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Molecular Cancer Therapeutics

Targeting Sarcoplasmic/Endoplasmic Reticulum Ca^{2+}-ATPase 2 by Curcumin Induces ER Stress-Associated Apoptosis for Treating Human Liposarcoma

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Abstract

Human liposarcoma is the most common soft tissue sarcoma. There is no effective therapy so far except for surgery. In this study, we report for the first time that curcumin induces endoplasmic reticulum (ER) stress in human liposarcoma cells via interacting with sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase 2 (SERCA2). Curcumin dose-dependently inhibited the cell survival of human liposarcoma cell line SW872 cells, but did not affect that of human normal adipose-derived cells. Curcumin-mediated ER stress via inhibiting the activity of SERCA2 caused increasing expressions of CHOP and its transcription target death receptor 5 (TRAIL-R2), leading to a caspase-3 and caspase-8 cascade-dependent apoptosis in SW872 cells *in vitro* and *in vivo*. Moreover, 70% of human liposarcoma tissues showed an elevated SERCA2 expression compared with normal adipose tissues. Curcumin dose-dependently inhibited the activity of SERCA2, and the interaction of molecular docking and colocalization in ER of curcumin with SERCA2 were further observed. These findings suggest that curcumin may serve as a potent agent for curing human liposarcoma via targeting SERCA2. *Mol Cancer Ther; 10(3); 461–71. ©2011 AACR.*

Introduction

Liposarcoma is the most common soft tissue sarcoma and accounts for at least 20% of all sarcomas in adults (1). The 2 major locations of liposarcoma are the extremities and the retroperitoneum (2). Surgery serves as the main effective therapy for localized liposarcoma so far and there are few effective treatment options (3). Patients with liposarcoma often suffer from a high risk of relapse locally in cases of incomplete resection. In some cases, the local relapse of liposarcoma may usually accompany dedifferentiation and metastasis. The key chemotherapeutic drugs for soft tissue sarcomas such as doxorubicin and its combinations (4) have been used as the mainstay over decades despite their substantial toxicity. On the other hand, the newly developed molecular-targeted agents have been hardly applied for the treatment of liposarcoma in the present time. A basic research of the targeted drug sorafenib found that liposarcoma cell lines were completely resistant to sorafenib treatment (5).

Curcumin is a polyphenol that is derived from the dietary spice turmeric. The compound shows wide-ranging antiinflammatory and anticancer properties (6). The abilities of curcumin to induce apoptosis of cancer cells (7, 8), as well as to inhibit angiogenesis and cell adhesion (9), have been reported to contribute to its efficacy in the treatment of cancer. The phase I and phase II clinical trials indicate that curcumin is quite safe and exhibits therapeutic efficacy in patients with progressive advanced cancers (10). However, to our knowledge, the underlying mechanism of the anticancer effect of curcumin remains to be investigated.

In this study, to find an effective drug therapy for liposarcoma, we report for the first time that curcumin induced endoplasmic reticulum (ER) stress in human liposarcoma cell line SW872 cells via inhibiting the activity of sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase2 (SERCA2), which then resulted in CHOP-mediated apoptosis. The elevated expression of SERCA2 in SW872 as well as human liposarcoma tissues may be considered as a target of curcumin.

Materials and Methods

Reagents

Curcumin (>98% purity) was purchased from Shanghai R&D Centre for Standardization of Traditional Chinese Medicine and the stock solution was prepared with dimethyl sulfoxide. Nutlin-3 was purchased from Sigma and dissolved in dimethyl sulfoxide at the concentration of 20 mmol/L to produce a stock solution. Doxorubincin

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and cisplatin were purchased from Sigma and dissolved with PBS at 5 mg/mL and 1 mmol/L, respectively. Sorafenib and sunitinib (Pfizer) were dissolved in DMSO to a concentration of 20 mmol/L as stock solution.

Animals

Six-week-old female homozygous ICR SCID mice were purchased from the Shanghai Laboratory Animal Center. They were maintained in pathogen-free condition at 22 ± 2°C and kept on a 12-hour light–dark cycle. Animal welfare and experimental procedures were carried out strictly in accordance with the Guide for the Care and Use of Laboratory Animals (The Ministry of Science and Technology of China, 2006) and the related ethical regulations of our university. All efforts were made to minimize the animals' suffering and to reduce the number of animals used.

