Mol. Cells *27*, 283-289, March 31, 2009
DOI/ 10.1007/s10059-009-0036-9

Molecules and Cells

Curcumin Induces Apoptosis and Inhibits Growth of Human Burkitt's Lymphoma in Xenograft Mouse Model

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Curcumin, a natural compound extracted from rhizomes of curcuma Curcuma species, has been shown to possess potent anti-inflammatory, anti-tumor and anti-oxidative properties. However, the mechanism of action of the compound remains poorly understood. In this report, we have analyzed the effects of curcumin on the cell proliferation of Burkitt's lymphoma Raji cells. The results demonstrated that curcumin could effectively inhibit the growth of Raji cells in a dose- and time-dependent manner. Further studies indicated that curcumin treatment resulted in apoptosis of cells. Biochemical analysis showed that the expression of Bax, Bid and cytochrome C were up-regulated, while the expression of oncogene c-Myc was down regulated after curcumin treatment. Furthermore, poly (ADP-ribose) polymerase (PARP) cleavage was induced by the compound. Interestingly, the anti-apoptotic Bcl-2 expression was not significantly changed in Raji cells after curcumin treatment. These results suggested that the mechanism of action of curcumin was to induce mitochondrial damage and therefore led to Raji cell apoptosis. We further investigated the *in vivo* effects of curcumin on the growth of xenograft tumors in nude mice. The results showed that curcumin could effectively inhibit tumor growth in the xenograft mouse model. The overall results showed that curcumin could suppress the growth of Burkitt's lymphoma cells in both *in vitro* and *in vivo* systems.

INTRODUCTION

Burkitt's lymphoma, a highly aggressive malignancy of B cells, is associated with chromosomal translocations that dysregulates the expression of the c-Myc gene (Cutrona et al., 2003; Zirong et al., 2003). Although much effort has been focused on the development of therapeutic treatments for Burkitt's lymphoma, these therapies have severe side effects. Furthermore, relapses may happen, particularly among the higher-risk and older patients (Bowman et al., 1996; Cairo et al., 2003; Ferry, 2006). Therefore, development of safe and effective therapeutic drugs is desirable for the treatment of Burkitt's lymphoma.

Curcumin, commonly known as the spice turmeric, is derived from the rhizome of *Curcuma longa* in South and Southeast tropical Asia (Somasundaram et al., 2002), where its common uses are as a flavoring, a coloring agent and a food preservative (Aggarwal et al., 2003; Fang et al., 2005). The compound has also been used for centuries in indigenous medicine for the treatment of a variety of inflammatory conditions and other diseases (Salvioli et al., 2007). Epidemiologic studies have shown that people in India who consume large amount of curcumin have a low incidence of colon cancers (Moos et al., 2004; Rao et al., 1995). Several recent studies have also shown that curcumin is a potential inhibitor of tumor growth *in vivo* (Balasubramanyam et al., 2004; Kunnumakkara et al., 2007; Odot et al., 2004) and that it demonstrates anti-proliferative activities against tumor cells *in vitro* (Balasubramanyam et al., 2004; Delettre et al., 2006). These potent anti-tumor and anti-inflammatory effects of curcumin may be induced by interaction of this compound with cellular processes that trigger cellular apoptosis.

Mitochondrial-mediated apoptosis is initiated by the release of cytochrome C, apoptosis- inducing factor (AIF) and other factors from mitochondria (Delettre et al., 2006; Cregan et al., 2002; Gajate et al., 2003). Cytochrome C can activate caspase-9 via Apaf1 and which then leads to the cleavage of caspase-3 and poly-adenosine diphosphate (ADP)-ribose polymerase (PARP) (Boulares et al., 1999; 2007), leading eventually to a caspase-dependent apoptosis. On the other hand, AIF, a caspase-independent apoptosis-inducing factor, is released into the cytoplasm, and then is translocated into the nucleus to directly promote DNA degradation and trigger apoptosis (Liu et al., 2005; Wang et al., 2002). How these pathways might be triggered by curcumin and the molecular mechanisms underlying curcumin effects still remain elusive (Kroemer et al., 2007; Martin, 2006; Schimmer et al., 2001; Sun et al., 2004).

