potential diagnostic and therapeutic target. Much evidence suggests that they act as oncogenes or tumor suppressor genes relying on their target genes' function in tumor cells [28], because miRNAs bind with the 3′-untranslated region of their target mRNA, which causes their target gene's double-stranded mRNA degradation [29]. The target genes that have been demonstrated as miR-21 include K-ras, TPM1, PDC4, etc., and miR-21 can bind to mRNAs of these factors, thereby affecting protein expression [7]. Thus, suitable inhibitors could modulate the growth of cancer by miR-21, which regulates the expression of protein levels in a number of cancer cells.

Curcumol is a monomeric compound extracted from *Curcumae Rhizoma* which is used as traditional Chinese medicine for thousands of years. It has a wide range of biological activities, including proliferation inhibition and apoptosis induction of a variety of cancer cells, such as curcumol can target EEF1A1 to inhibit tumor cell metastasis in MDA-MB-231 cells, and plays a role in inhibiting the growth of nasopharyngeal carcinoma cells by targeting nucleolin, the proliferation of MGC-803 cells was inhibited by down-regulating IDH1 [22,24,26]. In fact, curcumol is a multi-target natural anticancer substance, and the main target involved in different cancers is also different. When it comes to CRC, we found that curcumol could inhibit the proliferation of CRC cells through PI3K/Akt pathways [25], but the reports on miRNAs related to curcumol have not been seen so far. Previous studies have shown that the factors of PI3K and Akt activated in cancer are commonly associated with high expressed miR-21 [15,20,30]. Based on the above, it was hypothesize that curcumol exerts its anti-tumor activity which maybe associated with the inhibition of miR-21 in CRC cells. According to our research , curcumol had different inhibition effect on HCT-116, LoVo and SW480 cells; curcumol had the strongest effect on the highest expression of miR-21 in these three cell lines. In addition, the expression of miR-21 decreased, as the concentration of the curcumol increased in CRC cells' and animal models.

Next, we investigated the regulation of miR-21 on PTEN/PI3K/Akt signal pathways in human CRC cells. It has been already proven that miR-21 participates in the process of cell division, differentiation, and

apoptosis in gastric cancer, lung cancer, and breast cancer [30–32], and it works on PI3K/Akt signal pathways to influence the biological behaviors of malignant cells [33,34]. Although many studies have reported thatmiR-21 is also a target of PTEN [14,33,35], which is the upstream of the PI3K/Akt signal pathways and negatively regulate cancer cells' proliferation as a tumor suppressor gene [36], but the role in CRC cells is rarely reported. In our study, we found that miR-21 can significantly reduce the expression of PTEN and increase the activation of PI3K, Akt and its downstream NF-κB. We also found that in the cells inhibited miR-21, the expression of PTEN was improved and the expression of p85, p-p85, p-Akt and NF-κB were down-regulated after transfection with miR-21-SP.

Furthermore, we verified that whether the anti-proliferation activity of curcumol in CRC cells was correlated with its effect on the expression of miR-21 and the PTEN/PI3K/Akt signaling pathways. Our results suggested that curcumol could affect the level of miR-21 in CRC cells and over-expressed miR-21 could counter-affect the proliferation inhibition of curcumol. Meanwhile, curcumol's effects on the factors of PTEN/PI3K/Akt signaling pathway was found to be reversed by miR-21, but the inhibitory effect of curcumol significantly increased when we knocked down miR-21 in SW480 cells. Compared with the normal group, over-expression miR-21significantly enhance the expression of p85, p-p85, p-Akt, NF-κB and decreased the expression of PTEN, Moreover, curcumol's effects on p85, p-p85, p-Akt, NF-κB and PTEN were inhibited inmiR-21 over-expressed group, while the effect of curcumol on the PI3K/Akt pathways was enhanced in the miR-21 knockdown group. These results have confirmed our hypothesis that curcumol may be an inhibitor of miR-21 in the way of inhibiting the activity of the PI3K/AKT pathway and the proliferation of CRC cells. These results have indicated that curcumol could modulate PTEN/PI3K/Akt signaling by repressing the expression of miR-21; accordingly, curcumol could act on the miR-21 to transmit information in order to block the PI3K/AKT pathways.

In conclusion, our experiments confirmed that curcumol targeting miR-21 could debilitate CRC cells' proliferation via inhibiting activation of the PI3K/AKT/PTEN pathway. Although it is yet known whether curcumol directly or indirectly interacted with miR-21, this current research has provided a new direction for the targeted preparation of miRNAs and also provided new insights for the application of natural medicines in cancer treatment.

Acknowledgements

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Conflict of interest

The authors declare no conflict of interest in this study.

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