Curcumin induces cell apoptosis in human chondrosarcoma through extrinsic death receptor pathway

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Abstract

Chondrosarcoma is a soft tissue sarcoma with a poor prognosis that is unresponsive to conventional chemotherapy. Surgical treatment leads to severe disability with high rates of local recurrence and life threat. Curcumin, an active compound in turmeric and curry, has been proven to induce tumor apoptosis and inhibit tumor proliferation, invasion, angiogenesis, and metastasis of cancer cells. In this study, we investigated the anticancer effects of curcumin in human chondrosarcoma cells. Curcumin induced apoptosis in human chondrosarcoma cell lines (JJ012 and SW1353) but not in primary chondrocytes. Curcumin induced upregulation of Fas, FasL, and DR5 expression in chondrosarcoma cells. Transfection of cells with Fas, FasL, or DR5 siRNA reduced curcumin-induced cell death. In addition, p53 involved in curcumin-mediated Fas, FasL, and DR5 expression and cell apoptosis in chondrosarcoma cells. Most importantly, animal studies revealed a dramatic 60% reduction in tumor volume after 21 days of treatment. Thus, curcumin may be a novel anticancer agent for the treatment of chondrosarcoma.

Running title: Curcumin induces apoptosis in chondrosarcoma

Key words: Chondrosarcoma; Curcumin; Extrinsic pathway; Chinese Herb; Apoptosis

Introduction

Chondrosarcomas are malignant tumors showing cartilage differentiation, and it is the third most common primary bone malignancy after myeloma and osteosarcoma. Due to its resistance to both ionizing radiation and chemotherapy, chondrosarcoma is making the management of chondrosarcoma a complicated challenge [1]. Clinically, surgical resection remains the primary mode of therapy for chondrosarcoma. In the absence of an effective adjuvant therapy, this mesenchymal malignancy has a poor prognosis and novel and adequate therapies are needed [2].

Apoptosis is an intracellular suicide program possessing morphologic characteristics and biochemical features, including chromatin condensation, nuclear DNA fragmentation, cell shrinkage, membrane blebbing, and the formation of apoptotic bodies [3, 4]. Apoptosis is a physiological mechanism for eliminating malignant cells or cancer cells without eliciting damage to normal cells or surrounding tissues. Thus, induction of apoptosis in target cells is a key mechanism by which anti-cancer therapy works. To date, two major apoptotic pathways have been described as follows: the extrinsic death receptor-mediated pathway and the intrinsic mitochondrion-initiated pathway. The extrinsic apoptotic pathway originates at membrane death receptors (DRs) such as Fas, DR4, and DR5 and then engages the intracellular apoptotic machinery involving adaptor molecules and proximal caspase-8 as well as distal executioner caspases [5].

p53 is a proapoptotic gene [6]. It has been reported that p53 triggers apoptosis through both DR and mitochondrial apoptotic pathways [7]. DRs such as DR4, DR5, and Fas are increased by p53-dependent transcriptional activation [8]. Interaction of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), a member of the tumor necrosis factor family of proteins, with DR4 and DR5 leads to recruitment of the adaptor protein FADD and initiator caspase-8 to the death-inducing signaling complex [9]. This results in enzymatic activation of caspase-8, which in turn activates a downstream caspase cascade in the presence or absence of mitochondrial amplification machinery [10, 11].

Curcumin [1,7-bis(4-hydroxy-3methoxyphenyl)-1,6-heptadien-3,5-dione] is the primary bioactive component isolated from turmeric, a dietary spice made from the rhizome of Curcuma longa [12]. It possesses wide-ranging anti-inflammatory and anticancer properties [13, 14]. The abilities of curcumin to induce apoptosis of cancer cells and to inhibit angiogenesis and cell adhesion contribute to its chemotherapeutic potential in the treatment of cancer [15, 16]. Several phase I and phase II clinical trials indicate that curcumin is quite safe and may exhibit therapeutic efficacy in patients with progressive advanced cancers [17]. However, the roles of curcumin in human chondrosarcoma remain largely undefined. To the best of our knowledge, this study is the first to attempt to determine the apoptosis activity of curcumin in human chondrosarcoma cell lines. Our data provide evidence that curcumin reduced cells survival and tumor growth in human chondrosarcoma cells *in vitro* and *in vivo*.