Curcumin-induced apoptosis and resultant anticancer properties could operate through either mitochondrial-mediated or nonmitochondrial-mediated pathways (Hussain et al., 2005; Shankar and Srivastava, 2007). In this report, we demonstrated that curcumin could induce the activity of apoptotic molecules

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Received March 27, 2008; revised December 8, 2008; accepted December 19, 2008; published online March 19, 2009

Keywords: apoptosis, curcumin, lymphoma, mitochondria, proliferation, xenograft

and suppress the growth of Raji cells from Burkitt's lymphoma *in vitro* and *in vivo*. Although many studies have shown that curcumin is able to inhibit tumor cell proliferation *in vitro*, only a few studies have examined its effects on cancer cells *in vivo*. Our recent studies demonstrated that curcumin could effectively inhibit the growth of Raji lymphoma cells *in vivo* using a nude mouse xenograft mouse model.

MATERIALS AND METHODS

Antibodies and chemicals

Curcumin (purity 98%) was purchased from Yousi (China), and Hoechst 33258 was from Beyotime Institute of Biotechonolgy (China). Cell Counting Kit-8 was purchased from Dojindo (Japan) and Annexin V-FITC Apoptosis Detection Kit II was from Biosciences Pharmigen (USA). Trizol RNA kit and cDNA synthesis kit were from Invitrogen (USA). TaqTM Hot Start Version kit was purchased from TaKaRa (China). Polyclonal rabbit anti-human c-Myc and Polyclonal mouse anti-human cytochrome C antibodies were from Pharmigen (USA). The monoclonal anti-human PARP antibody was from Upstate (USA), and the monoclonal anti-human AIF antibody was from Santa Cruz Biotechnology (USA). Rabbit anti-human β-actin was purchased from Biosynthesis Biotechnology (China). Peroxidase-conjugated goat-anti-rabbit and goat- anti-mouse secondary antibodies were purchased from GenScript Corporation (USA).

Cell culture

Burkitt's lymphoma Raji cell line (ATCC CCL 86) was purchased from Shanghai Institute Cell Resources Bank. The characteristics of the cells have been previously described (Fabre et al., 1986; Trenfield et al., 1978). The cells were maintained in RPMI1640 medium (GIBCO/Invitrogen, USA) containing 10% heat-inactivated fetal calf serum (PAA), sodium bicarbonate (1.5 g/L) high glucose (4.5 g/L), 1 mM glutamine, 10 mM HEPES, 0.05 mM 2-mercaptoethanol and a 1× penicillin-streptomycin solution. Cells were cultured in a humidified incubator in 5% CO_2 at 37°C.

Measurement of cell viability

Curcumin was dissolved in the organic solvent dimethyl sulfoxide (DMSO, Sigma Chemical Co., USA) to make a 500 mM stock solution and stored at -20°C. The compound was further diluted to a 1 mM working solution in RPMI1640 medium. The final concentrations of curcumin in the Raji cell culture were 4-64 μM. The cells were treated for 0, 12, 24, 36 and 48 h and cell viability assays were carried out according to the procedure described (Chen et al., 2007). Briefly, the Raji cells (5×10^4/well) were plated in 0.1 ml medium (RPMI-1640 with 10% FBS) in 96-well plates with or without addition of different concentrations of curcumin. At the end of the incubation, cell viability was measured by the Cell Counting Kit-8 (Japan) according to the manufacturer's protocol. The optical densities at 450 nm were measured using a 96-well multiscanner autoreader (TECH, Japan) and the cell viability was calculated.

Hoechst 33258 staining

Cells were harvested and fixed with 4% formaldehyde in PBS for 10 min and stained with Hoechst 33258 according to manufacturer's protocol. The stained cells were observed using fluorescence microscopy.

Detection of apoptosis

For the analysis of apoptotic cells, the sample was prepared using an Annexin V-FITC Apoptosis Detection kit II according to the manufacturer's instructions, with minor modification. After being treated with curcumin for 36 h, the cells were washed two times with 1× PBS and resuspended to a final concentration of 5×10^5 cells/ml in 1× binding buffer. Then 97.5 μl cell suspension was mixed evenly with 2.5 μl annexin V-FITC in a new tube and incubated for 10 min at room temperature. 500 μl of 1× binding buffer was added and the tubes were centrifuged at 1,000 × *g* for 5 min to pellet the cells. The cells were resuspended in 100 μl of 1× binding buffer and stained with 0.2 μl of Cy5®-streptavidin and 0.1 μl of calcein according to the Agilent Technology protocol. The cells were incubated in dark for 10 min at room temperature, washed once with 500 μl of 1× binding buffer and then resuspended in cell buffer for detection of apoptosis by an Agilent 2100 Bioanalyzer.

