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Curcumin induces apoptosis via simultaneously targeting AKT/mTOR and RAF/MEK/ERK survival signaling pathways in human leukemia THP-1 cells

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Background: Curcumin is a multi-targeted anti-cancer agent. However, there are few studies on its anti-leukemia activity in human acute monocytic leukemia. Here, we study the effect and mechanisms of curcumin on acute monocytic leukemia. *Methods:* The acute monocytic leukemia cell line THP-1 was used as *in vitro* cell model to explore the anti-leukemia effects and mechanisms of curcumin. Cell proliferation was measured by MTT assay, cell apoptosis bodies were observed using a light microscope, cell apoptosis rate was evaluated by flow cytometry, and the expression alterations of growth-sinaling proteins were detected by Western blotting. *Results:* Curcumin inhibited cell proliferation and induced cell apoptosis in time- and dose- dependent manner in THP-1 cells. Curcumin significantly inhibited the activations of AKT/mTOR and RAF/MEK/ERK signaling pathways simultaneously. *Conclusion:* This study demonstrates that curcumin inhibits proliferation and induces apoptosis in THP-1 cells *via* inhibiting the activations of AKT/mTOR and RAF/MEK/ERK signaling pathways simultaneously. Our data suggest that curcumin is a promising anti-tumor agent in acute monocytic leukemia.

1. Introduction

Acute myeloid leukaemia is a clonal disorder which comprises a group of clonal malignant diseases characterized by a deregulated proliferation of myeloid haematopoietic stem/progenitor cells (Steffen et al. 2005). The main therapy strategies for AML patients are aggressive chemotherapeutic regimens and HSCT. Though most AML patients who receive intensive chemotherapy can achieve complete remission, the frequency of relapse is high and the overall five year survival rate is only 20% (Rowe 2008). The main characteristic of AML is the uncontrolled proliferation/survival of immature myeloid progenitors which undergo a differentiation block at various maturation steps, resulting in the accumulation of leukemic cells in the bone marrow and inhibition of normal hematopoiesis (Gilliland 2004). Deregulation of the signaling pathways including PI3K/AKT, RAF/MEK/ERK, STAT3/5 (Steelman et al. 2008), Wnt/β-catenin (De et al. 2006; Valencia et al. 2009), and NF-kB (Guzman et al. 2001) enhance the survival and proliferation of hematopoietic progenitor cells which is the basis of development of AML. So it has been postulated that effectively targeting some of these pathways could have a considerable impact on AML therapy. Much interest has focused on specific inhibitors such as mTOR inhibitor, MEK inhibitor, PI3Kinhibitor, AKT inhibitor and so on. While AML is usually the result of dysregulation of several signaling pathways, so these specific inhibitors used as monotherapy which only target one or two components of one signaling pathway have not translated into substantial clinical benefit in early phase trials (Chapuis et al. 2010; Yee et al. 2006; Rizzieri et al. 2008). The current paradigm for most treatments is to either combine several specific inhibitors or design drugs that modulate multiple targets. Many natural drugs are multi-target agents, and is one of the most intriguing of these natural drugs, and has been shown to suppress multiple signaling pathways and inhibit cell proliferation, invasion, metastasis, and angiogenesis of many cancer cells. Using the acute myelogenous leukemia cell line THP-1, we investigated whether curcumin could act as multi-target agent in acute myelogenous leukemia cells, then induced the inhibition of proliferation and apopto-

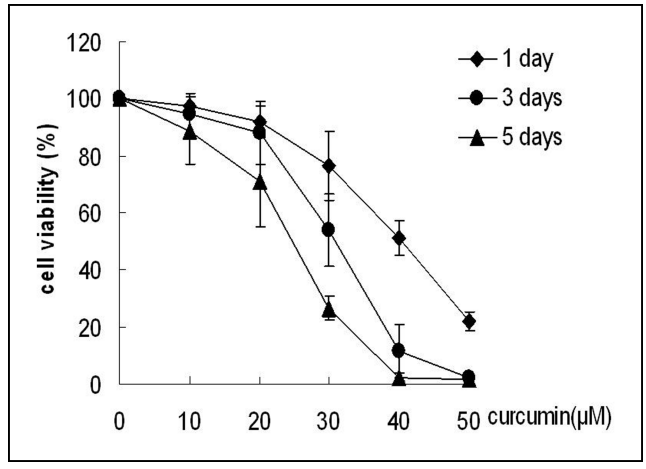

Fig. 1: Curcumin inhibited proliferation of THP-1 cells in a time- and dose-dependent manner. THP-1cells were incubated with 0, 10, 20, 30, 40,50 μM curcumin for 1 day, 3 day and 5 day. At the end of incubation, the cell survival rates were determined by MTT methods. Cell viability is expressed as the percentage of cell survival compared to the control. The results shown were representative of three independent experiments.