Cells and cell culture

Human liposarcoma cell line SW872 cells and K562 cells were purchased from the American Type Culture Collection. The SW872 cell line was initiated by A. Leibovitz in 1974 from a surgical specimen of a patient. The histopathology evaluation reported an undifferentiated malignant tumor consistent with liposarcoma. Cells were maintained in L-15 Medium supplemented with 10% fetal bovine serum (FBS, Life Technologies), 100 U/mL penicillin, and 100 μg/mL streptomycin and incubated at 37°C. The K562 cell line was a human-immortalized myelogenous leukemia line that was maintained in Iscove's Modified Dulbecco's Medium supplemented with 10% fetal bovine serum (FBS, Life Technologies), 100 U/mL penicillin, and 100 μg/mL streptomycin and incubated at 37°C in a humidified atmosphere containing 5% CO_2. Fat tissues from donors were washed extensively with ice-cold PBS to remove debris and blood cells. Then the tissues were treated with 0.15% collagenase (type I) containing 0.1% BSA in PBS for 30 minutes at 37°C with gentle agitation. The adipose-derived cells digested from tissues was then resuspended in Low Glucose Dulbecco's Modified Eagle's Medium supplemented with 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin, 1 ng/mL human recombined bFGF, and incubated at 37°C in a humidified atmosphere containing 5% CO_2. The authentication of adipose-derived cells was determined by Oil Red staining after differentiated for 8 days (Supplementary Fig. S1).

Stable overexpression of SERCA2b in SW872 human liposarcoma cells

Full length of human SERCA2b was amplified with the primers as follows: sense: 5′-GCGGCTAGCATGGAGAACGCGCACACC-3′, antisense: 5′-GCGGGTACCTCAAGACCAGAACATATCG-3′, which was subcloned into pcDNA3.1(+) (Promega). After confirming the sequence of SERCA2b, SW872 cells were transfected with pcDNA3.1 and pcDNA3.1-SERCA2b for 48 hours, respectively. Then the cells were passaged to a 100-mm dish and geneticin (G418 sulfate; Sigma Chemical Co.) was added to final concentration of 400 μg/mL. Resistant cells were allowed to grow for 2 weeks.

RNA interference

Chemically synthesized 21-nucleotide sense and antisense RNA oligonucleotides were obtained from Invitrogen. SW872 cells were plated on 6-well plates at 3×10^5 cells per well and transfected with 100 pmol of siRNA duplex per well using Oligofectamine (Invitrogen). CHOP siRNA sequences were as follows: siRNA-1: UUCAUCUGAAGACAGGACCUCUUGC; siRNA-2: UUGAGCCGUUCAUUCUCUUCAGCUA. SERCA2 siRNA sequences were: 5′-CAAAGUUCCUGCUGAUAUA(dTdT)-3′. Luciferase siRNA was used as described before (11).

