



Anchorage of curcumin onto PVP enhances anti-tumor effect of curcumin

Lifang Guo¹ · Mingbiao Shi² · Nan Song³ · Zirui Wan¹ · He Liu¹ · Lihong Liu¹

Received: 10 November 2018 / Accepted: 16 February 2019 / Published online: 18 March 2019
© Springer Science+Business Media, LLC, part of Springer Nature 2019

Abstract

Curcumin, a natural polyphenol compound, exhibits wide range of pharmacological activities. However, its poor aqueous solubility, weak stability, and rapid metabolism limit its potential development. In this study, solid dispersion of curcumin with PVP was developed. This study aimed to evaluate the loading capacity, stability, solubility, and anti-tumor activity of PVP-Cur solid dispersion. Scanning electron microscope (SEM), differential scanning calorimetry (DSC), X-ray powder diffraction (XRPD), and fourier transform infrared (FTIR) spectroscopy were used to investigate physical characterization of PVP-Cur. Loading capacity, stability, and solubility of PVP-Cur were evaluated by spectrophotometer. The biological effects of PVP-Cur on tumor cell proliferation and apoptosis were analyzed using CCK-8 assay and 7-AAD/AnnexinV-APC assay, respectively. PVP-cur-induced activation of caspase-3 and caspase-9 were evaluated by in vitro caspase activity assay and western blotting. It was interesting to note that PVP: curcumin in the ratios of 6:1 or 8:1 exhibited the highest loading capacity. Anchorage of curcumin onto PVP increased its solubility and stability. Compared with curcumin alone, PVP-Cur displayed an increased ability in abrogating tumor cell proliferation and inducing tumor cell apoptosis via mitochondria signaling, which was attributed by the enhanced cellular uptake of PVP-Cur. Anchorage of curcumin onto PVP was an effective way to increase the solubility and stability of curcumin. PVP-Cur increased anti-tumor ability and cellular uptake relative to curcumin, indicating that PVP-Cur is a promising candidate for anti-cancer drug development.

Keywords Curcumin · Polyvinyl pyrrolidone · Solubility · PVP-Cur

These authors contributed equally: Lifang Guo, Mingbiao Shi, Nan Song

✉ He Liu
liuheliuhe@126.com

✉ Lihong Liu
liulihong@bjcyh.com

- ¹ Pharmacy Department of Beijing Chao-Yang Hospital, Capital Medical University, No.8 Gongren Tiyuchang Nanlu, Chaoyang District, Beijing 100020, P. R. China
- ² Central Hospital of Jiaozuo coal industry Refco Group Ltd, No. 1 Health Road, Jiefang District, Jiaozuo, Henan, P. R. China
- ³ Beijing Institute of Tropical Medicine, Beijing Friendship Hospital, Capital Medical University, No.95 Yong'an Road, Xicheng District, Beijing 100005, P. R. China

Introduction

Curcumin (Cur), low-molecular-weight polyphenol, is extracted from the rhizome of *Curcuma longa*. It has been widely used as a yellow pigment or spice in the food industry. Curcumin exhibits a wide range of pharmacological activity on account of its capability to regulate multiple cell signaling pathways (Dong et al. 2016; Mulik et al. 2012; Rahmani et al. 2014; Ranjan et al. 2004; Seo et al. 2016; Wang et al. 2015; Ye et al. 2015), and is effective in managing various diseases including cancer (Shanmugam et al. 2015; Troselj and Kujundzic 2014; Zang et al. 2014), infection (Ferreira et al. 2015; Padmanaban and Rangarajan 2016), Alzheimer (Samy et al. 2016; Wang et al. 2013), cardiovascular (Wongcharoen and Phrommintikul 2009), diabetes (Castro et al. 2014; Nabavi et al. 2015). Especially it has little obvious toxicity or side effects for wide spectrum of actions against tumors, including breast cancer (Nagaraju et al. 2012), head and neck cancers (Gao et al. 2012; Wilken et al. 2011), prostate cancer (Chen 2015; Jordan et al. 2016), glioblastoma (Sordillo et al. 2015), liver

cancer (Darvesh et al. 2012), lung cancer (Howells et al. 2014; Mehta et al. 2014; Yao et al. 2015; Ye et al. 2012) and so on, which have been considered as one of a remarkable promise of clinical implications in cancer treatment.

Despite all these extraordinary properties, curcumin suffers from low solubility in aqueous solution and undergoes rapid degradation that results in low systemic bioavailability, and greatly hampers its *in vivo* efficacy. To improve curcumin application as therapeutic agents, a diverse range of efforts were made and different formulation techniques have been employed for curcumin, including polyvinyl pyrrolidone (PVP) (Paradkar et al. 2004), polymeric nanoparticles (Gao et al. 2015; Liu et al. 2015; Mahmood et al. 2015; Sun et al. 2012), liposomes (Hasan et al. 2014; Nayak et al. 2016; Song et al. 2014; Sou et al. 2008), chitosan (Karewicz et al. 2013), cyclodextrins (CDs)-based inclusion compound (Cutrignelli et al. 2014; Swaminathan et al. 2016; Yallapu et al. 2010), PLGA and conjugates (Najlah et al. 2017; Tabatabaei Mirakabad et al. 2016), which was a consequence of enhanced aqueous solubility, extended blood circulation, improved permeability, and resistance to degradation or metabolic processes, but these strategies suffered from a poor loading efficiency of the covalently linked curcumin, larger particle size or bad curative effect.

PVP consists of a synthetic linear chain homopolymer with no branching obtained by polymerization of the monomer N-vinylpyrrolidone (NVP). It is divided into several grades according to the viscosity, ranging from low to high mean molecular weight (2500–3,000,000 g/mol), characterized by the K value in the pharmacopeias. As the molecular weight of PVP is K30, the aqueous solubility and viscosity of the polymer become suitable for circulation system of the human body (Knopp et al. 2016). Meanwhile, it has no obvious toxicity, which is appropriate for drug applications (Jahangiri et al. 2015; Li et al. 2013; Sadeghi et al. 2016; Thongnopkoon and Puttipipatkachorn 2016).

In this study, solid dispersion of curcumin with PVP was developed. Then, we comprehensively compared the stability, solubility, and anti-tumor ability of PVP-Cur and curcumin, and therefore provide a basis for better understanding the bioactivities and druggability of PVP-Cur.

Materials and methods

Reagents, antibodies, and cell culture

Curcumin (96%, purity) was acquired from Sigma-Aldrich Co. LLC (Shanghai, China). Polyvinyl pyrrolidone K30

(PVP, MW: 40,000) was purchased from Solarbio Science & Technology Co. Ltd (Beijing, China). Ethyl alcohol and dimethylsulfoxide (DMSO) were obtained from Sinopharm Chemical Reagent Co. Ltd (Beijing, China). Dulbecco's modification of eagle's medium (DMEM), fetal bovine serum (FBS), glutamine and penicillin–streptomycin were purchased from Sigma-Aldrich (St. Louis, MO). Antibodies against Cleaved caspase 3, Cleaved caspase 9, and cytochrome c were purchased from Abcam (Cambridge, UK), Antibody against β -actin was obtained by Santa Cruz Biotechnology (Santa Cruz, CA, USA). Human lung cancer cell lines A549, Human hepatoma cell lines HepG₂ and the human prostate cancer cell lines PC₃ were supplied by Cell Resource Center of Peking Union Medical College and cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (Hyclone, Logan, UT, USA).