Materials and methods

Materials

Horseradish peroxidase-conjugated anti-mouse and anti-rabbit IgG, and rabbit polyclonal antibodies specific for Fas, FasL, TRAIL, DR4, DR5, p53, caspase-8, caspase-3, caspase-7, caspase-9, and PARP were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). All other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Cell culture

The human chondrosarcoma cell line JJ012 was kindly provided by Dr. Sean P Scully (University of Miami School of Medicine, Miami, FL, USA). The human chondrosarcoma cell line SW1353 was obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured in DMEM/α-MEM which were supplemented with 10% fetal bovine serum (FBS) and maintained at 37°C in a humidified atmosphere of 5% CO_2.

Primary cultures of human chondrocytes were isolated from articular cartilage as we previously described [18]. The cells were grown in plastic cell culture dishes in 95% air-5% CO_2 in DMEM supplied with 20 mM HEPES, 10% heat-inactivated FBS, 2 mM-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin.

MTT assay

Cell viability was determined with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [19, 20]. After treating with curcumin for 24 or 48 h, cultures were washed with PBS. Then MTT (0.5 mg/ml) were added to each well and the mixture was incubated at 37°C for 2 h. To dissolve formazan crystals, culture medium was then replaced with an equal volume of DMSO. After the mixture was shaken at room temperature for 10 min, absorbance of each well was determined at 550 nm using a microplate reader (Bio-Tek, Winooski, VT, USA).

Colony assay

To determine the long-term effects of curcumin, cells (1000 per well) were treated with curcumin at various concentrations for 3 h simultaneously. After rinsing with fresh medium, cells were allowed to form colonies for 7 days before being stained with crystal violet (0.4 g/l). After washing three times with ddH_2O, acetic acid was added to a final concentration of 33% (v/v), and the absorbance was measured at 550 nm.

Quantification of apoptosis by flow cytometry

Apoptosis was assessed by using Annexin V, a protein that binds to phosphatidylserine (PS) residues exposing on the cell surface of apoptotic cells, as previously described [21]. Cells ($1 \text{ X } 10^5$ cells) were treated with vehicle or curcumin for the indicated times, washed twice with PBS, and resuspended in staining buffer containing 1 μg/ml Propidium iodide (PI) and 0.025 μg/ml Annexin V-FITC. Double-labeling was performed at room temperature for 10 min in the dark, and cells were immediately analyzed by FACScan and the Cellquest program (Becton Dickinson; Lincoln Park, NJ, USA).

Quantitative assessment of apoptotic cells was also assessed by examining the cell cycle. Cells were collected by centrifugation. Pre-chilled ethanol was added to 0.5 ml of cell suspensions and the mixture was incubated at 4 °C for 30 min. Ethanol was then removed by centrifugation, and cellular DNA was stained with 100 μg/ml PI (in PBS containing 0.1% Triton-X 100, and 1 mM EDTA) in the presence of an equal volume of DNase-free RNase (200 μg/ml). After staining, cells were analyzed immediately with a FACScan and Cellquest program. The extent of apoptosis was determined by measuring the DNA content of cells below sub G_1 peak [22].

TUNEL assay

Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick

endlabeling (TUNEL) assay was used to examine the cell apoptosis by using BD ApoAlert™ DNA Fragmentation Assay Kit. Briefly, cells were incubated with curcumin for the indicated times. The cells were trypsinized, fixed with 4% paraformaldehyde, and permeabilized with 0.1% Triton-X-100 in 0.1% sodiumcitrate. After being washed, the cells were incubated with the reaction mixture for 60 min at 37 °C. The stained cells were then analyzed with flow cytometer

Western blot analysis

Cellular lysates were prepared as we described [23, 24]. Proteins were resolved on SDS-PAGE and transferred to Immobilon polyvinyldifluoride membranes. The blots were blocked with 4% BSA for 1 h at room temperature, and then probed with rabbit anti-human antibodies against Fas, FasL, caspas-8, caspase-3, caspase-9, or PARP (1:1000 dilution) for 1 h at room temperature. After washed three times, the blots were incubated with a peroxidase-conjugated donkey anti-rabbit secondary antibody (1:1000 dilution) for 1 h at room temperature. The signals were visualized by enhanced chemiluminescence with Kodak X-OMAT LS film (Eastman Kodak, Rochester, NY, USA).