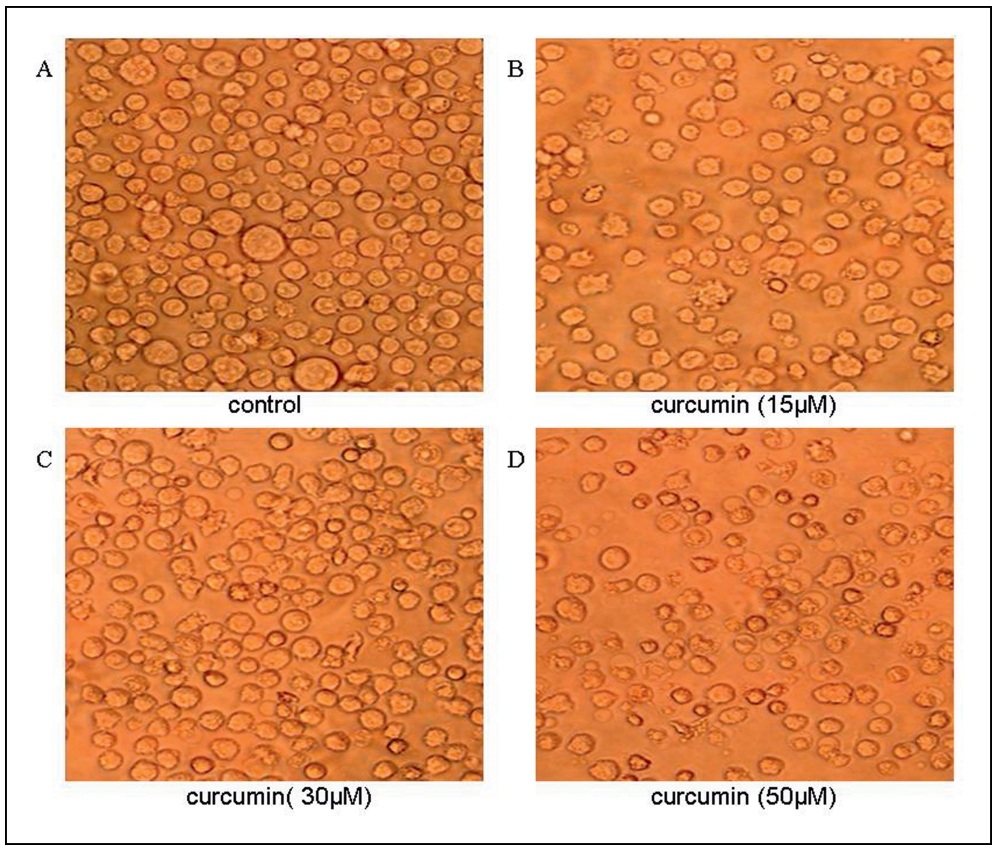

Fig. 2: Curcumin induced the production of apoptosis bodies in THP-1 cells.The cells were treated with 15, 30, 50 μM curcumin for 24 h, the cellular morphologic changes were observed using a light microscope (Olympus). The amount of apoptosis bodies increased with the augment of concentration of curcumin.