RT-PCR and real-time PCR

RNA samples were treated by DNase and subjected to semiquantitative RT-PCR. First-strand cDNAs were generated by reverse transcription using oligo (dT). The cDNAs were amplified by PCR for 28 cycles (94°C for 30 seconds, 58°C for 30 seconds, and 72°C for 30 seconds) using TaqDNA polymerase (Promega Corp.,). The PCR products were electrophoresed on a 2% agarose gel and visualized by ethidium bromide staining. The Gel Imaging and Documentation DigiDoc-It System (version 1.1.23; UVP, Inc.) was used to scan the gels and the intensity of the bands was assessed using Labworks Imaging and Analysis Software (UVP, Inc.). Quantitative PCR was done with the ABI Prism 7000 sequence detection system (Applied Biosystems) using SYBR Green I dye (Biotium, Inc.), and threshold cycle numbers were obtained using ABI Prism 7000 SDS software version 1.0. Conditions for amplification were 1 cycle of 94°C for 5 minutes followed by 35 cycles of 94°C for 30 seconds, 59°C for 35 second, and 72°C for 45 seconds. The primer sequences used in this study were as follows: GAPDH forward, 5′-AACAGCGACACCCACTCCTC-3′; GAPDH reverse, 5′-GGAGGGGAGATTCAGTGTGGT-3′; SERCA1 forward, 5′-GTGATCCGCCAGCTAATG-3′; SERCA1 reverse, 5′-CGAATGTCAGGTCCGTCT-3′; SERCA2a forward, 5′- CTGTCCATGTCACTCCACTTCC-3′, SERCA2a reverse, 5′- AGCGGTTACTCCAGTATTGCAG-3′; SERCA2b forward, 5′-CGCTACCTCATCTCGTCCA-3′; SERCA2b reverse, 5′-TCGGGTATGGGGATTCAA-3′; SERCA2c forward, 5′- CTGGAACCTGTTCTTAGCTCAG-3′; SERCA2c reverse, 5′- TCTAGAGCAGCAGAGCAGGAGCCTT -3′;SERCA3 forward, 5′-GATGGAGTGAACGACGCA-3′; SERCA3 reverse, 5′-CCAGGTATCGGAAGAAGAG-3′.

Western blot

The Western blot was done as described before (11). Briefly, the cells were collected and lysed (50 mmol/L Tris, pH 8.0, 150 mmol/L NaCl, 1% NP-40, 0.1% SDS, 5 mmol/L EDTA, 0.1 mmol/L PMSF, 0.15 U/mL aprotinin, 1 μg/mL pepstatin, and 10% glycerol). The proteins were

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fractionated by SDS-PAGE and electrotransferred to nitrocellulose membranes. Antibodies to PARP, cleaved PARP, caspase-9, caspase-8, caspase-3, Fas, FADD, phosphorylated elf-2a, elf-2a, CHOP (Cell Signaling Technology, Inc.), antibody to α-tubulin, GAPDH, ATF4, and SERCA2 (Santa Cruz), respectively, were used for blotting, and detection was done by enhanced chemiluminescence (Amersham Pharmacia Biotech).

Immunohistochemistry and tunnel assay

TUNEL assay was done to detect apoptotic cells using the *In situ* Cell Death Detection kit from Roche Applied Science according to the manufacturer's instructions. Immunostaining of SERCA2 and CHOP was done using Real Envision Detection kit from Gene Tech Company according to the manufacturer's instructions. H&E staining in tumor tissues were done following manufacturer's protocol.

Growth of human liposarcoma cells in SCID mice

Cultured SW872 cells were washed with and resuspended in ice-cold PBS. Portions of the suspension (3×10^6 cells in 0.1 mL) were injected into the left inguinal area of SCID mice. Four weeks after injection, the mice-bearing tumors were distributed into 2 groups. Curcumin, dissolved in olive oil, was given daily for 40 days by intraperitoneal injection at dose of 100 mg/kg. Tumor volumes were measured every 3 days and calculated by the following formula: $0.5236 \times L1 \times (L2)^2$, where L1 and L2 are the long and short diameters of the tumor mass, respectively.

Statistical analysis

Data were expressed as mean ± SEM. Student's *t*-test was used to evaluate the difference between 2 groups. Kaplan–Meier method was used to evaluate the survival test. $P < 0.05$ was considered to be significant.

Results

Curcumin inhibits human liposarcoma cell growth without affecting normal adipose-derived cells, distinct from current drugs for cancer

The structure of curcumin is presented in Fig. 1A. Human liposarcoma cell line SW872 cells and adipose-derived cells were exposed to various concentrations of curcumin, chemotherapeutic agents doxorubicin and cisplatin, molecular-targeted drugs sorafenib and sunitinib, and the MDM2 antagonistic agent nutlin-3, respectively, for 48 hours. The results revealed that doxorubicin, cisplatin, and sunitinib showed a dose-dependent inhibition of both liposarcoma cells and adipose-derived cells whereas sorafenib and nutlin-3 did not show any inhibition of both cells at the concentrations used. However, unlike the others, curcumin showed a dose-dependent inhibition of human liposarcoma cells, whereas it did not affect adipose-derived cells (Fig. 1B).