Caspase activity assay

The assay is based on the ability of active enzyme to cleave chromophore from enzyme substrate Ac-IETD-pNA (for caspase-8) and Ac-DEVD-pNA (for caspase-3). Cell lysates were prepared and incubated with anti-caspase-8 and -3. Immunocomplexes were incubated with peptide substrate in assay buffer (100 mM NaCl, 50 mM 4-(2-hydroxyethyl)-1-piperazine- ethanesulphonic acid [HEPES], 10mM dithiothreitol, 1mM EDTA, 10% glycerol, 0.1% 3-[(3-cholamidopropyl)dimethylammonio]-1- propanesulfonate [CHAPS], pH 7.4) for 2 h at 37°C. The release of *p*-nitroaniline was monitored at 405 nm. Results are the percent change in activity compared to untreated control.

siRNA transfection

siRNA against human Fas, FasL, DR5, p53, and control siRNA were purchased from Santa Cruz Biotechnology. Cells were transfected with siRNA (at a final concentration of 2 μg/ml) using Lipofectamine 2000 (Invitrogen Life, Carlsbad, CA, USA) according to the manufacturer's instructions.

***In vivo* tumor xenograft study**

Male SCID mice (6 weeks old; BALB/cA-nu [nu/nu]) were purchased from the National Science Council Animal Center (Taipei, Taiwan), and they were maintained in pathogen-free conditions. JJ012 cells (1×10^6 in 200 μl) were injected subcutaneously into the flanks of SCID mice, and tumors were allowed to develop until they reached a size of approximately 100 mm^3 (~14 days). The mice were treated with vehicle or with 1 or 2 mg/kg (i.p.; total volume 200 μl) curcumin every day for 21 days (10 mice/group). The volume of implanted tumors in the dorsal side of the mice was determined twice a week with a caliper and the formula $V = LW^2/2$, where V =is volume (mm^3), L is = largest diameter (mm), and W is = smallest diameter (mm). All mice were manipulated in accordance with Animal Care and Use Guidelines of the China Medical University (Taichung, Taiwan) under a protocol approved by the Institutional Animal Care and Use Committee.

Statistics

The values reported are means ± SEM. Statistical analysis between two samples was performed using Student's *t*-test. Statistical comparisons of more than two groups were performed by using one-way analysis of variance (ANOVA) with Bonferroni's *post-hoc* test. In all cases, $P < 0.05$ was considered significant.

Results

Curcumin induces cell apoptosis in human chondrosarcoma cells

To investigate the potential for curcumin to induce cell death in human chondrosarcoma cells, we first examined the effect of curcumin on cell survival in human chondrosarcoma cells by using the MTT assay. Treatment of cells with curcumin for 24 or 48 h induced cell death in chondrosarcoma (JJ012 and SW1353 cells) but not primary chondrocytes (Fig. 1A). The IC_{50} values of curcumin for 48 h treatment were 9.1 and 20.8 μM for JJ012 and SW1353 cells, respectively. The anti-cancer activities of curcumin were further assessed with clonogenic assays, which correlated very well with previous *in vivo* assays of tumorigenicity in nude mice. Treatment of cells with curcumin reduced colony formation dose-dependently (Fig. 1B). We next investigated whether curcumin induces cell death through an apoptotic mechanism by TUNEL staining, Annexin V/PI, and PI assay. Treatment of cells with curcumin significantly increased the TUNEL fluorescence intensity by using flow cytometry (Fig. 1C). Annexin V/PI double-labeling was used to detect PS externalization, a hallmark of the early phase of apoptosis. Compared to vehicle-treated cells, a high proportion of annexin V labeling was detected in cells treated with curcumin (Fig. 2A). Because dead cell population (Q2 region) increased dramatically when treated with curcumin (Fig 2A), we then used cytotoxicity assay kit to examine the necrosis effect of curcumin. Treatment of cells with curcumin (30 μM) did not increase lactate dehydrogenase release (data not shown). Therefore, the curcumin on cell necrosis in human chondrosarcoma can be rule out. In addition, treating cells with curcumin induced a concentration-dependent increase in cell death, resulting in an increase in the percentage of cells in the sub G1 phase (Fig. 2B). These data indicate that curcumin induced cell death through an apoptosis mechanism.