RNA extraction and real-time quantitative RT-PCR

Total RNA was isolated from the cells using a Trizol RNA kit as described (Anke et al., 2007). The purified total RNA (1 μg) was reverse transcribed to cDNA using a cDNA synthesis kit (Promega, USA). Two microliters of the cDNA were used as template in a reaction mixture consisting of 0.8 μl forward (F) and reverse (R) primer at final concentrations of 10 μM, 0.2 μl of 10× SYBR Green I (Roche, Basel, Switzerland), and 0.2 μl of Prime STAR™ HS DNA polymerase (2.5 U/μl, Mg^{2+} plus), 4 μl of 5 × PrimeSTAR™ buffer, 1.6 μl of 2.5 mM dNTP, and ddH_2O was added for a total volume of 20 μl. The specific primers were chosen corresponding to the GenBank™ sequence of human Bax (F: 5′-TGACGGCAACTTCAACT G-3′and R: 5′-TTCTTCCAGATGGTGA GTGA-3′), BCL-2 (F: 5′-ATGTGTGT-GGAGAGCGT CA-3′ and R: 5′-GACAGCCA-GGAGAA ATC-AAA-3′), Bid (F: 5′-TGGACTGTGAGGTCAAC AAC-3′ and R: 5′-TCTATTCTTCCCAA GCGG-3′), β-actin (F: 5′-TTGCTGA-TCCACATCTG CTG-3′ and R: 5′-GACAGGAT-GCAGAAGGA GAT-3′). Quantitative real-time PCR was performed using a DNA Engine Opticon® 2 Continuous Fluorescence Detection System. The reaction was carried out with an initial denaturizing step at 95°C for 1 s and then 34 cycles at 95°C for 30 s, 55°C for 15 s, and 72°C for 60 s. The generated product specificity was verified by 1% gel electrophoresis. All PCR reactions were performed in duplicate. Relative gene expression was calculated with the $2^{-\Delta\Delta Ct}$ method, as described previously (Budhia et al., 2007).

Western blot analyses

Cell lysates were prepared by resuspending 1×10^6 cells in 100 μl of lysis buffer containing 137 mM NaCl, 15 mM EGTA, 0.1 mM sodium orthovanadate, 15 mM MgCl2, 0.1% Triton X-100, 25 mM MOPS, 100 μM phenylmethylsulfonyl fluoride and 20 μM leupeptin, pH 7.2. The cells were disrupted with gentle vortex and incubated at 4°C for 30 min. Lysates were centrifuged at 12,000 × *g* at 4°C for 10 min to obtain the supernatants (cytosolic extracts free of mitochondria) and the pellets (fraction that contains mitochondria) (Jung et al., 2005). Protein concentration of cytosolic fraction in the supernatants was measured as described (Beyotime Institute of Biotechonolgy, China). Equal amounts of protein were resolved by electrophoresis on SDS-PAGE and transferred onto nitrocellulose membranes. The membranes were blocked with 5% skim milk in TBST. For immunodetection, the blocked membranes were incubated with primary antibody at a dilution of 1:300 and with peroxidase labeled anti-rabbit or anti-mouse secondary antibody at a dilution of 1:10000. The immunoreactive bands were visualized using the ECL system. Band intensities were quantified by densitometry (VersaDoc Imaging System 3000, Bio-rad). Fold changes versus control values were calculated by normalizing