Preparation of PVP-Cur

The aqueous dispersed curcumin was prepared by polymer inclusion method. In brief, PVP K30 (0.6 g) were dissolved completely in anhydrous ethanol (50 mL), then the solution was transferred to curcumin (0.1 g) and was stirred constantly for 30 min without light-exposure under the normal temperature (25 °C) to make a good combination of curcumin. Then the yellow solution was moved to the RE-52AA rotary evaporation apparatus (Shanghai Ya Rong Biochemical Instrument Co. Ltd., Shanghai, China) and boiled ethanol away with 80 r/min (−0.1 MPa) at 50 °C. The products were obtained and recycled for the application.

Morphological analysis of samples

A scanning electron microscope (FEI Quanta 200, America) was used to investigate the morphologies of curcumin, PVP-Cur and PVP. Curcumin, PVP-Cur and PVP samples were coated with a thin gold-palladium layer by sputter coater (HITACH E1010, America) prior to observation.

Differential scanning calorimetry (DSC)

DSC analysis was conducted using DSC 8000 (PerkinElmer, America) equipped with a refrigerated cooling system to study the thermal behaviors of the samples. The instrument was calibrated using indium standard. 2–3 mg samples were placed in aluminum pans sealed with a lid and were scanned from 20 to 200 °C (0–700 °C?) at a scanning rate of 10 °C/min (100 °C/min?) under nitrogen gas at a flow rate of 80 mL/min.

X-ray powder diffraction studies (XRPD)

X-ray powder diffraction patterns of the selected samples were obtained by an X-ray diffractometer (PANalytical X'Pert Powder, Netherlands). Each sample was scanned in the range of 5–60°(2 θ) with a step size of 0.01°ata scan rate of 0.04/s.

Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared spectroscopy spectra of pure Cur, PVP K30, and PVP-Cur solid complexes were obtained on a Nicolet iS5 series FTIR spectrometer (Thermo Fisher Scientific, Inc., USA) to demonstrate the possibility of any chemical interaction between drugs and polymers. The scanning range was 400–4000 cm⁻¹ and the resolution was 1 cm⁻¹.

Loading efficiency and stability

The varied weight ratios of PVP and curcumin were prepared to determine curcumin loading efficiency. The obtained PVP-Cur was dissolved in 10 mL deionized water at 25 °C, then, the solution was filtered through a 0.22 μ m filter to remove uncombined and insoluble curcumin. The filtered fluid was measured at the maximum absorbance wavelength by DU-800 UV/Vis Spectrophotometer (Beckman Coulter, Inc., USA). To study the stability, Cur and PVP-Cur containing equal amounts of curcumin were dissolved in phosphate buffer (pH 7.4) with or without 1% dimethylsulfoxide (DMSO), without light-exposure under the normal temperature. The samples were analyzed for curcumin content using UV-visible spectrophotometer at predetermined time intervals (0, 1, 2, 3, 4, and 5 h). The degradation of curcumin was plotted to study its stability.

CCK-8 assay

Cells were seeded at 5 \times 10³ cells/well in 96-well plate overnight and treated with PBS, PVP, Curcumin, and PVP-cur. After 48 and 72 h, 10 μ l of CCK-8 solution was added to each well and incubated for 3 h at 37 °C. Then, the absorption was measured by the microplate at 450 nm (Bio-Rad Laboratories, Hercules, CA, USA).

Cell apoptosis analysis

Cells (1 \times 10⁵ cells/well) were cultured in six-well plate overnight and treated with PBS, PVP, Curcumin, and PVP-cur for 48 h. Then, cells were harvested and washed with PBS, resuspended in 500 μ l binding buffer with 5 μ l 7-AAD (PI) and 5 μ l anti-Annexin V-APC antibody. Apoptosis was analyzed with a FACScalibur flow cytometer (BD, USA).

Western blotting

The harvested cells were washed by PBS and lysed with protein lysis buffer. Protein lysates were boiled for five min and the same amount of protein samples were separated on 12% SDS-PAGE gels and transferred to polyvinylidene fluoride membranes. and then incubated with primary antibody at 4 °C overnight. After washed with TBST for three times and incubated with the second antibody at room temperature for one hour. Then the expression of protein was detected by electrochemiluminescence (ECL) assay.

Cell fractionation assay

PBS, PVP, Cur, or PVP-cur treated cells were fractionated by differential centrifugation. Briefly, PBS, PVP, Cur, or PVP-cur treated cells were homogenized in a buffer containing 0.25 M sucrose and 10 mM HEPES (pH 7.4) supplemented with protease inhibitor cocktail (Thermo Fisher Scientific, USA). The homogenate was then centrifuged at 800 \times g for 10 min to remove nuclei and unbroken cells, and then the supernatant was centrifuged at 12,000 \times g for 20 min to get the mitochondria. The cytosolic cytochrome c was thereby detected by immunoblotting assay with cytochrome c monoclonal antibody.

In vitro caspase activity assay

PBS, PVP, Cur, or PVP-cur treated cells were lysed. Then, equal amounts of protein samples were transferred into a 96-well microtiter plate with substrates. Caspase-3, -9 activity were measured by the cleavage of DEVD-*p*NA and LHED-*p*NA, respectively. (BioVision, Inc., USA). The absorbance was measured in a microplate reader at 405 nm (Bio-Rad Laboratories, Hercules, CA, USA). The activity of caspases was indicated by comparing the absorbance of PBS, PVP, Cur, or PVP-cur -treated samples with PBS control.

Immunofluorescence

Cells were seeded in 6-well plates in 2 mL medium. After cells were attached to the plate, media was added with indicated reagents. After washing with PBS three times, cells were examined under an Olympus BX 51 fluorescence microscope (Olympus, Center Valley, PA).

Statistical analysis

All experiments were performed in triplicate and analyzed by GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). Where appropriate, the data are presented as the mean \pm S.D.