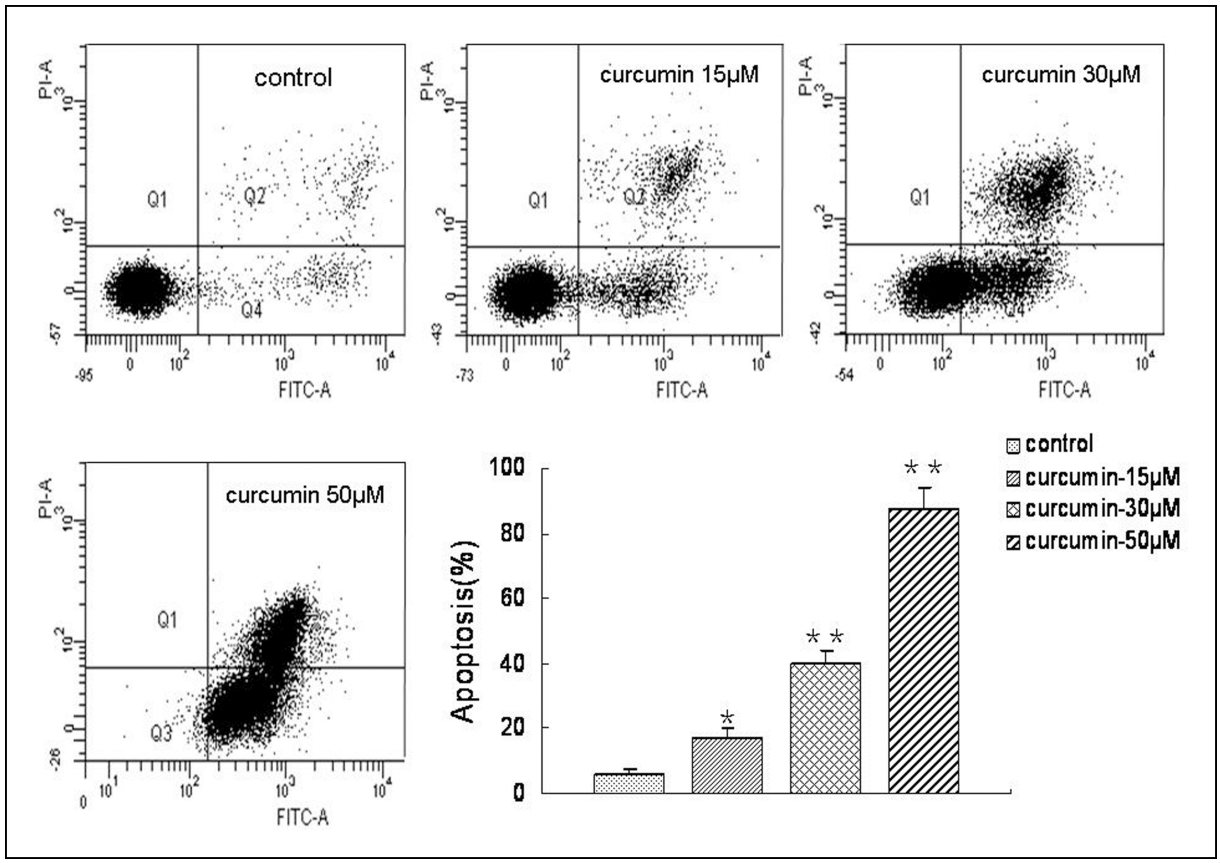

Fig. 3: Curcumin induced apoptosis in THP-1 cells. To further confirm the curcumin-mediated apoptosis in THP-1 cells, the percent of apoptotic cells was examined by flow cytometry using the Annexin V-FITC /PI apoptosis detection kit. Data were obtained from three independent experiments and expressed as mean ± SE. * indicates $p < 0.05$ compared with control,** $p < 0.01$ compared with control.

sis. The present study demonstrated that curcumin inhibits the proliferation and induces the apoptosis of acute myelogenous leukemia cells *via* simultaneously targeting AKT/mTOR and RAF/MEK/ERK signaling pathways.

2. Investigations and results

2.1. The anti-proliferative effect of curcumin on THP-1 cells

The results of MTT assay showed that curcumin inhibited cell growth in time- and dose- dependent manner in THP-1 cells (Fig. 1). After 72 h of treatment, IC_{50} value (the concentrations of oridonin which caused 50% inhibition of cell growth) was 27.13 ± 1.49 μM.

2.2. Curcumin-mediated growth inhibition via induction of apoptosis in THP-1 cells

To determine whether curcumin-mediated growth inhibition is associated with apoptosis, THP-1 cells were treated with 15 μM, 30 μM, 50 μM curcumin for 24 h, then the morphologic changes were observed using a light microscope. As shown in Fig. 2, 24 h curcumin-tretment induced the appearance of apoptosis bodies, and the amount of the apoptosis bodies increased with the increase of the concentration of curcumin. To further confirm the curcumin-mediated apoptosis in THP-1 cells, cell apoptosis rate was performed by FCM. Our results revealed that apoptotic rate of SUP-B15 cells which, without drug treatment, was 5.90 ± 1.77 %, increased to 17.47 ± 2.55 %, 40.13 ± 4.01% and 87.37 ± 6.41% respectively following treatment with 15 μM, 30 μM, 50 μM curcumin (Fig. 3). These results suggested that curcumin suppresses the growth of THP-1 cells *via* inducing cell apoptosis.