Extrinsic pathway is mediated curcumin-induced cell apoptosis in human chondrosarcoma cells

One of the hallmarks of the apoptotic process is the activation of cysteine

proteases, which include both initiators and executors of cell death. Treatment with curcumin resulted in the induction of cleaved forms of caspase-3, -7, and -8, but not -9 (Fig. 3A), indicating activation of the extrinsic apoptotic pathway. In addition, curcumin also increased caspase-3 and -8 activities time-dependently (Fig. 3B&C). Pretreatment of cells with the specific caspase-3 inhibitor (z-DEVD-FMK) or the specific caspase-8 inhibitor (z-IETD-FMK) reduced curcumin-induced cell death, as shown by PI-staining (Fig. 3D). On the other hand, curcumin also increased cleaved-PARP (Fig. 3A). These data indicate that curcumin induced cell death through an extrinsic signaling pathway.

Fas, FasL, and DR5 up-regulation are mediated curcumin-induced cell apoptosis in human chondrosarcoma cells

Because curcumin induced the cleavage of initiator caspase-8, we speculated that its proapoptotic response could be mediated via the death receptor-signaling pathway. As shown in Fig. 4A, curcumin induced an increase in Fas, FasL, and DR5 but not TRAIL and DR4 expression. To further evaluate the role of Fas, FasL, and DR5 in curcumin-induced apoptotic signaling in chondrosarcoma cells, the Fas, FasL, and DR5 siRNA were used. Transfection of cells with Fas, FasL, or DR5 siRNA reduced curcumin-induced cell apoptosis in JJ012 cells (Fig. 4B). Taken together, these results suggested that Fas, FasL, and DR5 up-regulation play an important role in curcumin-induced apoptosis in human chondrosarcoma cells.

p53 is involved in curcumin-induced Fas, FasL, and DR5 up-regulation in chondrosarcoma cells

It has been reported that p53, a transcriptional factor, could activate the extrinsic DRs signaling pathway. Next, we examine whether p53 is involved in curcumin-enhanced cell death in chondrosarcomas. Treatment of cells with curcumin increased p53 protein expression (Fig. 5A). To further investigation whether curcumin

induced apoptosis through p53, we then transfected p53 siRNA to JJ012 cells for 24 h. Then the results show that siRNA transfection inhibited curcumin-induced cell death in human chondrosarcoma cells (Fig. 5B). In addition, transfection of cells with p53 siRNA also decreased curcumin-induced Fas, FasL, and DR5 expression (Fig. 5C). Therefore, p53 expression is mediated curcumin-induced Fas, FasL, and DR5 expression and cell apoptosis in chondrosacoma cells.

Curcumin inhibits tumor growth in the mouse xenograft model of JJ012 cells

On the basis of the curcumin-induced apoptotic effect exhibited *in vitro*, we decided to detect whether curcumin possessed antitumor activities *in vivo*. Therefore, we established xenografts of JJ012 cells in SCID mice; as tumors reached 100 mm^3 in size, the mice were divided into three groups and treated with either vehicle or curcumin. Curcumin induced a dose-dependent inhibition of tumor growth (Fig. 6A&B). Moreover, in these two animal models, body weights were not significantly affected by curcumin (Fig. 6C). Finally, *ex vivo* analysis of tumors excised from mice showed significantly increasing Fas, FasL, p53, and DR5 expression in the curcumin-treated group compared with that in the control group, as shown by Western blot (Fig. 6D). Taken together, these results suggest that curcumin inhibits tumor growth by inducing JJ012 cell apoptosis *in vivo*.

Discussion

Unlike other mesenchymal malignancies, such as osteosarcoma and Ewing's sarcoma, which are dramatic increase in long-term survival with the advent of systemic chemotherapy, chondrosarcoma continue to have a poor prognosis due to absence of an effective adjuvant therapy [25]. The development of novel therapeutic agents targeting the malignant behavior of chondrosarcoma cells is important to improve the prognosis. Curcumin, a major yellow pigment and spice in turmeric and curry, exhibits anticarcinogenic effects [26]. The therapeutic values of curcumin have been proven in human clinical studies [27]. More importantly, curcumin is safe in humans even at a dose of 10 g/day [12]. However, the anti-cancer effects of curcumin on chondrosarcoma cells are mostly unknown. We found that curcumin induced cell death in human chondrosarcoma cell lines but not primary chondrocytes. In this study, we identified curcumin as a potential lead base on anticancer activity in human chondrosarcoma cells with good pharmacological properties. Our results revealed that curcumin induced a significant concentration-dependent induction of chondrosarcoma apoptosis. We also showed that the extrinsic death receptor signaling pathway is involved in curcumin-mediated cell death. On the other hand, we found that curcumin induced cytochrome c release (data not shown). Therefore, the mitochondrion-initiated pathway involved in curcumin-induced cell death in chondrosarcoma cells.