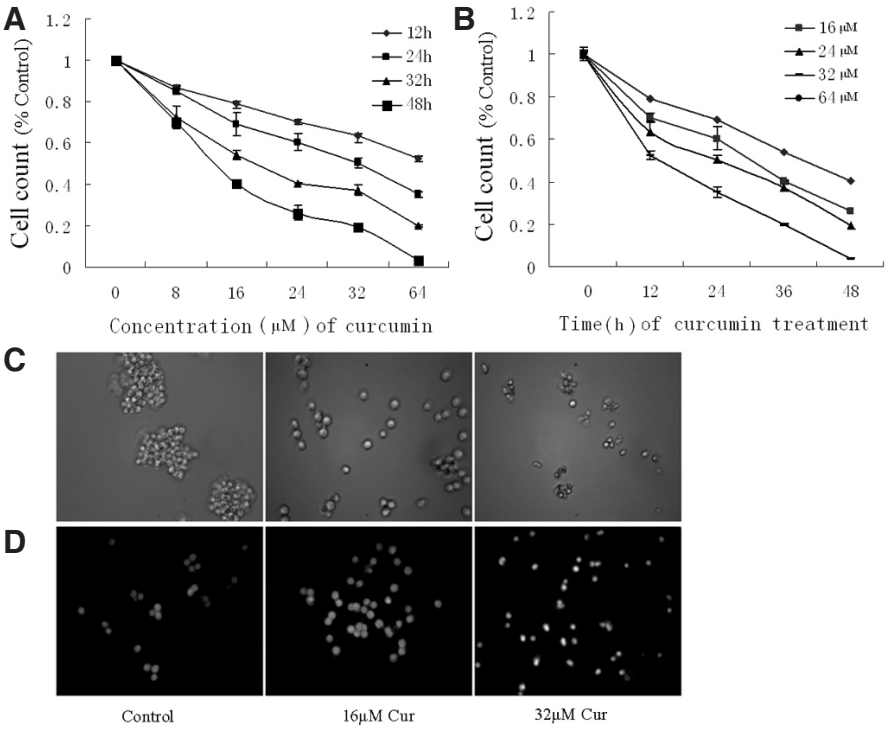


Fig. 1. Effect of curcumin on morphological changes and viability of Burkitt's lymphoma Raji cells. (A, B) CCK-8 assays were performed on Raji cells. Data shown are means ± SD from three separate experiments ($P < 0.05$ vs control). (C) Raji cells were treated with various doses of curcumin for 36 h, Magnification, ×400. (D) Cells were stained with Hoechst 33258 (blue), representative photos were recorded at ×400 magnification.

densities from the protein samples with those obtained for β-actin (VersaDoc Imaging System 3000, Bio-rad).

In vivo effects of curcumin

The *in vivo* studies were carried out following the procedures approved by ECNU Institutional Animal Care and Use Committee. Burkitt's lymphoma Raji cells were washed once with PBS resuspended in RPMI 1640 containing 5% fetal bovine serum at a concentration of 5×10^7 cells/100 μl. For each 4-week-old female athymic nu/nu mice (BALB/c), 100 μl of Cells/ (RPMI 1640) mixture was injected on the dorsal surface. Tumor volumes were measured and calculated using the formula of (length × width^2) π/6 (Li et al., 2002). After about 4 weeks, the xenograft tumors (0.2 cm^3) were separated from the tumor-loading mice. The tumors were cut into 0.01 cm^3 tumor fragments and transplanted into the dorsal regions subcutaneously in 4-week-old female athymic nu/nu mice. The xenograft tumors were grown for 15 d to a volume of 0.18 cm^3 in average and were injected daily, on two sides of each tumor for 20 d, with 0.2 ml of either saline or 200 mM curcumin. In addition, an interaperitoneal injection was performed to the xenograft mice with the same dosage of curcumin. Tumor size was measured weekly. At the end of 6 weeks, animals were euthanized, blood samples were taken, and organs were harvested for toxicology analysis.

Toxicology studies

Blood samples taken from the mice before euthanasia were tested for alanine aminotransferase isoenzymes (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), triglyceride (TG), total bilirubin (TBL), total protein (TP), albumin (ALB), urea (UR), creatinine (CR), uric acid (UA), direct bilirubin (DBIL) and lactate dehydrogenase (LDH). Skeletal muscle, thyroid, salivary gland, pancreas, brain, liver and gastrointestinal organs were examined to determine curcumin effects on inflammatory, hyperplastic and neoplastic changes.

Statistical analysis

The experimental data were analyzed by both ANOVA and two-group *t*-tests.