2.3. Curcumin inhibits activation of Akt/mTOR and Raf/MEK /ERK signaling pathways in THP-1 cells

To study the mechanisms of anti-leukemia activity of curcumin, we first examined activation levels of Akt/mTOR and Raf/MEK/ERK signaling pathways in THP-1 cells. Western blot analysis showed that THP-1 cells without curcumin treatment expressed constitutively the phosphorylated form of Akt (Ser473), GSK3β (ser9), mTOR (ser2448), 4EBP1 (Thr37/46), p70S6K (Tyr389)proteins, Raf (ser338), MEK1/2 (ser217/221) and ERK 1/2 (Thr202/Tyr204) proteins. After treatment with curcumin, not only levels of the phosphorylated forms of Akt(Ser473), GSK3β(ser9), mTOR (ser2448), 4EBP1 (Thr37/46), p70S6K (Tyr389), Raf (ser338), MEK1/2 (ser217/221) and ERK 1/2 (Thr202/Tyr204) but also the total protein level of Akt, mTOR, 4EBP1, p70S6K were down-regulated in a time- and dose-dependent manner (Fig. 4, 5).

3. Discussion

In this study, curcumin exhibited a strong anti-leukemia effect in AML cell line THP-1 *in vitro*. Our results showed that curcumin inhibited the spontaneous growth of THP-1 cells in a time- and dose-dependent manner. To further investigate the mechanisms of anti-leukemia activity of curcumin, we analyzed the effect of curcumin on apoptosis and activation of Akt/mTOR and Raf/MEK/ERK signaling pathways which were constitutively activated in THP-1 leukemia cells. Our results showed that curcumin induced apoptosis by down-regulating Akt/mTOR and Raf/MEK/ERK signaling pathways.

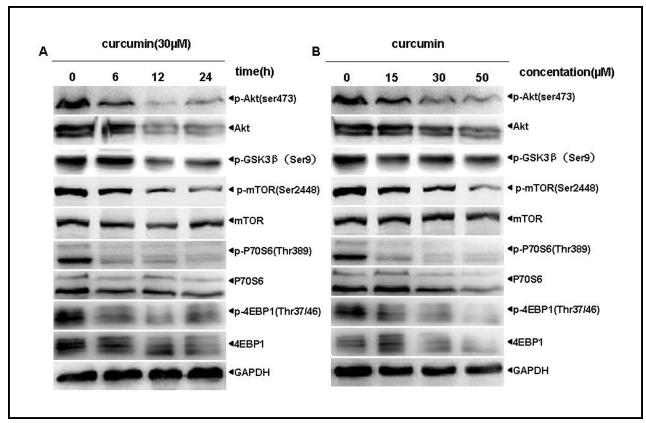

Fig. 4: Curcumin inhibited the constitutive activation of AKT/mTOR signaling pathway in THP-1 cells. The cells were incubated with 30 μM curcumin for 6, 12, 24 h or with 15, 30, 50 μM curcumin for 24 h, cells were then harvested and total proteins were extracted. Equal amounts of protein from each sample were separated on 6–15%SDS-PAGE and immunoblotted with indicated antibodies, GAPDH was used as a loading control. Curcumin inhibited the constitutive activation of Akt/mTOR signaling pathway in a time- and dose-dependent manner.