The process of apoptosis is controlled by two diverse cell signals, which can be initiated by two major pathways: intrinsic and extrinsic pathway [28]. Within the extrinsic apoptotic pathway, caspase-8 is the most proximal caspase that transmits apoptotic signals originating at the membrane DRs [29]. In this study we found that curcumin increased the expression and activity of caspase-8. Pretreatment of cells with caspase-8 inhibitor reduced curcumin-induced cell death. These data suggests that extrinsic death receptor pathway is involved in curcumin-induced cell apoptosis. The extrinsic pathway induces activation of caspase-8, and caspase-3 through DRs [30]. The interaction between Fas and FasL results in the formation of the

death-inducing signaling complex, which contains the FADD and caspase-8. Here, we found that curcumin increased Fas and FasL expression. In addition, transfection of cells with Fas or FasL siRNA reduced curcumin-increased cell apoptosis. Therefore, Fas and FasL are involved in curcumin-induced cell death.

In this study, we also examine the expression of DR4 and DR5 after curcumin treatment. The data indicate that curcumin increased the expression of DR5, but not DR4 in human chondrosarcoma cells. On the other hand, TRAIL induces apoptosis by activating caspase-8 via its receptors DR4 and/or DR5 [11]. Recent studies, however, indicate that DR levels can be enhanced by endogenous induction or exogenous overexpression. Several genotoxic and nongenotoxic agents can induce apoptosis by increasing endogenous DR5 [31]. We demonstrated that curcumin-induced apoptosis is coupled with DR5 induction without changes in the expression of its ligand, TRAIL. It is therefore possible that curcumin activated DR5 to induce cell death in a ligand-independent manner. Furthermore, transfection of cells with DR5 siRNA reduced curcumin-induced cell death. Therefore, DR5 also involved in curcumin-increased cell apoptosis in human chondrosarcoma cells. In this study, curcumin induced Fas, FasL, and DR5 begins at least 6 h and peaks at 24 h, while p53 up-regulated at 1 to 3 h and back toward the basal level by 6 h. Furthermore, transfection of cells with p53 siRNA decreased curcumin-induced Fas, FasL, and DR5 expression. Therefore, p53 expression is upstream molecule on curcumin-induced Fas, FasL, and DR5 expression and cell apoptosis in chondrosacoma cells.

Natural product drugs have been suggested to play a dominant role in pharmaceutical care [32]. Natural products are one of the important sources of potential cancer chemotherapeutic and chemopreventive agents [32]. Curcumin itself is a potent anticancer agent. Phase III clinical trials are undergoing to evaluate the effects of curcumin against pancreatic cancer and colon cancer [33]. Possible antitumor activity of curcumin includes induction of tumor apoptosis and inhibition of

tumor proliferation, invasion, angiogenesis, and metastasis [33]. Numerous targets regulated by curcumin have been reported, consisting of kinases, enzymes, growth factors, cytokines, and transcription factors [27]. Among them, as many as 33 different proteins can physically bind to curcumin [27]. However, the precise molecular mechanism of exhibited anti-tumor activity by curcumin in human chondrosarcoma is not well understood. Thus, the results of this study provide evidences for the anti-tumor activity of curcumin in chondrosarcoma cells, and more importantly, the molecular basis for its effect. The present study has demonstrated that curcumin causes apoptosis in chondrosarcoma cells *in vitro* and *in vivo*. Curcumin induced cell apoptosis in chondrosarcoma cells through the extrinsic death receptor pathway. It have been show that curcumin induced cell apoptosis through extrinsic death receptor pathway in prostate and ovarian cancer cells [34, 35]. In best of our knowledge, this study is the first to determine the extrinsic death receptor is involved in curcumin-induced cell apoptosis in chondrosarcoma. The proposed working models for the molecular basis would provide valuable insights for approaches to the development of effective chemotherapy by targeting appropriate signal transducers.