RESULTS

Growth inhibition of lymphoma cells with curcumin

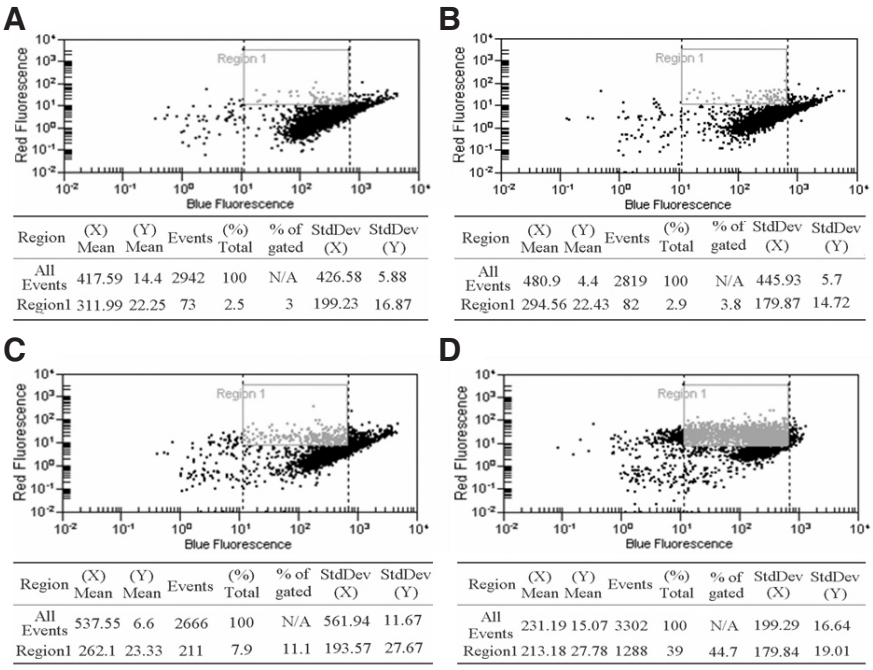
After treatment with different concentrations of curcumin for 12, 24, 36 and 48 h, in dose- and time-dependent inhibition of Raji cell proliferation was seen (Figs. 1A and 1B). Similar results were also observed in a jurkat cell line (data not shown). ANOVA analysis indicated that the inhibitory effects of curcumin were statistically significant ($P < 0.05$. vs control) (Figs. 1A and 1B).

Morphological changes in the Hoechst 33258 stained cells showed typical features of apoptosis including chromatin condensation and smaller cellular bodies after the treatment of the cells with curcumin (Figs. 1C and 1D). Fluorescence-activated cell sorting analysis further confirmed that curcumin treatment significantly induced the apoptosis of Raji cells (Fig. 2).

Effect of curcumin on the expression of apoptosis related genes

Mitochondrial-mediated apoptosis was attributed to the aberrant changes in the expression of the proapoptotic gene Bax and the antiapoptotic gene Bcl-2 (Yi et al., 2003). The activation of caspase-8 and the cleavage of Bid, which could promote expression of the Bax gene, were found in human myeloid leukemic cell line (HL-60) with curcumin treatment (Anto et al., 2002). In Raji cells quantitative RT-PCR analysis revealed that mRNA levels of Bax and Bid were significantly up-regulated after curcumin treatment (Figs. 3A and 3B), whereas no effect was seen in the expression of antiapoptotic gene Bcl-2 (Fig. 3C). Thus the expression of Bax/Bcl-2 ratio (Yi et al., 2003) remarkably increased with curcumin treatment (Figs. 3A and 3C).

The c-Myc oncoprotein, a transcription factor involved in cel-



Region	(X) Mean	(Y) Mean	Events	(%) Total	% of gated	StdDev (X)	StdDev (Y)
All Events	417.59	14.4	2942	100	N/A	426.58	5.88
Region1	311.99	22.25	73	2.5	3	199.23	16.87

Region	(X) Mean	(Y) Mean	Events	(%) Total	% of gated	StdDev (X)	StdDev (Y)
All Events	480.9	4.4	2819	100	N/A	445.93	5.7
Region1	294.56	22.43	82	2.9	3.8	179.87	14.72

Region	(X) Mean	(Y) Mean	Events	(%) Total	% of gated	StdDev (X)	StdDev (Y)
All Events	537.55	6.6	2666	100	N/A	561.94	11.67
Region1	262.1	23.33	211	7.9	11.1	193.57	27.67

Region	(X) Mean	(Y) Mean	Events	(%) Total	% of gated	StdDev (X)	StdDev (Y)
All Events	231.19	15.07	3302	100	N/A	199.29	16.64
Region1	213.18	27.78	1288	39	44.7	179.84	19.01

Fig. 2. Fluorescence-activated cell sorting analysis of Burkitt's lymphoma Raji cells treated with curcumin. Raji cells were treated with curcumin for 36 hours, stained with calcein and annexinV-Cy5 and analyzed on the Agilent 2100 bioanalyzer. Left, representing dead or damaged cells; bottom right are live cells, and early apoptotic cells are shown in yellow. (A) untreated sample; (B) 16 μM curcumin-treated sample; (C) 32 μM curcumin-treated sample; (D) 64 μM curcumin-treated sample.