Different from normal cells, leukemia cells often express constitutively active survival-signaling pathways such as Akt/mTOR, RAF/MEK/ERK, Jak/STAT and NF-κB signaling pathways because of gene mutations, rearrangements and chromosomal translocations, and these survival-signaling pathways play vital roles in tumorigenesis, proliferation, anti-apoptosis, drug resistance (Karin et al. 2002; Talapatra et al. 2001; Teachey et al. 2009). The more growth-signaling pathways are constitutively active in acute myelogenous leukemia, the poorer is the prognosis (Kornblau et al. 2006). Our results showed that in acute monoblastic leukemia cell line THP-1, both Akt/mTOR and RAF/MEK/ERK signal transduction pathways

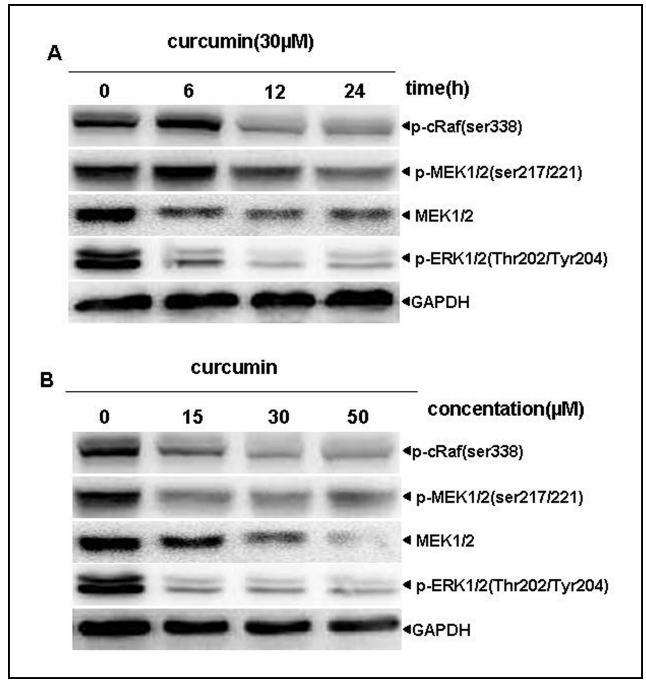

Fig. 5: Curcumin inhibited the constitutive activation of RAF/MEK/ERK signaling pathway in THP-1 cells. The cells were incubated with 30 μM curcumin for 6, 12, 24 h or with 15, 30, 50 μM curcumin for 24 h, cells were then harvested and total proteins were extracted. Equal amounts of protein from each sample were separated on 6–15%SDS-PAGE and immunoblotted with indicated antibodies, GAPDH was used as a loading control. Curcumin inhibited the constitutive activation of RAF/MEK/ERK signaling pathway in a time- and dose-dependent manner.

were constitutively active simultaneously, this may be one of reasons why acute monoblastic leukemia is more aggressive, and the prognosis is poorer than that of other types of acute myelogenous leukemia. Since Akt/mTOR and RAF/MEK/ERK signaling pathways were simultaneously active in THP-1 cells, we speculate that agents inhibiting activation of Akt/mTOR, Raf/MEK/ERK would create anti-leukiemia effects, especially the inhibitors targeting simultaneously the two pathways may prove more effective in THP-1 cells.

Curcumin has been proven to be a powerful therapeutic drug (Anand et al. 2008; Aggarwal et al. 2009). Curcumin induces apoptosis and inhibits proliferation in a variety of cancer cells by targeting multiple cellular signaling pathways (Kunnumakkara et al. 2008). It was reported that curcumin induced apoptosis and inhibited survial of pancreatic and lung adenocarcinoma cells *via* inhibition of Erk1/2 activity (Lev-Ari et al. 2006). Shishodia et al. (2005) found curcumin induced apoptosis and suppressed proliferation in mantle cell lymphoma by inhibiting constitutive NF-kappaB activation. Beevers et al. (2006) reported that curcumin inhibited the mammalian target of rapamycin-mediated signaling pathways in cancer cells and Choi et al. (2008) found that curcumin down-regulated the multidrug-resistance mdr1b gene by inhibiting the PI3K/Akt pathway. Prasad et al. (2009) found that curcumin inhibited proliferation in human breast cancer cells by modulating Wnt/beta-catenin signaling. Wang et al. (2006) reported that curcumin induced apoptosis and inhibited growth in pancreatic cancer cells by down-regulating Notch-1 signaling pathways. Since curcumin acts as multi-target agent inhibiting proliferation in a variety of cancer cells, we hypothesized that curcumin could induce proliferation inhibition in THP-1 cells *via* targeting the constitutively active survival signaling pathways. In consistence with our hypothesis, curcumin induced apoptosis, then inhibited the proliferation of THP-1 cells in a time- and dose-dependent manner by inhibiting the activation of Akt/mTOR and RAF/MEK/ERK pathways simultaneously. Unlike Yang's findings, curcumin induced THP-1 cells apoptosis *via* the activation of MAPK/AP1 pathways (Yang et al. 2012). We think that different cell culture environments account for this difference.