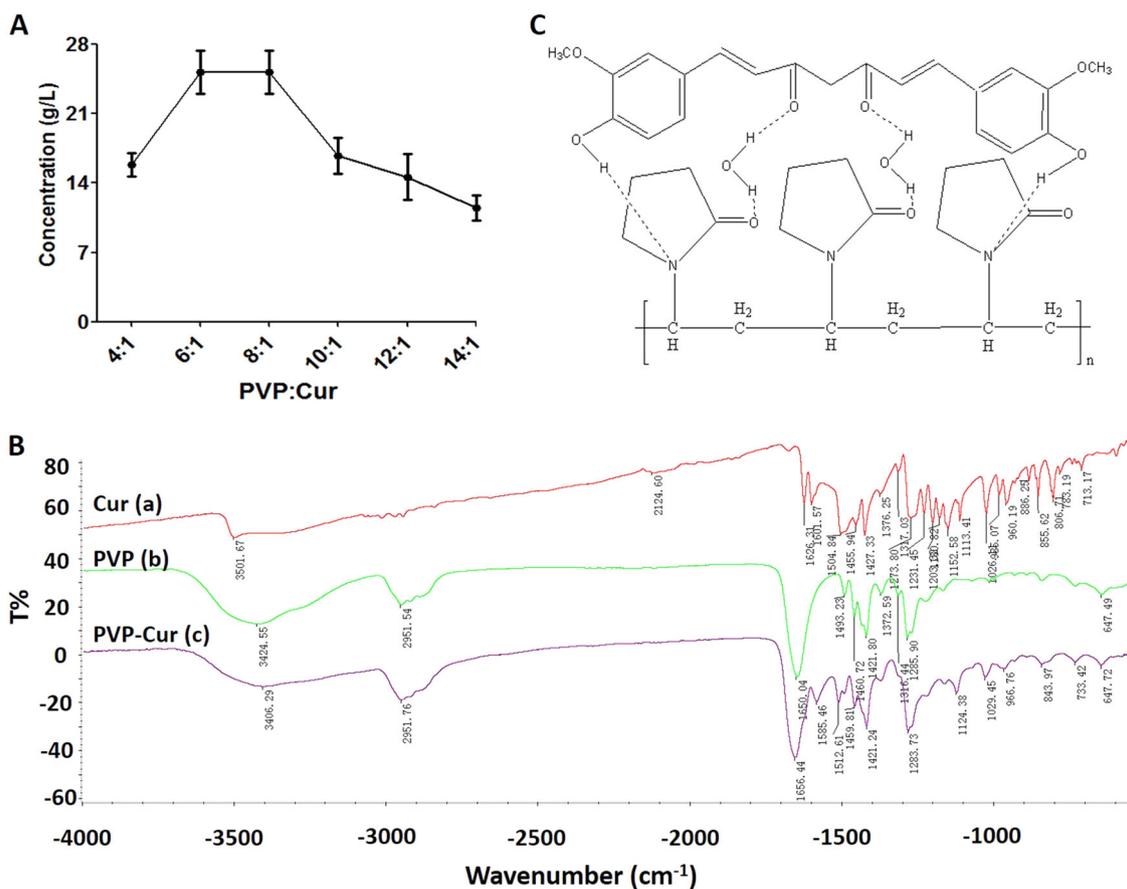


Fig. 1 Encapsulation of curcumin onto PVP. **a** Curcumin loading capacity in the ratio of 4:1, 6:1, 8:1, 10:1, 12:1, 14:1. **b** FTIR spectra of Cur(a), PVP K30(b) and PVP-Cur(c) solid dispersion, Cur: curcumin, PVP: polyvinyl pyrrolidone. **c** Putative schematic structures of PVP-Cur

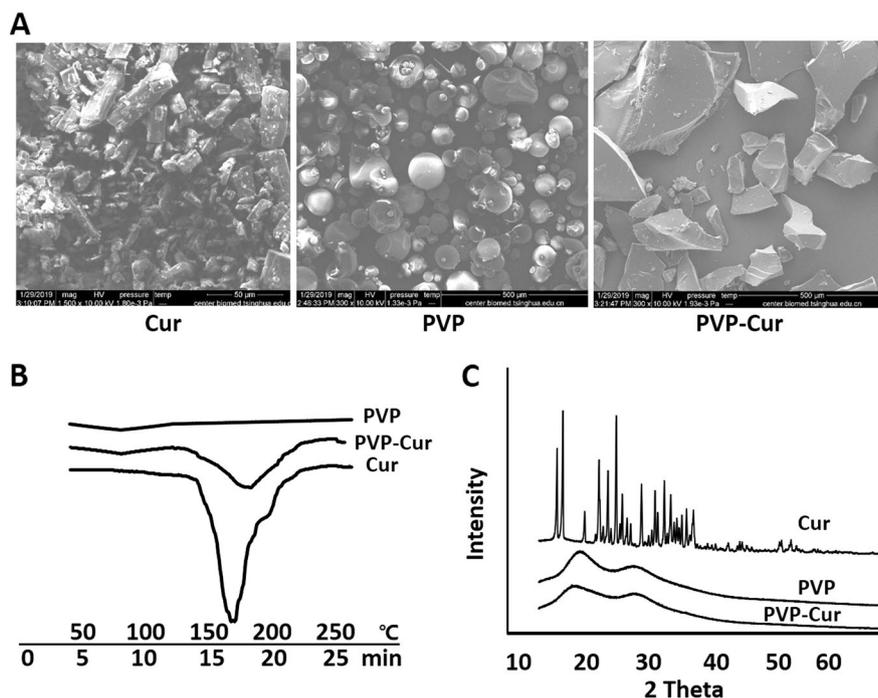
Results

Anchorage of curcumin onto PVP

Curcumin is a hydrophobic polyphenol compound. To increase the solubility of curcumin in aqueous solution, PVP-Cur solid dispersions in different ratios (4:1, 6:1, 8:1, 10:1, 12:1 and 14:1) were prepared. As shown in Fig. 1a, PVP: curcumin in the ratio of 6:1 or 8:1 exhibited the highest loading capacity. Then, fourier transform infrared (FTIR) spectroscopy was applied to observe the difference among PVP, Cur, PVP-Cur, the FTIR spectrum of Curcumin (Cur a) exhibited an absorption band at 3501 cm⁻¹ attributed to the phenolic O-H stretching vibration (Fig. 1b). Additionally, absorption bands at 1601 cm⁻¹ and 1504 cm⁻¹ (stretching vibrations of benzene ring, aromatic C=C), 1626 cm⁻¹ (C=O stretching vibrations), 1427 cm⁻¹ (olefinic C-H bending vibration), 1273 cm⁻¹ (aromatic C-O stretching vibrations), and 1027/855 cm⁻¹ (C-O-C stretching vibrations) were noticed. In the case of PVP K30 spectrum (PVP b), A broadband was visible at 3424 cm⁻¹ (O-H stretching vibration) that was attributed to the presence of water

(Sarisuta et al. 1999). The important bands at 2951, 1650, 1421, and 1285 cm⁻¹ corresponded to C-H stretching vibrations, C=O stretching vibrations, C-H bending vibration and C-N stretching vibrations. The chemical structure of PVP had two proton acceptor groups (C-N and C=O) that could potentially form a hydrogen bond with Curcumin. The spectrum of PVP-Cur (PVP-Cur c) showed bands at 3406 cm⁻¹ (O-H stretching vibration of PVP), 2951 cm⁻¹ (C-H stretching vibrations of PVP), 1656 cm⁻¹ (C=O stretching vibrations of PVP), 1585 cm⁻¹ (N-H bending vibration), 1421 cm⁻¹ (alkanes C-H bending vibration of PVP), 1283 cm⁻¹ (C-N stretching vibrations of PVP) and 1124/1029 cm⁻¹ (C-O-C stretching vibrations of Cur). The presence of (PVP-Cur c) 1585 cm⁻¹ (N-H bending vibration) and the shift of 3406 cm⁻¹ (O-H stretching vibration of PVP), 1656 cm⁻¹ (C=O stretching vibrations of PVP), 1283 cm⁻¹ (C-N stretching vibrations of PVP) were indicative of interaction with curcumin via hydrogen bonding. Considering chemical structures of Cur and PVP, we speculate that hydrogen bonding might be anticipated between the O-H, C=O groups of Cur and the C-N, C=O groups of PVP (Fig. 1c).

Fig. 2 **a** SEM of Cur, PVP, and PVP-Cur(1:6); **b** DSC data for PVP, PVP-Cur(1:6) and Cur; **c** XRPD data for Cur, PVP, PVP-Cur(1:6)



Characterization of PVP-Cur

To further explore the characters of PVP-Cur, SEM, DSC, and XRPD were used. SEM of curcumin exhibits granular crystals, and PVP exhibits a spherical shape. However, PVP-Cur exhibits an amorphous structure with no clear geometry (Fig. 2a). These findings indicate that PVP is capable of preventing crystal growth in crystalline media. DSC of PVP-Cur sample showed a broad peak at 70–80 °C, 140–220 °C, which may be due to the presence of PVP, and the lack of a transition peak of PVP-Cur indicates that PVP-Cur is in an amorphous state in the precipitated sample. Cur endotherms at 170 °C may be attributed to the melting of curcumin. In addition, a sharp peak was observed in the XRPD pattern of curcumin. The XRPD pattern on PVP and PVP-Cur shows a halo pattern with no diffraction peaks (Fig. 2c).