Curcumin induces apoptosis via caspase-3/-8 pathway with an increased DR5 expression in SW872 cells

In order to detect the mechanism of the unique inhibition of curcumin toward human liposarcoma cells, the cell extracts of SW872 cells were prepared as described in Materials and Methods for immunoblotting after 24 hours incubation with various concentrations of curcumin. As shown in Fig. 2A, curcumin increased the active form of caspase-8, which then cleaved its substrate bid and caspase-3 in both dose- and time-dependent manners. Consistently, we also detected a dose-dependent increase in both caspase-3 and caspase-8 activities of SW872 cells after curcumin treatment (Fig. 2B). Caspase-8 is a key mediator of apoptotic signals triggered by death receptors such as Fas, TNFR1, and TRAIL-R1/TRAIL-R2. In SW872 cells, however, no change in either Fas or FADD at the protein level was found after curcumin treatment (Fig. 2C). Interestingly, we detected a dramatic increase in DR5 (TRAIL-R2) but not DR4 (TRAIL-R1) at the protein level (Fig. 2C). Consistently, the cell surface expression of DR5 but not Fas could also be induced by curcumin (Fig. 2C, lower panel). The apoptotic changes in morphology in SW872 cells were also observed by curcumin treatment (Fig. 2D).

Curcumin induces ER stress in SW872 cells

It has been reported that CHOP acted as a trigger transcript factor of DR5 under the condition of ER stress. After 24-hour exposure to curcumin, a significant increase in the phosphorylation of elf-2α was found. The expression of ATF4 and CHOP in SW872 cells was also elevated in both dose- and time-dependent manners at both protein (Fig. 3A) and mRNA levels (Fig. 3B), indicating that curcumin induced ER stress in SW872 cells. Knocking down of CHOP expression with siRNA reversed curcumin-induced apoptosis as well as the inhibition of cell survival (Fig. 3C and D). ER stress could be induced by unfolded protein response or imbalance of Ca^{2+} transfer. However, we found no obvious changes of GRP78 or PDI expression at the protein level (Supplementary Fig. S2). Interestingly, as a noncompetitive inhibitor of SERCA, thapsigargin also caused the cleavage of PARP, capsase-8, caspase-3, and bid and increased the CHOP expression in SW872 cells as curcumin (Fig. 3E). Like the apoptosis induction mode of curcumin, thapsigargin treatment caused an increase in the expression of DR5, but not Fas, in SW872 cells by flow cytometric analysis (Fig. 3F).

Curcumin binds to SERCA2 and inhibits its activity in SW872 cells

For the similar function mode of both curcumin and thapsigargin, we measured the activity of Ca^{2+}-ATPase in SW872 cells after incubation with various concentrations of curcumin for 24 hours. As shown in Fig. 4A, curcumin significantly inhibited the activity of Ca^{2+}-ATPase in a dose-dependent manner. There was no detectable SERCA1 and SERCA2a in adipose-derived