Compared to many specific inhibitors which hit a single target for the treatment of leukemia, curcumin may be more effective, because drug resistance emerges frequently because of the hyperactivation of alternative signaling pathways under treatment of a single target. While curcumin exhibited multi-targeted characteristics, we expect that resistance to curcumin in acute monoblastic leukemia may rarely occur and response to curcumin may be higher, response duration may be longer.

It is worth noting that curcumin had no major toxicities in phase I and II clinical studies at doses of up to 8 g/day (Lao et al. 2006; Dhillon et al. 2008).

Taken together, curcumin simultaneously inhibits Akt/mTOR and Raf/MEK/ERK pathways which are constitutively activated in acute monoblastic leukemia cell line, THP-1 cells, then induces apoptosis and inhibits proliferation. Curcumin is a promising therapeutic agent for acute monoblastic leukemia.

4. Experimental

4.1. Materials

Curcumin was obtained from Sigma Chemical Co. The stock solution of curcumin (50 mM) was prepared by dissolving curcumin in dimethyl sulfoxide (DMSO) and stored at -20 °C. Antibodies against Akt, p-Akt (ser473), p-GSK3β(ser9), mTOR, p-mTOR (ser2448), 4EBP1, p-4EBP1 (Thr37/46), P70S6, p-P70S6 (Thr389),p-cRaf (ser338), MEK1/2, p-MEK1/2 (ser217/221), p-ERK1/2(Thr202/Tyr204), and goat anti-rabbit horseradish peroxidase (HRP) conjugate were obtained from Cell Signaling Technologies. AnnexinV-FITC appotosis detection kit was purchased from KeyGen Biotech.CO., Ltd. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) was obtained from Sigma Chemical Co.

4.2. Cell culture conditions

The human monoblastic leukemia cell line THP-1 was obtained from American Type Culture Collection and cultured in RPMI-1640 medium supplemented with 10% FBS, 100 U/ml penicillin and 100 g/ml streptomycin and in 5% Co_2 incubator at 37 °C.

4.3. MTT assay

The antiproliferative effects of curcumin on THP-1 cells were determined by MTT assay. Briefly, the cells (1×10^5 cells/well) were incubated in triplicate in a 96-well plate in the presence or absence of indicated concentration curcumin in a final volume of 100 μL for 1,3,5 days at 37 °C. Then, 20 μL MTT solution (5 mg/mL in PBS) were added to each well. After an 4–6 h incubation at 37 °C, 100 μL SDS-isobutanol-HCl solution was added and incubation was continued overnight at room temperature. Thereafter the optical density (OD) was measured using μQuant MQX200 Microplate Spectrophotometer (Biotek) at a wavelength of 570 nm. The cell viability was expressed as a percentage: OD(experiment samples)-OD(blank)/(OD (control)-OD(blank)) × 100%. The percentage of cell growth inhibition was calculated as follows: OD(control)-OD(experiment samples)/(OD(control)-OD(blank)) × 100%.

4.4. Light microscopy

THP-1 cells (2×10^6) in RMPI-1640 with 10% FBS were seeded into 6-well culture plates and cultured with indicated concentration of curcumin for 24 h. The cellular morphologic changes were observed using a light microscope (Olympus).

4.5. Annexin V-FITC/PI staining for evaluation of apoptosis

THP-1 cells were stained using KeyGen Apoptosis Kit according to manufacturer's recommended protocol. Briefly, cells treated with indicated curcumin for 24 h, 2×10^5 cells were harvested, washed twice with cold PBS and resuspended in 100 μl of binding buffer. 5 μl of annexin VFITC were added, 15 min later 5 μl of PI were added, cells were incubated at room temperature for 15 min in the dark. Then the samples were diluted with 400 μl of binding buffer and the stained cells were analyzed in Cytomics FC500 flow cytometer equipped with CXP software within 1 h. For each analysis 10,000 events were recorded.