Anchorage of curcumin onto PVP increased its solubility and stability

To determine whether incorporation of curcumin onto PVP increase its solubility, an identical amount of curcumin (curcumin alone or PVP-Cur) was mixed in an equal volume of PBS. As showed in Fig. 3a, PVP-Cur group, but not curcumin free group, showed clear and transparent. After the mixtures (curcumin alone or PVP-Cur) were placed on ice for 30 min, the concentration of curcumin in the supernatant was determined by DU-800 UV/Vis Spectrophotometer. The curcumin concentration in the PVP-Cur

group was 0.1 mg/L, no curcumin was detected in curcumin alone group (Fig. 3b–e). These results demonstrate that anchorage of curcumin onto PVP can dramatically increase the solubility of curcumin. In addition, curcumin is relatively unstable and easy to degrade in aqueous solution. To determine whether anchoring of curcumin onto PVP can increase the stability of curcumin, PVP-Cur or curcumin alone was incubated at 37 °C over a period of 5 h. As shown in Fig. 3f, curcumin in PVP-cur group almost had no degradation, whereas curcumin in PBS degraded quickly and only 20% remained after 5 h incubation. Collectively, these results demonstrate that anchoring of curcumin onto PVP significantly increase its solubility and stability.

PVP-cur inhibited cell proliferation and induced cell apoptosis

It was well reported that curcumin exerts its anti-tumor activity in a broad spectrum of human cancers including prostatic cancer, hepatic carcinoma, and lung cancer. To estimate the anti-cancer viability of PVP-Cur, CCK-8 assay was used to measure the growth viability in human prostate cancer cell line PC3, human lung cancer cell line A549, and human hepatoma cell line HepG₂. PC3, A549, and HepG₂ were treated with different concentrations of curcumin or PVP-Cur for 48 h. As shown in Fig. 4a–c, anchoring of curcumin onto PVP significantly increased the anti-proliferation activity of curcumin. The IC₅₀ of PVP-cur that caused 50% inhibition of cell growth at 48 h for PC3 cells was 7.24 mg/L, for A549 was 15.85 mg/L, and for

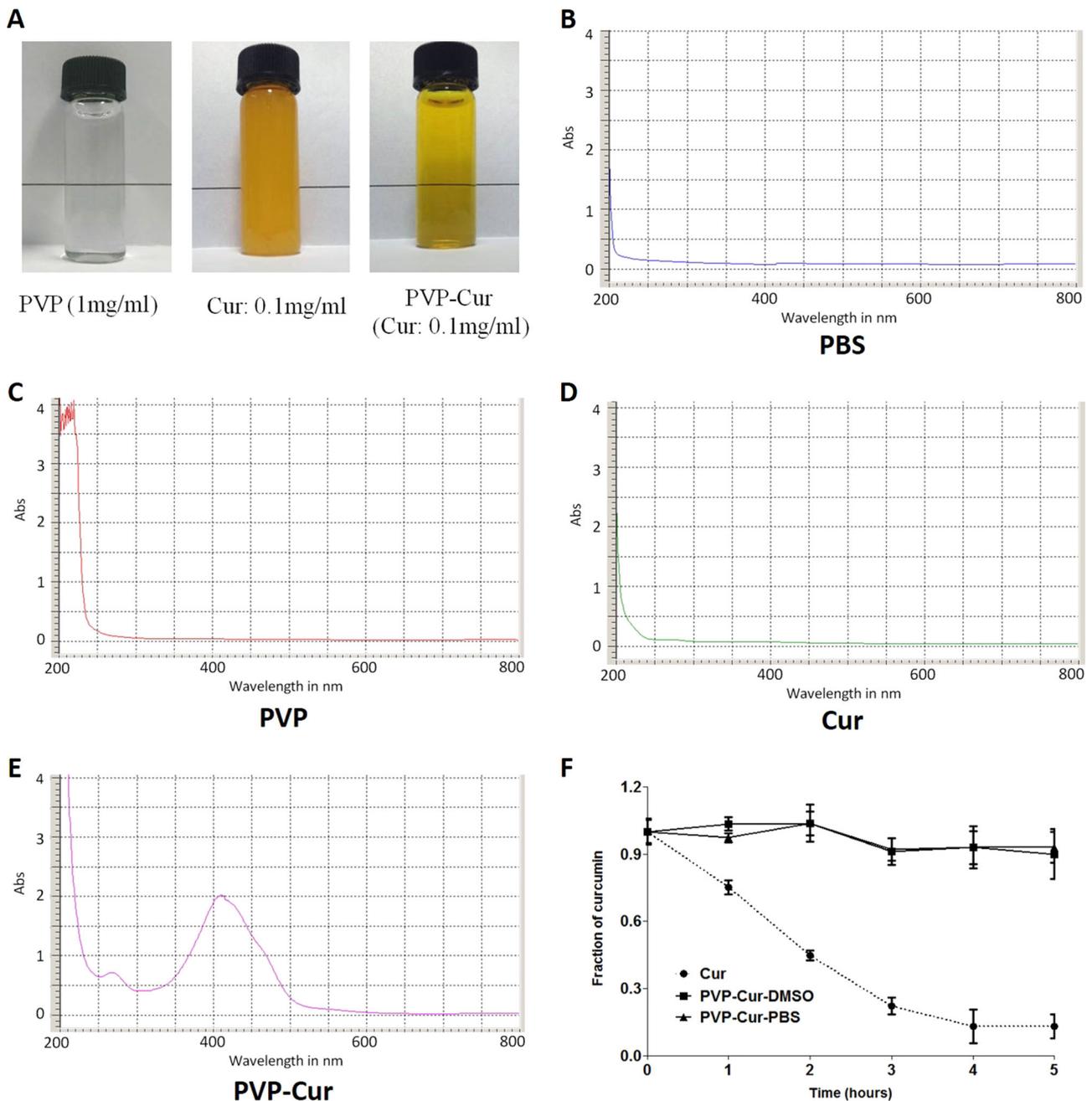


Fig. 3 Incorporation of curcumin onto PVP can increase the solubility, and stability of curcumin. **a** PVP, curcumin (Cur, 0.1 mg/ml) and PVP-Cur (Cur, 0.1 mg/ml) were added to 2 ml PBS, representative photo of PVP, Cur (curcumin), PVP-Cur is shown. **b–e** PBS, PVP, curcumin (Cur, 0.1 mg/ml) and PVP-Cur (Cur, 0.1 mg/ml) were added to 2 ml PBS, the spectrographs were obtained using a DU 800 UV/Vis spectrophotometer. **f** Curcumin (Cur) and PVP-Cur containing equal amounts of curcumin were dissolved in phosphate buffer (pH 7.4) with

(PVP-Cur-DMSO) or without 1% dimethylsulfoxide (DMSO) (PVP-Cur-PBS), and incubated in the dark in a 37 °C water bath. At 1, 2, 3, 4, and 5 h, 100 µl of each sample was taken to determine the concentration of curcumin. The concentrations of curcumin or PVP-Cur at the beginning were set as 1.00. The fold reduction of the concentration at each time point compared to the beginning is shown. The experiments were repeated three times for each time point (**P* < 0.05, ***P* < 0.01)

HepG₂ was 14.13 mg/L (Fig. 4e). Among them, PC3 cell line was noteworthy. Then, we explored whether PVP-cur could trigger prostatic cell apoptosis. 7-AAD/AnnexinV-APC assay was applied to evaluate apoptotic cells after PC3

treated with PVP-cur or curcumin for 48 h. Compared to curcumin alone group, PVP-cur could dramatically induced PC3 cell apoptosis (Fig. 5a–c), indicating that PVP-cur treatment led to apoptosis in prostatic cell.