lular transformation as well as apoptotic cell death (Pelengaris et al., 2003), is largely presented in Burkitt's lymphoma (Kelly et al., 2007). It has been reported that the cell death-promoting gene Bax was involved in a pathway downstream of c-Myc mediated apoptosis (Evan et al., 1992). Indeed, overexpression of c-Myc could induce the expression of Bax and lead to mitochondrial- dependent apoptosis (Mitchell et al., 2000). Western blot analysis showed that the expression of c-Myc protein was significantly reduced after 6 h curcumin treatment with dose-dependent manner in Raji cells (Figs. 4A and 4B). Furthermore, we found that curcumin could significantly enhance cytochrome C release from the mitochondria into the cytoplasm (Figs. 4A and 4C). In addition, curcumin activated PARP cleavage in dose-dependent manner in Raji cells (Figs. 4A and 4D). However, the protein level of antiapoptotic Bcl-2 was not markedly changed (Figs. 3C, 4A and 4B).

Apoptosis-inducing factor (AIF), known to be involved in the induction of apoptotic cell death through a caspase-independent pathway (Kroemer et al., 2000; Susin et al., 1999), is released and translocated to the nucleus and causes nuclear condensation and cellular apoptosis in response to death stimuli. Curcumin treatment had no effects on the distribution of AIF in Raji cells (Figs. 4A and 4C).

Inhibition of tumor growth with curcumin

Inhibition of tumor growth was examined in Burkitt's lymphoma Raji xenografts mice after intraperitoneal injection of curcumin. Although i.p. injection of the drug had no effect on tumor growth (data not shown), injections of curcumin on either side of an existing tumor resulted in the inhibition of tumor growth (Fig. 5A).

We did not find significant side effects of curcumin in the drug-treated mice. The activity of aspartate aminotransferase, alanine aminotransferase and isoenzymes was not affected, indicating that the liver function was normal. However, lactate dehydrogenase was significantly decreased (*$p < 0.05$ vs. control) (Fig. 5B). Finally, there were no abnormalities in skeletal muscle, thyroid, salivary gland, pancreas, brain, and gastrointestinal organs in curcumin treated mice.

DISCUSSION

Despite recent progress in treating children and adults with Burkitt's lymphoma, 40 to 70% of patients with intermediate- and high-grade lymphomas failed to achieve long-term disease-free survival. Significant side effects, including frequent myelosuppression, mucositis, neuropathy, and some treatment-related deaths, were observed in the patients treated with the present chemotherapy drugs now in use (Ferry, 2006; Lee et al., 2005; Voog et al., 2000). Therefore, development of novel and less toxic therapeutic agents for treating Burkitt's lymphoma is vital. Recently, compounds targeting apoptotic related proteins have begun to be developed for the treatment of this disease.

Curcumin can apparently effectively suppress the proliferation of a variety of tumor cells (Balasubramanyam et al., 2004; Bowman et al., 1996; Delettre et al., 2006; Kunnumakkara et al., 2007; Odot et al., 2004; Sharma et al., 2001; Somasundaram et al., 2002). However, mode of action of curcumin is not still definitive, only indirectly inferred. A number of studies have indicated that curcumin acts by inhibiting growth factor induced cell proliferation, suppressing the cell cycle, and activating apoptosis processes through changes of its specific signal molecules, which may be gene products such as NF-κB, p53, JNK, ERK, p38 Jak/Stat, caspase, BCl-2 family and others. However, curcumin responses are not identical in every tumor cell (Han et al., 1999; Morin et al., 2001; Rashmi et al., 2004). For Burkitt's lymphoma Raji cells, a jurkat cell line and normal peripheral blood mononuclear cells, curcumin is able to selectively inhibit the proliferation of Raji cells and jurkat cells, and yet it has low toxicity on normal peripheral blood mononuclear cells (data not shown). This is consistent with findings of normal mammary epithelial cell following curcumin treatment, and normal appearance of rat hepatocytes, which showed no superoxide generation and therefore no cell death after treatment with curcumin (Syng-Ai et al., 2004).