4.6. Western blotting analysis for protein expression

After curcumin-treatment for indicated time, 5×10^6 THP-1 cells were collected and washed twice with cold PBS. After centrifugation, RAPI lysis buffer (20 mM Tris, pH 7.4; 250 mM NaCl; 2 mM EDTA, pH 8.0; 0.1% Triton-X100; 0.01 mg/mL aprotinin; 0.005 mg/mL leupeptin; 0.4 mM PMSF; 4 mM NaVO4) was added. Cell lysis was carried out on ice for 15 min, after centrifugation at 12,000× g for 20 min, the supernatant was separated and stored at –70 °C until use. Protein extracts were quantitated and loaded on 6% to 15% sodium dodecyl sulfate–polyacrylamide gel. After electrophoresis, proteins were transferred to polyvinylidene difluoride blots at 250 mA for 30 min to 2 h. The blots were blocked for 1 h at room temperature in fresh blocking buffer (0.1% Tween 20 in Tris-buffered saline, pH 7.6, containing 5% non-fat powdered milk). Following three washes with TBST (TBS and 0.1% Tween 20), the blots were incubated with specific primary antibody overnight at 4 °C, then washed again three times with TBST and incubated with horseradish peroxidase (HRP)–conjugated secondary antibody for 1 h at room temperature, and finally detected by enhanced chemiluminescence (ECL) detection system (Bio-Rad Laboratories Inc.) according to manufacturers instruction. We used GAPDH as an endogenous control for standardizing the amount of the sample proteins.

4.7. Statistical analysis

Apoptosis rate were analyzed with one-way ANOVA and independent sample t test. P values less than 0.05 were considered statistically significant and were derived from 2-sided statistical tests. All statistical analysis was performed using the software SPSS 16.0 for windows.

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References

Aggarwal BB, Sung B (2009) Pharmacological basis for the role of curcumin in chronic diseases: an age-old spice with modern targets. Trends Pharmacol Sci 30: 85–94.

Anand P, Sundaram C, Jhurani S, Kunnumakkara AB, Aggarwal BB (2008) Curcumin and cancer: an "old-age" disease with an "age-old" solution. Cancer Lett 267: 133–164.

Beevers CS, Li F, Liu L, Huang S (2006) Curcumin inhibits the mammalian target of rapamycin-mediated signaling pathways in cancer cells. Int J Cancer 119: 757–764.

Chapuis N, Tamburini J, Green AS, Vignon C, Bardet V, Neyret A, Pannetier M, Willems L, Park S, Macone A, Maira SM, Ifrah N, Dreyfus F, Herault O, Lacombe C, Mayeux P, Bouscary D (2010) Dual inhibition of PI3K and mTORC1/2 signaling by NVP-BEZ235 as a new therapeutic strategy for acute myeloid leukemia. Clin Cancer Res 16: 5424–5435.

Choi BH, Kim CG, Lim Y, Shin SY, Lee YH (2008) Curcumin down-regulates the multidrug-resistance mdr1b gene by inhibiting the PI3K/Akt/NF kappa B pathway. Cancer Lett 259: 111–118.

De Toni F, Racaud-Sultan C, Chicanne G, Mas VM, Cariven C, Mesange F, Salles JP, Demur C, Allouche M, Payrastre B, Manenti S, Ysebaert L (2006) A crosstalk between the Wnt and the adhesion-dependent signaling pathways governs the chemosensitivity of acute myeloid leukemia. Oncogene 25: 3113–3122.

Dhillon N, Aggarwal BB, Newman RA, Wolff RA, Kunnumakkara AB, Abbruzzese JL, Ng CS, Badmaev V, Kurzrock R (2008) Phase II trial of curcumin in patients with advanced pancreatic cancer. Clin Cancer Res 14: 4491–4499.

Gilliland DG (2004) Targeted therapies in myeloid leukemias. Ann Hematol 83 (Suppl 1): S75–76.

Guzman ML, Neering SJ, Upchurch D, Grimes B, Howard DS, Rizzieri DA, Luger SM, Jordan CT (2001) Nuclear factor-kappaB is constitutively activated in primitive human acute myelogenous leukemia cells. Blood 98: 2301–2307.

Karin M, Cao Y, Greten FR, Li ZW (2002) NF-kappaB in cancer: from innocent bystander to major culprit. Nat Rev Cancer 2: 301–310.

Kornblau SM, Womble M, Qiu YH Jackson CE, Chen W, Konopleva M, Estey EH, Andreeff M (2006) Simultaneous activation of multiple signal transduction pathways confers poor prognosis in acute myelogenous leukemia. Blood 108: 2358–2365.