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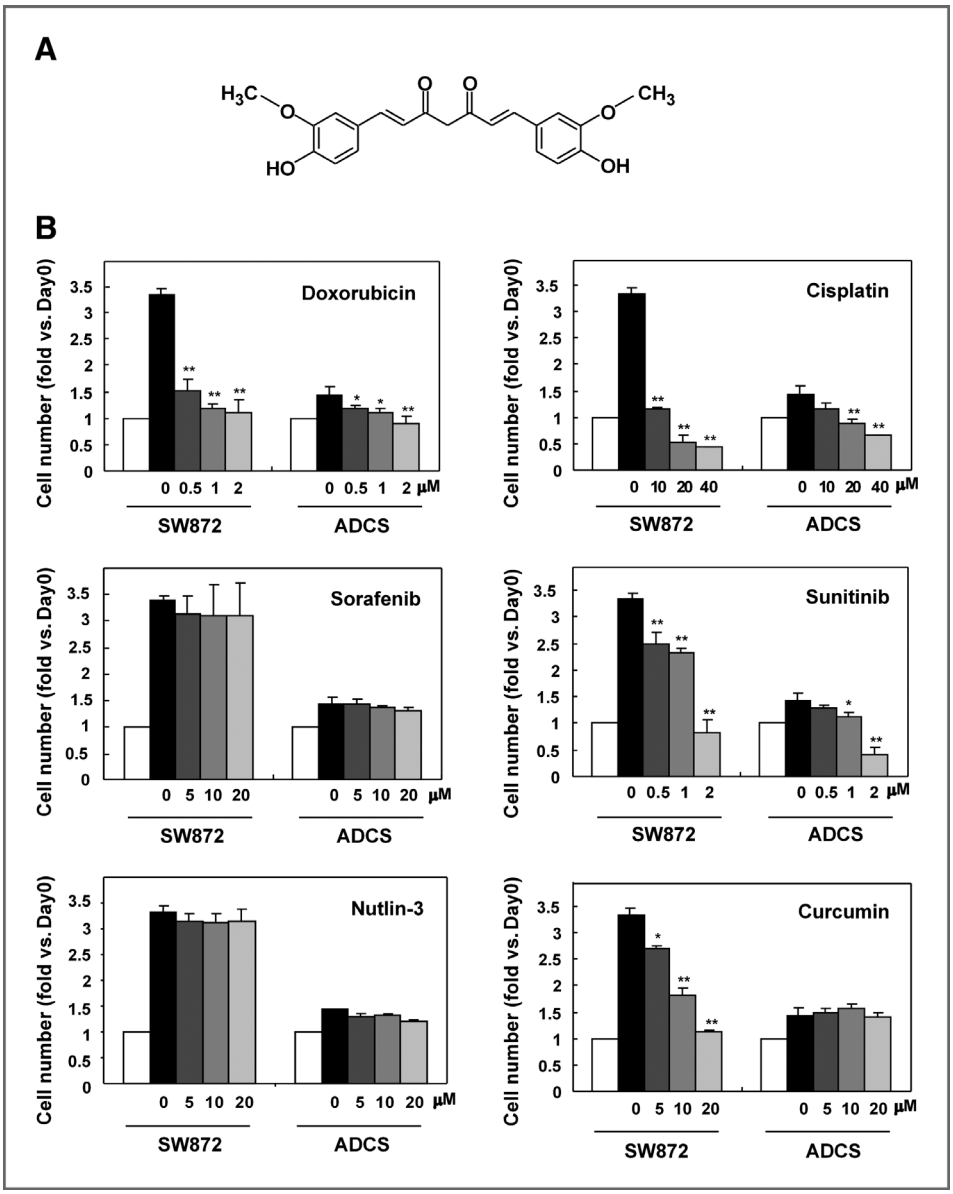

Figure 1. Curcumin inhibits human liposarcoma cell growth without affecting normal adipose-derived cells. A, structure of curcumin. B, SW872 cells and adipose-derived cells were seeded in 96-well plates and treated with various concentrations of doxorubicin, cisplatin, sorafenib, sunitinib, nutlin-3, and curcumin for 48 hours, respectively. The inhibition rate was determined by MTT assay. Data are mean ± SEM of 3 independent experiments, and each experiment includes triplicate sets. *, $P < 0.05$, **, $P < 0.01$ versus cells treated without drug.

cells or SW872 cells, compared with cDNA from human normal muscle tissue, and there was also no detectable SERCA3 expression in human adipose-derived cells or SW872 cells compared with cDNA from k562 cells. Interestingly, the expression of SERCA2b was dramatically elevated in SW872 cells, at both mRNA and protein levels (Fig. 4B). To confirm the possibility of SERCA2b as the most likely target of curcumin in SW872 cells, a docking study followed by a 2.5 ns molecular dynamics simulation suggests that curcumin has its 2 carbonyl oxygen atoms chelating the calcium cation, together with D59 and 4 water molecules. These water molecules also nearly bridge the interactions between calcium and some residues, namely D254, R264, and Q56 (Fig. 4C). Furthermore, we found that as a compound with fluorescence, curcumin located on the endoplasmic reticulum in SW872 cells, which was well merged with the reported localization of SERCA2b (Fig. 4D). Moreover, knocking down the expression of SERCA2 blocked the elevation of CHOP and cleaved PARP induced by curcumin (Fig. 4E, top panel). Stable overexpression of SERCA2b in SW872 cells reversed at least a part of the cell growth inhibition (Fig. 4E, middle panel). The CHOP elevation as well as the caspase-8 of the 3 cleavages induced by curcumin could also be reduced by SERCA2b overexpression (Fig. 4E, lower panel).