Bcl-2 family proteins are important regulators of apoptotic signaling, involving pathways that either inhibit or promote programmed cell death. The Bcl-2 protein is known to inhibit apop-

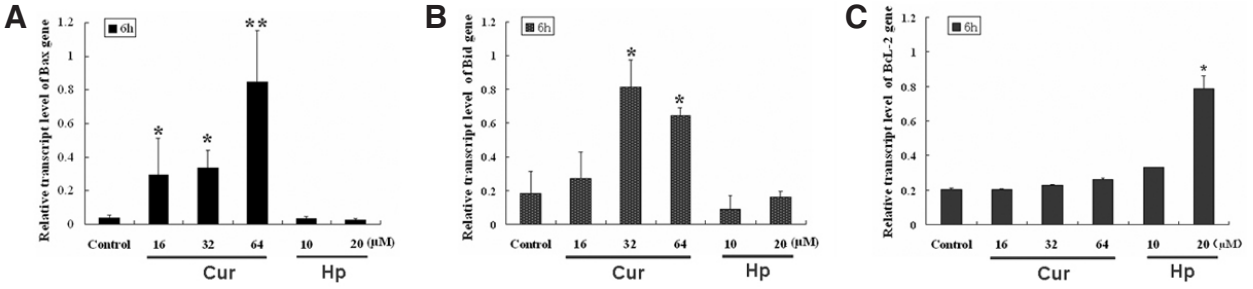


Fig. 3. Quantitative RT-PCR analysis. Relative mRNA levels of selected genes in Burkitt's lymphoma Raji cells treated with different concentrations of curcumin for 6 hours (3 replicates per experiment). The expression of Bax (A) and Bid (B) were analyzed. Human β-actin gene was used as endogenous reference. The relative mRNA levels were calculated using the comparative Ct method (see Materials and Methods). Statistical significance was tested by student *t*-test. Data are means ± SE (**P* < 0.05 and ***P* < 0.01. vs control). Cur, curcumin; Hp, Hydrogen peroxide.

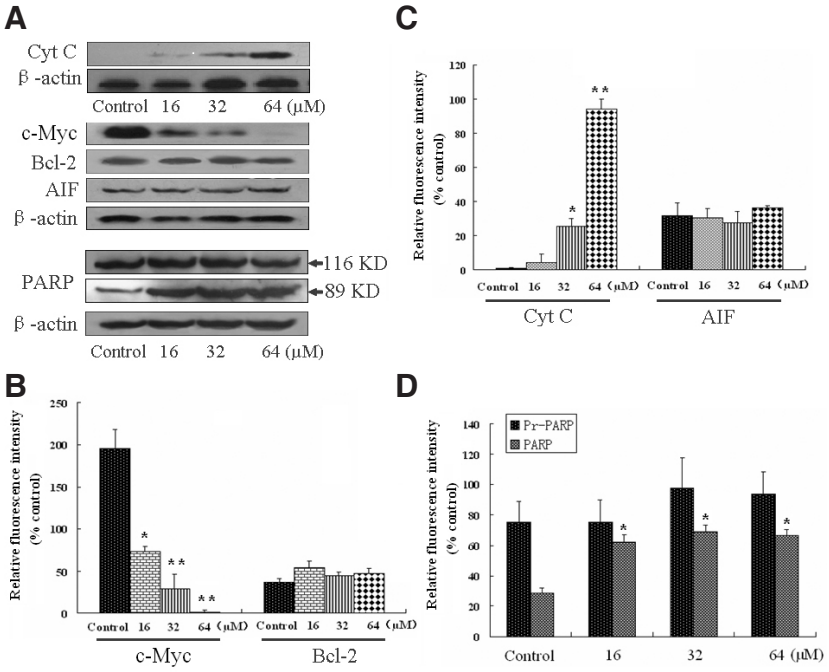


Fig. 4. Western blot analysis of the PARP cleavage, cytochrome C, AIF release, expression of Bcl-2, and c-Myc. Cells were treated with different concentrations of curcumin for 6 h (3 replicates per experiment). β-actin was used as a endogenous reference. Cyt C, cytochrome C (**P* < 0.05 and ***P* < 0.01. vs control).

tosis, while the Bax and Bid proteins promote it. Expression of Bid can led to apoptosis as it is processed into active forms of truncated Bid or tBid. Following processing, tBid translocates to the membrane-bound organellar fraction. Expression of Bcl-2 does not prevent Bid processing or tBid translocation but does inhibit tBid insertion into mitochondrial membranes, and also prevents Bid-induced Bax translocation from cytosol to the membrane-bound organellar fraction. This diminishes Bid-induced oligomerization of Bax within the membrane-bound organellar fraction. On the other hand, increasing Bax promotes its translocation in the cytosol, leading to mitochondrial membrane damage (Siu and Always, 2005; Figs. 3 and 4).