Kunnumakkara AB, Anand P, Aggarwal BB (2008) Curcumin inhibits proliferation, invasion, angiogenesis and metastasis of different cancers through interaction with multiple cell signaling proteins. Cancer Lett 269: 199–225.

Lao CD, Ruffin MT 4th, Normolle D, Heath DD, Murray SI, Bailey JM, Boggs ME, Crowell J, Rock CL, Brenner DE (2006) Dose escalation of a curcuminoid formulation. BMC Complement Altern Med 6: 10.

Lev-Ari S, Starr A, Vexler A, Karaush V, Loew V, Greif J, Fenig E, Aderka D, Ben-Yosef R (2006) Inhibition of pancreatic and lung adenocarcinoma cell survival by curcumin is associated with increased apoptosis, down-regulation of COX-2 and EGFR and inhibition of Erk1/2 activity. Anticancer Res 26: 4423–4430.

Prasad CP, Rath G, Mathur S, Bhatnagar D, Ralhan R (2009) Potent growth suppressive activity of curcumin in human breast cancer cells:Modulation of Wnt/beta-catenin signaling. Chem Biol Interact 181: 263–271.

Rizzieri DA, Feldman E, Dipersio JF, Gabrail N, Stock W, Strair R, Rivera VM, Albitar M, Bedrosian CL, Giles FJ (2008A) phase 2 clinical trial of deforolimus (AP23573, MK-8669), a novel mammalian target of rapamycin inhibitor, in patients with relapsed or refractory hematologic malignancies. Clin Cancer Res 14: 2756–2762.

Rowe JM (2008) Why is clinical progress in acute myelogenous leukemia so slow? Best Pract Res Clin Haematol 21: 1–3.

Shishodia S, Amin HM, Lai R, Aggarwal BB (2005) Curcumin (diferuloylmethane) inhibits constitutive NF-kappaB activation, induces G1/S arrest, suppresses proliferation, and induces apoptosis in mantle cell lymphoma. Biochem Pharmacol 70: 700–713.

Steelman LS, Abrams SL, Whelan J, Bertrand FE, Ludwig DE, Bäsecke J, Libra M, Stivala F, Milella M, Tafuri A, Lunghi P, Bonati A, Martelli AM, McCubrey JA (2008) Contributions of the Raf/MEK/ERK, PI3K/PTEN/Akt/mTOR and Jak/STAT pathways to leukemia. Leukemia 22: 686–707.

Steffen B, Müller-Tidow C, Schwäble J, Berdel WE, Serve H (2005) The molecular pathogenesis of acute myeloid leukemia. Crit Rev Oncol Hematol 56: 195–221.

Talapatra S, Thompson CB (2001) Growth factor signaling in cell survival: implications for cancer treatment. J Pharmacol Exp Ther 298: 873–878.

Teachey DT, Grupp SA, Brown VI (2009) Mammalian target of rapamycin inhibitors and their potential role in therapy in leukaemia and other haematological malignancies. Br J Haematol 145: 569–580.

Valencia A, Román-Gómez J, Cervera J, Such E, Barragán E, Bolufer P, Moscardó F, Sanz GF, Sanz MA (2009) Wnt signaling pathway is epigenetically regulated by methylation of Wnt antagonists in acute myeloid leukemia. Leukemia 23: 1658–1666.

Wang Z, Zhang Y, Banerjee S, Li Y, Sarkar FH (2006) Notch-1 down-regulation by curcumin is associated with the inhibition of cell growth and the induction of apoptosis in pancreatic cancer cells. Cancer 106: 2503–2513.

Yang CW, Chang CL, Lee HC, Chi CW, Pan JP, Yang WC (2012) Curcumin induces the apoptosis of human monocytic leukemia THP-1 cells via the activation of JNK/ERK Pathways. BMC Complement Altern Med 12: 22.

Yee KW, Zeng Z, Konopleva M, Verstovsek S, Ravandi F, Ferrajoli A, Thomas D, Wierda W, Apostolidou E, Albitar M, O'Brien S, Andreeff M, Giles FJ (2006) Phase I/II study of the mammalian target of rapamycin inhibitor everolimus (RAD001) in patients with relapsed or refractory hematologic malignancies. Clin Cancer Res 12: 5165–5173.