Curcumin suppresses the growth of human SW872 cells in SCID mice with apoptosis induction

SW872 cells (3×10^6) were inoculated s.c. into the right flank of SCID mice. Four weeks later when the tumors began to enlarge (about 50 mm^3), the mice were

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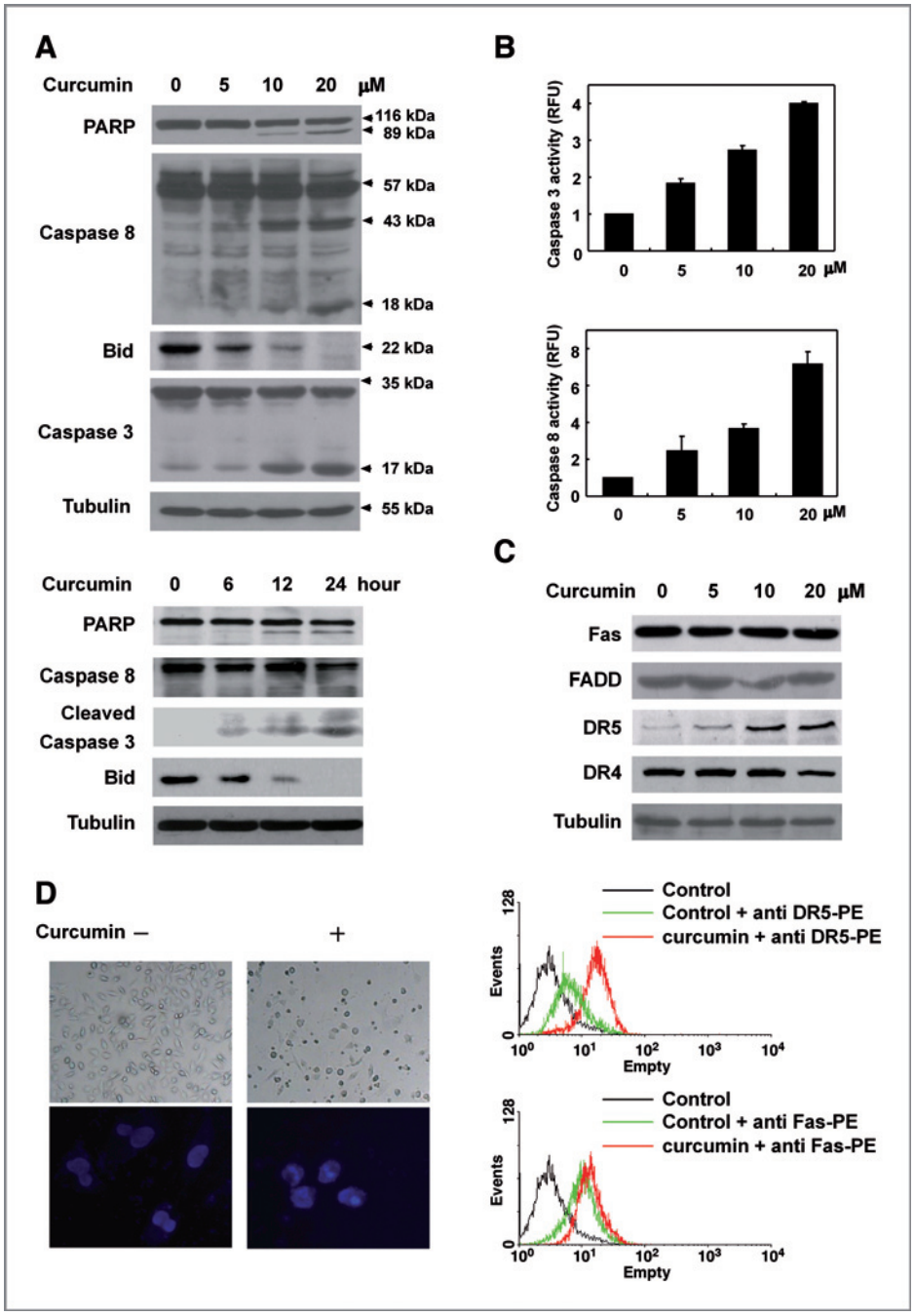