Fig. 4 Anchorage of curcumin onto PVP can increase anti-tumor proliferation of curcumin. Effect of PVP-Cur on cell growth in PC₃ cell **a**, in A549 **b**, and in HepG₂ **c** were detected by CCK-8 assay. **d** Effect of PVP-Cur, Cur (curcumin), PVP, and PBS on cell growth in PC₃ cell was detected by CCK-8 assay. The experiments were repeated three times for each time point (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). **e** The IC₅₀ of PVP-cur that caused 50% inhibition of cell growth at 48 h for PC₃, A549, and HepG₂

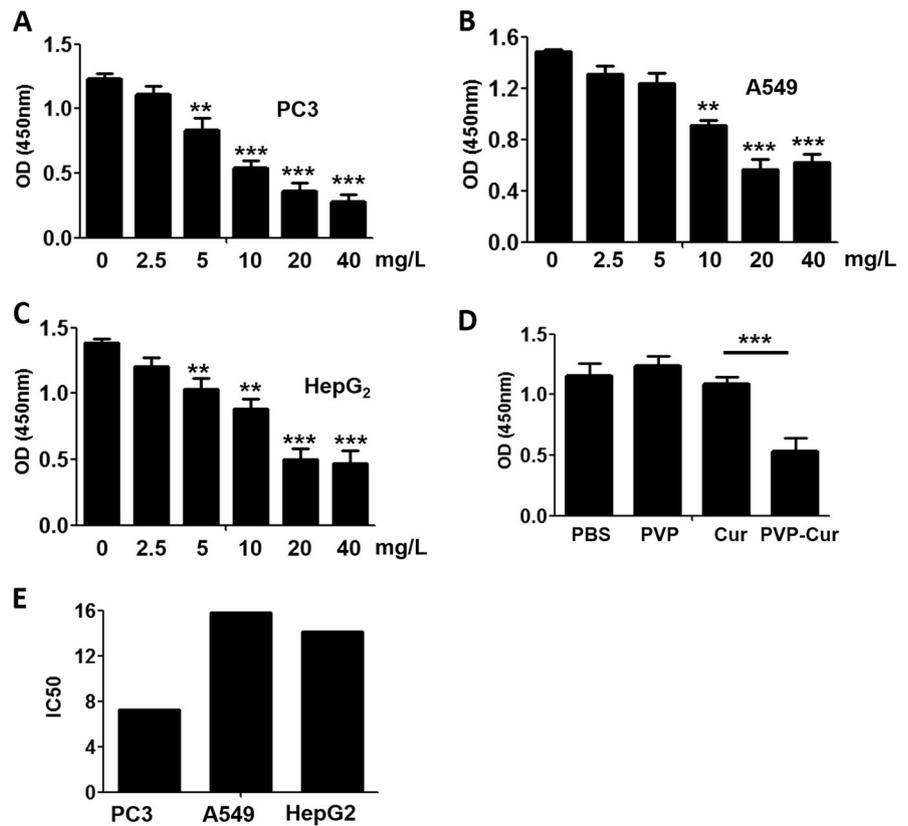
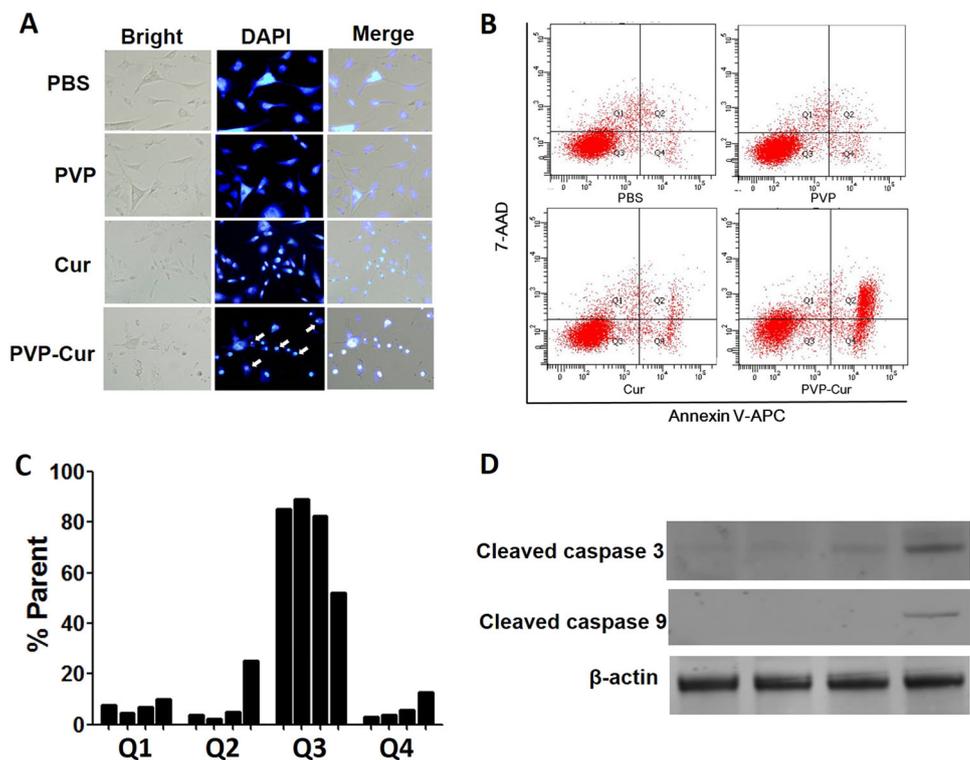


Fig. 5 Incorporation of curcumin onto PVP displayed an increase ability inducing tumor cell apoptosis of curcumin. **a** apoptotic body was observed bylympus BX 51 fluorescence microscope after PVP-Cur, Cur (curcumin), PVP, and PBS treatment. **b** Cell apoptosis in PC₃ cells treated with PVP-Cur, Cur (curcumin), PVP, and PBS was determined by Flow cytometry. **c** The quantitative result of **b**. **d** The expression of Cleaved caspase 3, and Cleaved caspase 9 were determined by western blotting analysis in PC₃ cells after PVP-Cur, Cur (curcumin), PVP, and PBS treatment