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Figure legends

Figure 1. The effect of curcumin on cell viability and colony formation in human chondrosarcoma cells. (A) JJ012, SW1353 and chondrocyte cells were incubated with various concentrations of curcumin for 24 or 48 h, and the cell viability was examined by MTT assay. (B) For the colony-forming assay, the clonogenic assay was performed as described in Materials and Methods. The quantitative data are shown. (C) Cells were treated with vehicle or curcumin for 24 h, the TUNEL positive cells were examined by flow cytometry. Results are expressed as the means ± S.E.M. of four independent experiments. *, $p<0.05$ as compared with control group.

Figure 2. Curcumin induces apoptosis of human chondrosarcoma cells. (A) JJ012 cells were treated with vehicle or curcumin for 24 h, and the percentage of apoptotic cells was also analyzed by flow cytometric analysis of Annexin V/PI double staining. (B) JJ012 cells were treated with vehicle or curcumin for 24 h, and the percentage of apoptotic cells was analyzed by flow cytometric analysis of PI-stained cells. Results are expressed as the means ± S.E.M. *, $p<0.05$ as compared with control group.

Figure 3. Curcumin induces the activation of caspases in human chondrosarcoma cells. (A) JJ012 cells were incubated with curcumin (10 μM) for different time intervals, and the PARP, caspase-3, caspase-7, caspase-8, and caspase-9 expression were examined by Western blot analysis. (B&C) JJ012 cells were incubated with curcumin (10 μM) for different time intervals, and then caspase-3 and caspase-8 activities were examined by caspase ELISA kit. (D) Cells were pre-treated for 30 min with z-DEVD-FMK (caspase 3 inhibitor) or z-IETD-FMK (caspase 8 inhibitor), and then followed by stimulation with curcumin (10 μM) for 24 h, and the percentage of apoptotic cells were analyzed by flow cytometric analysis of cell cycle assay. Results are expressed as the means ± S.E.M. *, $p<0.05$ as compared with

control group. #, $p < 0.05$ compared with curcumin-treated group.

Figure 4. Extrinsic pathway is involved in curcumin-induced cell apoptosis in human chondrosarcoma cells. (A) JJ012 cells were incubated with curcumin (10 μM) for different time intervals, the Fas, FasL, TRAIL, DR4, and DR5 expression were examined by Western blot analysis. (B; upper panel) JJ012 cells were transfected with Fas, FasL, DR5, or control siRNA for 24 h, and Fas, FasL, or DR5 expression was examined by Western blot analysis. (B; lower panel) JJ012 cells were transfected with Fas, FasL, DR5, or control siRNA for 24 h, and then followed by stimulation with curcumin (10 μM) for 24 h, and the percentage of apoptotic cells was analyzed by flow cytometric analysis of PI-stained cells. Results are expressed as the means ± S.E.M. *, $p < 0.05$ compared with control group. #, $p < 0.05$ compared with curcumin-treated group.

Figure 5. p53 is involved in curcumin-induced cell death. (A) JJ012 cells were incubated with curcumin (10 μM) for different time intervals, and p53 expression was examined by Western blot analysis. (B; upper panel) JJ012 cells were transfected with p53 or control siRNA for 24 h, and p53 expression was examined by Western blot analysis. (B; lower panel) JJ012 cells were transfected with p53 or control siRNA for 24 h, and then followed by stimulation with curcumin (10 μM) for 24 h, and the percentage of apoptotic cells was analyzed by flow cytometric analysis of PI-stained cells. (C) JJ012 cells were transfected with p53 or control siRNA for 24 h, and then followed by stimulation with curcumin (10 μM) for 24 h, and the Fas, FasL, and DR5 expression were examine by Western blot analysis. Results are expressed as the means ± S.E.M. *, $p < 0.05$ compared with control; #, $p < 0.05$ compared with curcumin-treated group.

Figure 6. Effects of curcumin on tumorigenicity and *in vivo* growth of

xenografts in SCID mice. (A&B) The chondrosacoma cells (1×10^6) were injected subcutaneously into the 5-week-old SCID mice. After the tumors reached 100 mm^3 in size, the animals treated with an intraperitoneal injection of curcumin (1 or 2 mg/kg) or vehicle were administered daily for 3 weeks. The mean tumor volume was measured at the indicated number of days after implantation (n = 8–10). (C) Mean body weight was measured at the indicated number of days after implantation. (D) Western blot analysis determined levels of Fas, FasL, p53, and DR5 expression in tumor with and without treatment. Results are expressed as the means ± S.E.M.