Figure 2. Curcumin activates caspase-3/-8 pathway via increasing DR5 expression in SW872 cells. SW872 cells were seeded in 6-well plate and incubated with 5, 10, and 20 μmol/L of curcumin for 24 hours or with 20 μmol/L curcumin for 6 to 24 hours, respectively. A, the protein levels of PARP, caspase-8, and caspase-3 were determined at least 3 times and representative data are shown. B, the caspase-3/-8 activity was measured according to the instructions of the caspase-3/-8 activity kit provided by BD Pharmingen. C, the protein levels of Fas, FADD, DR4, and DR5 were determined at least 3 times and representative data are shown (top panel), DR5 and Fas expressions in SW872 cells were determined by flow cytometric analysis after 24 hours' incubation with 20 μmol/L curcumin. D, the apoptotic morphologic changes as well as DAPI stained karyon of SW872 cells were photographed at 24 hours after exposure to 20 μmol/L curcumin.

randomized into 2 groups for treatment of olive oil control and curcumin (day 0). As shown in Fig. 5A, curcumin-treated tumors grew to 1.583 cm^3 as the average size against the 3.697 cm^3 in control 40 days after the daily treatment. The protein level of CHOP in the tumor tissues removed was greatly elevated in the curcumin treatment group, and the cleaved forms of PARP, caspase-3 and caspase-8 were also detected (Fig. 5B). Curcumin treatment could significantly inhibit Ca^{2+}-ATPase of SW872 cells *in vivo* (Fig. 5C). Consistent with the data in the Western blot, the immunohistochemical and tunnel staining assay also showed the elevated CHOP expression and apoptosis induction in tumor tissues from the curcumin-treated group against those from olive oil-treated group, respectively (Fig. 5D and E).

Elevated expression of SERCA2 is related to the malignant degree of different types of human liposarcoma

Paraffin section of human liposarcoma tissues were collected from 24 patients who underwent surgical resection for liposarcoma between 2008 and 2009. Of the cases,