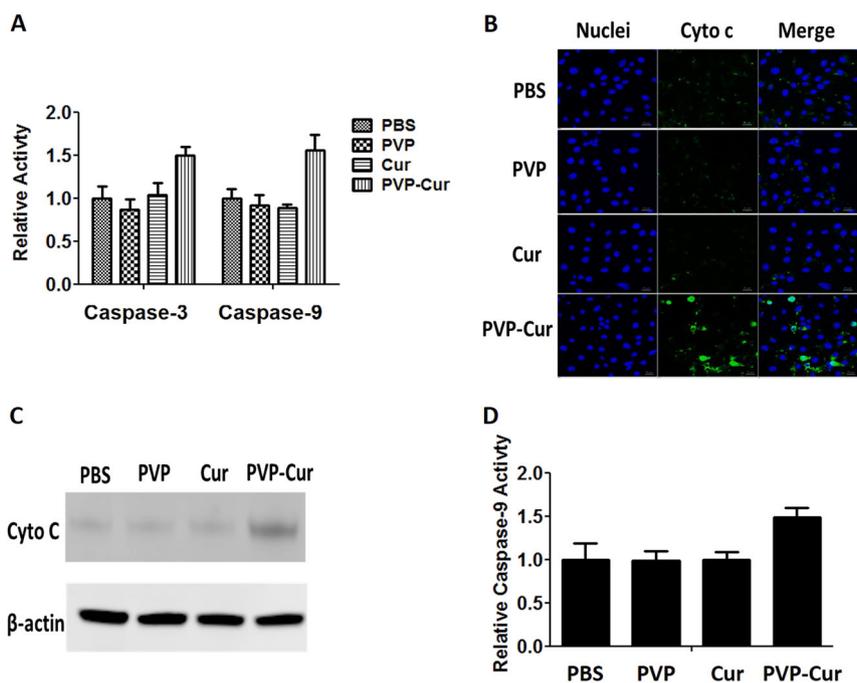


Fig. 6 PVP-cur induces PC3 cell apoptosis via the mitochondrial pathway. **a** Caspase-3, -9 cleavage activity assay. PC3 were treated with PBS, PVP, Cur, or PVP-Cur for 24 h, and the caspases were separately detected by the caspase activity assay kit. Data are means of 3 independent experiments. **b** the release of cytochrome c was detected by fluorescent microscopy (green). The nuclei were stained with DAPI

(blue), Scale bar 10 μ m. **c** cytochrome c released to the cytosol was subfractionated and examined by immunoblotting after PBS, PVP, Cur, or PVP-Cur treatment, β -actin was loaded as a control. **d** PC3 were treated with PBS, PVP, Cur, or PVP-Cur, and caspase-9 was detected by the caspase activity assay kit. Data are means of 3 independent experiments

PVP-cur induces PC3 cell apoptosis via mitochondrial pathway

Previously, Alithen group reported that Curcumin Analog DK1 but not Curcumin induces human osteosarcoma cells Apoptosis via mitochondria-dependent signaling (Aziz et al. 2018). To explore whether PVP-cur-induced cell apoptosis via mitochondria-dependent signaling, we measured the activation of caspases, hallmark of apoptosis. Immunoblotting assay showed that both caspase-3 and caspase-9 were activated on PVP-cur treatment group (Fig. 5d). PVP-cur-induced activation of caspase-3 and caspase-9 were further confirmed by DEVD-pNA and LHED-pNA cleavage activity assays, respectively (Fig. 6a). Since activation of caspase-9 is normally induced by cytochrome c released to the cytoplasm (14), we then detected released cytochrome c in the cytoplasm. Immunofluorescence and immunoblotting assays showed that PVP-Cur significantly promoted the release of cytochrome c to the cytoplasm when compared to PBS, PVP, or Cur group (Fig. 6b, c), which was consistent with the subsequent caspase-9 activation. Considering cytochrome c release and caspase-9 activation are both hallmarks of the mitochondria regulated apoptosis process, these results lead us to speculate that the

mitochondrial pathway is involved in the PVP-Cur-induced apoptotic process.

Anchorage of curcumin onto PVP enhanced cellular uptake of curcumin

Since anchorage of curcumin onto PVP increased the anti-proliferation activity of curcumin and enhanced PC3 cell apoptosis, we speculate that incorporation of curcumin into PVP might increase cellular uptake of curcumin in PC3 cells. PC3 cells were treated with PBS, PVP, Curcumin or PVP-cur for 4 h. As shown in Figs 6a and 7a, b, curcumin was only observed in PVP-Cur group, but not in curcumin alone group. Similar results were obtained when intracellular curcumin was detected. Collectively, our data suggest that anchorage of curcumin onto PVP can significantly enhance cellular uptake of curcumin.

Discussion

Curcumin exhibits anti-neoplastic, anti-inflammatory, and anti-virus activity. Several clinical trials dealing with cancers have addressed the pharmacokinetics, safety, and efficacy of

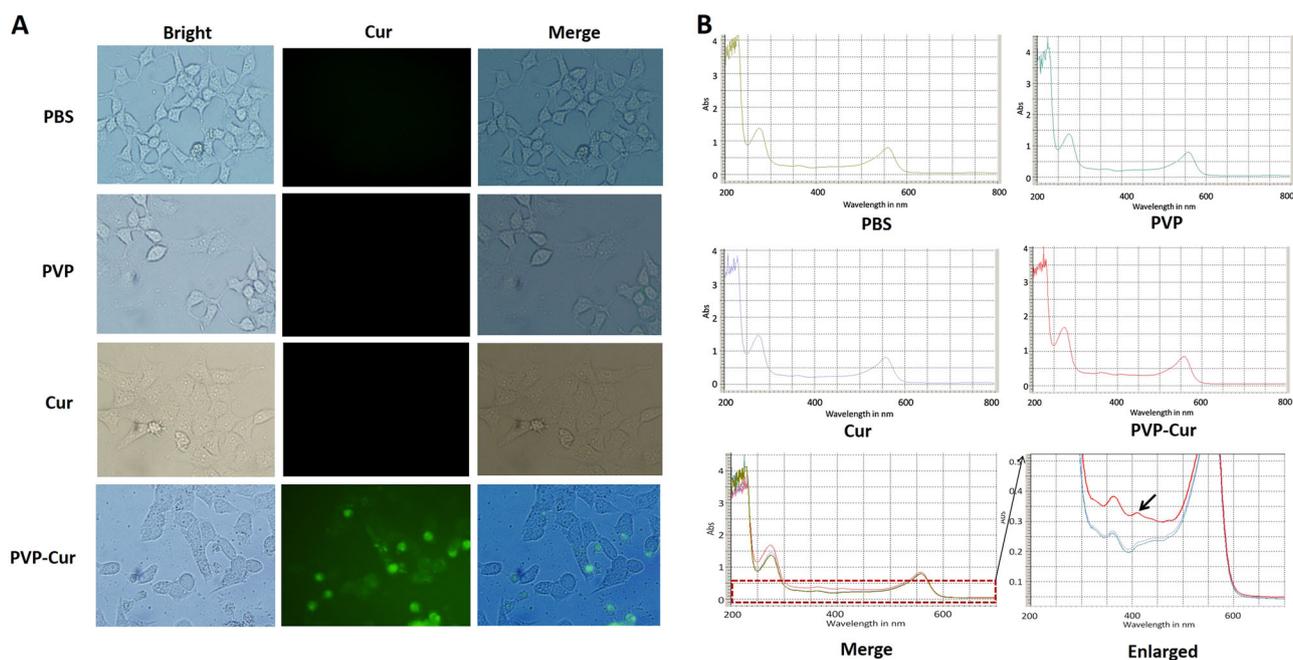


Fig. 7 Anchorage of curcumin onto PVP can promote cellular uptake of curcumin. **a** Immunofluorescence of Cur (curcumin) in PC3 cells treated with PVP-Cur, Cur (curcumin), PVP, and PBS. Digital images

were captured by Olympus BX 51 fluorescence microscope. **b** Spectrographs of Cur (curcumin) in PC3 cells treated with PVP-Cur, Cur (curcumin), PVP, and PBS

curcumin in humans (Ye et al. 2012). Although extensive research has been done for more than three decades, poor solubility of curcumin remains a major barrier in its bioavailability and clinical efficacy. To increase its solubility and bioavailability, many attempts have been made, including nanoparticles, liposomes, micelles, and phospholipid complexes etc. (Darvesh et al. 2012; Sun et al. 2012) Here, we employed a simple (with few steps) and environmental protection method to produce PVP-Cur and systematically demonstrated that anchorage of curcumin onto PVP enhances anti-tumor effect of curcumin. PVP: curcumin in the ratio of 6:1 or 8:1 exhibited highest loading capacity, incorporation of curcumin onto PVP could dramatically increase the solubility, and stability of curcumin. Compared with curcumin alone, PVP-Cur displayed an increased ability in abrogating tumor cell proliferation and inducing tumor cell apoptosis *via* mitochondria signaling, which was attributed by enhancing the cellular uptake of PVP-Cur.