In this study, biochemical analysis showed that the expression levels of Bid and Bax were significantly enhanced after curcumin treatment, but there was no effect on expression of Bcl-2 (Figs. 3C and 4A). This finding is consistent with curcumin-induced human hepatoma G_2 cell apoptosis (Cao et al., 2007). Thus, the ratio of Bax/Bcl-2 could be responsible for mitochondrial membrane damage and lead to the leakage of cytochrome C. Indeed, our results also suggested that curcumin-induced translocation of cytochrome C from the mitochondria to the cytosol provided a direct link between the mitochondria and the curcumin-induced apoptosis. The expression of Bcl-2 remained unchanged over the 6 hours of curcumin treatment, whereas Bid, Bax, cytochrome c release and PARP cleavage were significantly increased in Burkitt's lymphoma Raji cells.

Proto-oncogene c-Myc can improve cell proliferation, and its aberrant expression is a common molecular defect in human cancers, including acute myeloid leukemia (AML) (Adachi et al., 2001). Overexpression of c-Myc can promote up-regulation of Bax expression and lead to cell apoptosis through p53 activity (Juin et al., 2002). However, our studies showed that the expression of Bax gene was up-regulated by curcumin treatment, while c-Myc was down-regulated. One possible explanation for these paradoxical results was that Bax was not a transcriptional target effector of c-Myc; instead, the decrease of c-Myc was most likely associated with inhibition of NF-κB activity (Kanda et al., 2004). The increase of Bax expression was consistent with up-regulation of Bid in Raji Burkitt's cells following treatment

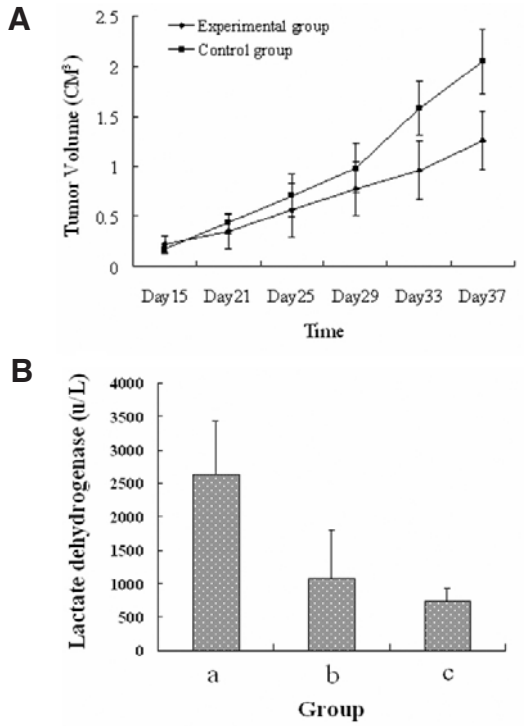


Fig. 5. Inhibition of xenograft tumors and lactate dehydrogenase in nude mice. (A) Application of curcumin at two sides of tumor and abdominal cavity resulted in a growth reduction in Burkitt's lymphoma (Raji). Five nude mice were used in the experimental group treated with curcumin and five nude mice in the control group. (B) Plasma taken from blood samples of mice was examined by 7020 Automatic analyzer (HITACHI, Japan). a, Control group (Burkitt's lymphoma) (n = 5); b, Curcumin-treatment group (n = 5; c, Normal group (n = 7).

with curcumin (Fig. 3B). The change of Bax expression could play an important role in apoptosis, although the phenomenon remains to be investigated. It is not clear whether the Bax gene is essential for maximum apoptotic response by curcumin (Shankar and Srivastava, 2007). Down-regulation of c-Myc could lead to the change of other critical signal molecules, such as proapoptotic Bak, CDK4, CDC25A, cyclins D1, D2, A and E (Beato et al., 2003; Nieminen et al., 2007).

In conclusion, we have shown that curcumin treatment of Burkitt's lymphoma Raji cells results in growth inhibition both *in vitro* and *in vivo*. We also demonstrated that the growth reduction is mediated through a mitochondrial dependent apoptotic pathway. Further studies should determine whether curcumin could be developed into a therapeutic or chemopreventive agent for treatment of Burkitt's lymphoma.

ACKNOWLEDGMENTS

This work is supported by the grants from Ministry of Science and Technology of China (973 project No2003CB716604), Shanghai Commission for Science and Technology, and Shanghai Commission for Education.

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