Anti-cancer therapies, such as chemotherapy has been shown to be aggressive approaches for treating cancer. Previously study reported that nearly 50% of cells treated with 5 $\mu\text{g}/\text{ml}$ curcumin lost viability in highly metastatic prostate cancer cells (Rivera et al. 2017). Consistently, we found that among PC3 cells (7.24 mg/L), A549 (15.85 mg/L) and HepG₂ (14.13 mg/L), the IC₅₀ of PVP-cur that caused 50% inhibition of cell growth is the lowest in PC3 cells (Fig. 4e), which indicated that PC3 cells was more sensitive to PVP-cur. Apoptosis, the cell's natural mechanism for death, is a promising target for anti-cancer

therapy. Both the intrinsic and extrinsic pathways use compass to carry out apoptosis through the cleavage of hundreds of proteins. Alitheen group reported that curcumin Analog DK1 induces human osteosarcoma cells apoptosis *via* mitochondria-dependent signaling (Aziz et al. 2018). Similarly, prostate cancer treated with curcumin, the upregulation of ER stress was measured, Chronic ER stress induction was concomitant with the upregulation of proapoptotic markers caspases 3 and 9. (Rivera et al. 2017) Consistent with previously results, we further confirm that PVP-cur trigger prostate cancer cell apoptosis *via* mitochondria signaling. Collectively, these findings suggest that PVP-Cur is a more suitable drug candidate in comparison with curcumin alone, and provides a basis for further curcumin-based drug development.

Acknowledgements This work was supported by the Natural Science Foundation of China (No. 81500336), Beijing Natural Science Foundation (No. 7151004, 7174307), and The China Postdoctoral Science Foundation (Special Fund 2015T81096).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

- Aziz MNM, Hussin Y, Che Rahim NF, Nordin N, Mohamad NE, Yeap SK, Yong CY, Masarudin MJ, Cheah YK, Abu N, Akhtar MN, Alitheen NB (2018) Curcumin analog DK1 induces apoptosis in human osteosarcoma cells in vitro through mitochondria-dependent signaling pathway. *Mol (Basel, Switz)* 23:pri: E75
- Castro CN, Barcala Tabarozzi AE, Winnewisser J, Gimeno ML, Antunica Noguero M, Liberman AC, Paz DA, Dewey RA, Perone MJ (2014) Curcumin ameliorates autoimmune diabetes. Evidence in accelerated murine models of type 1 diabetes. *Clin Exp Immunol* 177:149–160
- Chen QH (2015) Curcumin-based anti-prostate cancer agents. *Anti-Cancer Agents Med Chem* 15:138–156
- Cutrignelli A, Lopodota A, Denora N, Iacobazzi RM, Fanizza E, Laquintana V, Perrone M, Maggi V, Franco M (2014) A new complex of curcumin with sulfobutylether-beta-cyclodextrin: characterization studies and in vitro evaluation of cytotoxic and antioxidant activity on HepG-2 cells. *J Pharm Sci* 103:3932–3940
- Darvesh AS, Aggarwal BB, Bishayee A (2012) Curcumin and liver cancer: a review. *Curr Pharm Biotechnol* 13:218–228
- Dong Y, Yin S, Song X, Huo Y, Fan L, Ye M, Hu H (2016) Involvement of ROS-p38-H2AX axis in novel curcumin analogues-induced apoptosis in breast cancer cells. *Mol Carcinog* 55:323–334
- Ferreira VH, Nazli A, Dizzell SE, Mueller K, Kaushic C (2015) The anti-inflammatory activity of curcumin protects the genital mucosal epithelial barrier from disruption and blocks replication of HIV-1 and HSV-2. *PLoS ONE* 10:e0124903
- Gao M, Chen C, Fan A, Zhang J, Kong D, Wang Z, Zhao Y (2015) Covalent and non-covalent curcumin loading in acid-responsive polymeric micellar nanocarriers. *Nanotechnology* 26:275101
- Gao W, Chan JY, Wei WI, Wong TS (2012) Anti-cancer effects of curcumin on head and neck cancers. *Anti-Cancer Agents Med Chem* 12:1110–1116
- Hasan M, Belhaj N, Benachour H, Barberi-Heyob M, Kahn CJ, Jabbari E, Linder M, Arab-Tehrany E (2014) Liposome encapsulation of curcumin: physico-chemical characterizations and effects on MCF7 cancer cell proliferation. *Int J Pharm* 461:519–528
- Howells LM, Mahale J, Sale S, McVeigh L, Steward WP, Thomas A, Brown K (2014) Translating curcumin to the clinic for lung cancer prevention: evaluation of the preclinical evidence for its utility in primary, secondary, and tertiary prevention strategies. *J Pharmacol Exp Ther* 350:483–494
- Jahangiri A, Barzegar-Jalali M, Garjani A, Javadzadeh Y, Hamishkhar H, Rameshrad M, Adibkia K (2015) Physicochemical characterization and pharmacological evaluation of ezetimibe-PVP K30 solid dispersions in hyperlipidemic rats. *Colloids Surf B, Biointerfaces* 134:423–430
- Jordan BC, Mock CD, Thilagavathi R, Selvam C (2016) Molecular mechanisms of curcumin and its semisynthetic analogues in prostate cancer prevention and treatment. *Life Sci* 152:135–144
- Karewicz A, Bielska D, Loboda A, Gzyl-Malcher B, Bednar J, Jozkowicz A, Dulak J, Nowakowska M (2013) Curcumin-containing liposomes stabilized by thin layers of chitosan derivatives. *Colloids Surf B, Biointerfaces* 109:307–316
- Knopp MM, Nguyen JH, Becker C, Francke NM, Jorgensen EB, Holm P, Holm R, Mu H, Rades T, Langguth P (2016) Influence of polymer molecular weight on in vitro dissolution behavior and in vivo performance of celecoxib:PVP amorphous solid dispersions. *Eur J Pharm Biopharm* 101:145–151
- Li B, Konecke S, Wegiel LA, Taylor LS, Edgar KJ (2013) Both solubility and chemical stability of curcumin are enhanced by solid dispersion in cellulose derivative matrices. *Carbohydr Polym* 98:1108–1116
- Liu M, Du H, Zhai G (2015) The design of amphiphilic polymeric micelles of curcumin for cancer management. *Curr Med Chem* 22:4398–4411
- Mahmood K, Zia KM, Zuber M, Salman M, Anjum MN (2015) Recent developments in curcumin and curcumin based polymeric materials for biomedical applications: A review. *Int J Biol Macromol* 81:877–890
- Mehta HJ, Patel V, Sadikot RT (2014) Curcumin and lung cancer—a review. *Target Oncol* 9:295–310
- Mulik RS, Monkkonen J, Juvonen RO, Mahadik KR, Paradkar AR (2012) ApoE3 mediated polymeric nanoparticles containing curcumin: apoptosis induced in vitro anticancer activity against neuroblastoma cells. *Int J Pharm* 437:29–41
- Nabavi SF, Thiagarajan R, Rastrelli L, Daglia M, Sobarzo-Sanchez E, Alinezhad H, Nabavi SM (2015) Curcumin: a natural product for diabetes and its complications. *Curr Top Med Chem* 15:2445–2455
- Nagaraju GP, Aliya S, Zafar SF, Basha R, Diaz R, El-Rayes BF (2012) The impact of curcumin on breast cancer. *Integr Biol: Quant Biosci nano macro* 4:996–1007
- Najlah M, Ahmed Z, Iqbal M, Wang Z, Tawari P, Wang W, McConville C (2017) Development and characterisation of disulfiram-loaded PLGA nanoparticles for the treatment of non-small cell lung cancer. *Eur J Pharm Biopharm* 112:224–233
- Nayak AP, Mills T, Norton I (2016) Lipid based nanosystems for curcumin: past, present and future. *Curr Pharm Des* 22:4247–4256
- Padmanaban G, Rangarajan PN (2016) Curcumin as an adjunct drug for infectious diseases. *Trends Pharmacol Sci* 37:1–3
- Paradkar A, Ambike AA, Jadhav BK, Mahadik KR (2004) Characterization of curcumin-PVP solid dispersion obtained by spray drying. *Int J Pharm* 271:281–286
- Rahmani AH, Al Zohairy MA, Aly SM, Khan MA (2014) Curcumin: a potential candidate in prevention of cancer via modulation of molecular pathways. *BioMed Res Int* 2014:761608
- Ranjan D, Chen C, Johnston TD, Jeon H, Nagabhushan M (2004) Curcumin inhibits mitogen stimulated lymphocyte proliferation, NFκappaB activation, and IL-2 signaling. *J Surg Res* 121:171–177
- Rivera M, Ramos Y, Rodriguez-Valentin M, Lopez-Acevedo S, Cubano LA, Zou J, Zhang Q, Wang G, Boukli NM (2017) Targeting multiple pro-apoptotic signaling pathways with curcumin in prostate cancer cells. *PLoS ONE* 12:e0179587
- Sadeghi F, Ashofteh M, Homayouni A, Abbaspour M, Nokhodchi A, Garekani HA (2016) Antisolvent precipitation technique: A very promising approach to crystallize curcumin in presence of polyvinyl pyrrolidone for solubility and dissolution enhancement. *Colloids Surf B, Biointerfaces* 147:258–264
- Samy DM, Ismail CA, Nassra RA, Zeitoun TM, Nomair AM (2016) Downstream modulation of extrinsic apoptotic pathway in streptozotocin-induced Alzheimer's dementia in rats: Erythropoietin versus curcumin. *Eur J Pharmacol* 770:52–60
- Sarisuta N, Kumpugdee M, Muller BW, Puttipatkhachorn S (1999) Physico-chemical characterization of interactions between erythromycin and various film polymers. *Int J Pharm* 186:109–118
- Seo JA, Kim B, Dhanasekaran DN, Tsang BK, Song YS (2016) Curcumin induces apoptosis by inhibiting sarco/endoplasmic reticulum Ca²⁺ ATPase activity in ovarian cancer cells. *Cancer Lett* 371:30–37
- Shanmugam MK, Rane G, Kanchi MM, Arfuso F, Chinnathambi A, Zayed ME, Alharbi SA, Tan BK, Kumar AP, Sethi G (2015) The multifaceted role of curcumin in cancer prevention and treatment. *Molecules* 20:2728–2769
- Song Z, Zhu W, Liu N, Yang F, Feng R (2014) Linolenic acid-modified PEG-PCL micelles for curcumin delivery. *Int J Pharm* 471:312–321

- Sordillo LA, Sordillo PP, Helson L (2015) Curcumin for the Treatment of Glioblastoma. *Anticancer Res* 35:6373–6378
- Sou K, Inenaga S, Takeoka S, Tsuchida E (2008) Loading of curcumin into macrophages using lipid-based nanoparticles. *Int J Pharm* 352:287–293
- Sun M, Su X, Ding B, He X, Liu X, Yu A, Lou H, Zhai G (2012) Advances in nanotechnology-based delivery systems for curcumin. *Nanomedicine* 7:1085–1100
- Swaminathan S, Cavalli R, Trotta F (2016) Cyclodextrin-based nanosponges: a versatile platform for cancer nanotherapeutics development. *Wiley Interdiscip Rev Nanomed nanobiotechnol* 8:579–601
- Tabatabaei Mirakabad FS, Akbarzadeh A, Milani M, Zarghami N, Taheri-Anganeh M, Zeighamian V, Badrzadeh F, Rahmati-Yamchi M (2016) A Comparison between the cytotoxic effects of pure curcumin and curcumin-loaded PLGA-PEG nanoparticles on the MCF-7 human breast cancer cell line. *Artif Cells, Nanomed, Biotechnol* 44:423–430
- Thongnopkoon T, Puttipipatkachorn S (2016) New metastable form of glibenclamide prepared by redispersion from ternary solid dispersions containing polyvinylpyrrolidone-K30 and sodium lauryl sulfate. *Drug Dev Ind Pharm* 42:70–79
- Troselj KG, Kujundzic RN (2014) Curcumin in combined cancer therapy. *Curr Pharm Des* 20:6682–6696
- Wang L, Ye X, Cai X, Su J, Ma R, Yin X, Zhou X, Li H, Wang Z (2015) Curcumin suppresses cell growth and invasion and induces apoptosis by down-regulation of Skp2 pathway in glioma cells. *Oncotarget* 6:18027–18037
- Wang Y, Yin H, Wang L, Shuboy A, Lou J, Han B, Zhang X, Li J (2013) Curcumin as a potential treatment for Alzheimer's disease: a study of the effects of curcumin on hippocampal expression of glial fibrillary acidic protein. *Am J Chin Med* 41:59–70
- Wilken R, Veena MS, Wang MB, Srivatsan ES (2011) Curcumin: A review of anti-cancer properties and therapeutic activity in head and neck squamous cell carcinoma. *Mol Cancer* 10:12
- Wongcharoen W, Phrommintikul A (2009) The protective role of curcumin in cardiovascular diseases. *Int J Cardiol* 133:145–151
- Yallapu MM, Jaggi M, Chauhan SC (2010) beta-Cyclodextrin-curcumin self-assembly enhances curcumin delivery in prostate cancer cells. *Colloids Surf B, Biointerfaces* 79:113–125
- Yao Q, Lin M, Wang Y, Lai Y, Hu J, Fu T, Wang L, Lin S, Chen L, Guo Y (2015) Curcumin induces the apoptosis of A549 cells via oxidative stress and MAPK signaling pathways. *Int J Mol Med* 36:1118–1126
- Ye MX, Li Y, Yin H, Zhang J (2012) Curcumin: updated molecular mechanisms and intervention targets in human lung cancer. *Int J Mol Sci* 13:3959–3978
- Ye M, Zhang J, Zhang J, Miao Q, Yao L, Zhang J (2015) Curcumin promotes apoptosis by activating the p53-miR-192-5p/215-XIAP pathway in non-small cell lung cancer. *Cancer Lett* 357:196–205
- Zang S, Liu T, Shi J, Qiao L (2014) Curcumin: a promising agent targeting cancer stem cells. *Anti-Cancer Agents Med Chem* 14:787–792