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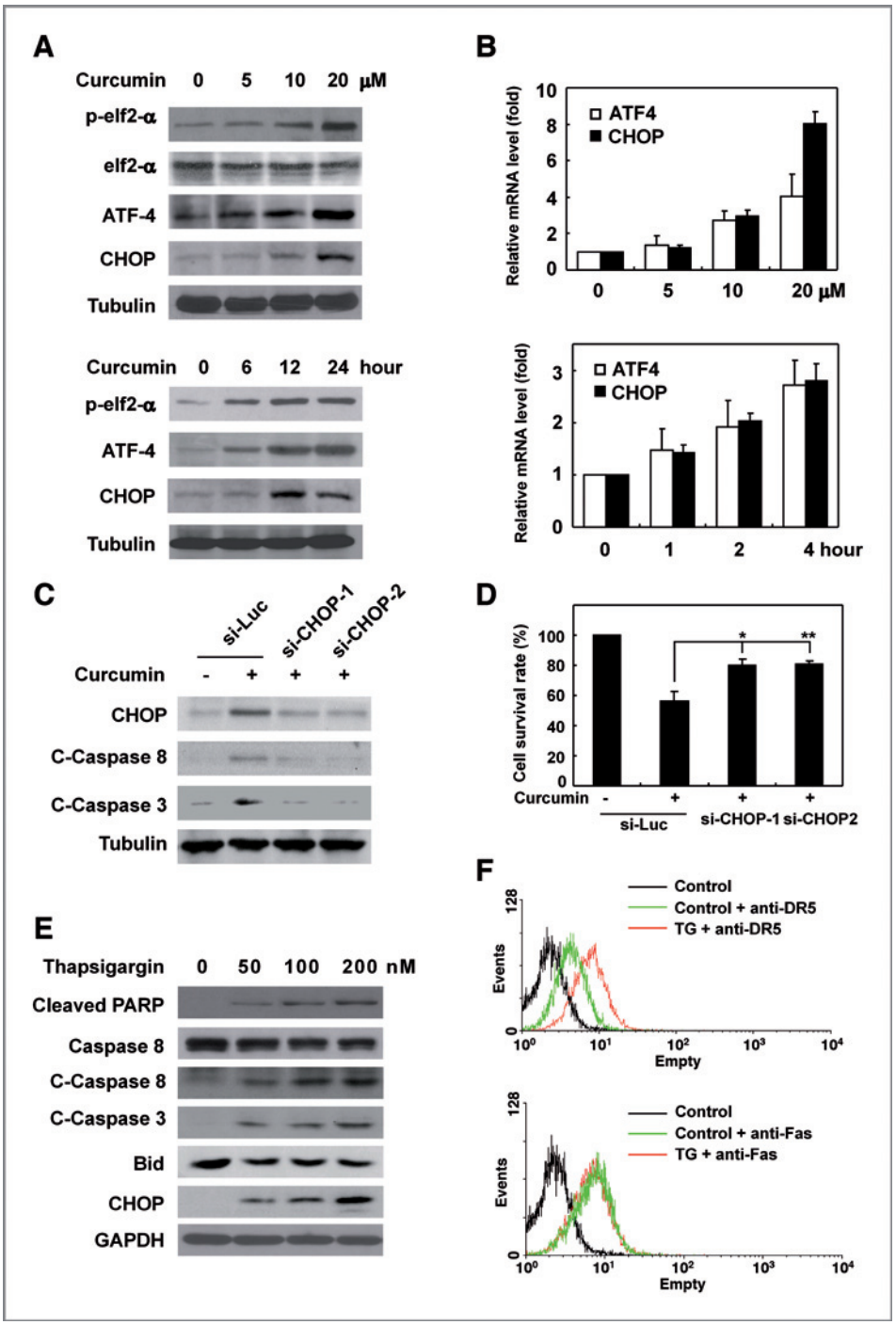

Figure 3. Curcumin induces ER stress in SW872 cells. SW872 cells were seeded in 6-well plate and incubated with various concentrations of curcumin or thapsigargin for 24 hours, or with 20 μmol/L curcumin for different hours, respectively. A, the protein levels of phosphorylated elf2-α, total elf2-α, ATF4, and CHOP were determined at least 3 times and representative data are shown. B, the mRNA level of ATF4 and CHOP above were measured by real-time PCR. GAPDH was used as an invariant control. C, SW872 cells were transfected with CHOP specific siRNA, and luciferase siRNA was used as control for 12 hours, respectively. After incubation with various concentrations of curcumin for an additional 24 hours, the whole cell lysis was prepared, and the expressions of CHOP, cleaved capase-8, and cleaved caspase-3 were detected by immunoblotting. D, SW872 cells transfected with CHOP siRNA and luciferase siRNA were treated with curcumin for 48 hours and the cell survival was determined by MTT assay. E, after treatment with various concentrations of thapsigargin for 24 hours, the protein levels of cleaved PARP, pro-caspase 8, cleaved caspase 8, cleaved caspase 3, bid, and CHOP were determined at least 3 times and representative data are shown. F, DR5 and Fas expressions in SW872 cells were determined by flow cytometric analysis after 24 hours' incubation with 100 nmol/L thapsigargin.

SERCA2 expression was detected in 16 patient samples (66.7%) by immunohistochemical assay with brown cytoplasmic, but not nuclear staining. The positive expression rate in high malignant liposarcoma including pleomophic liposarcoma (Fig. 6A), round cell liposarcoma, (Fig. 6B) and dedifferentiated liposarcoma (Fig. 6C) was significantly higher (83.3%) than that in low malignant liposarcoma samples (50.0%) such as well-differentiated liposarcoma (Fig. 6D). The percentage data are shown in Fig. 6E.

Discussion

So far, there is no effective therapy for human liposarcoma except surgery, and the molecular events involved in the pathogenesis remain unknown. Although Singer and colleagues have reported that MDM2 could be a potent drug target for human liposarcoma by using gene expression profiling between well-differentiated and dedifferentiated liposarcoma

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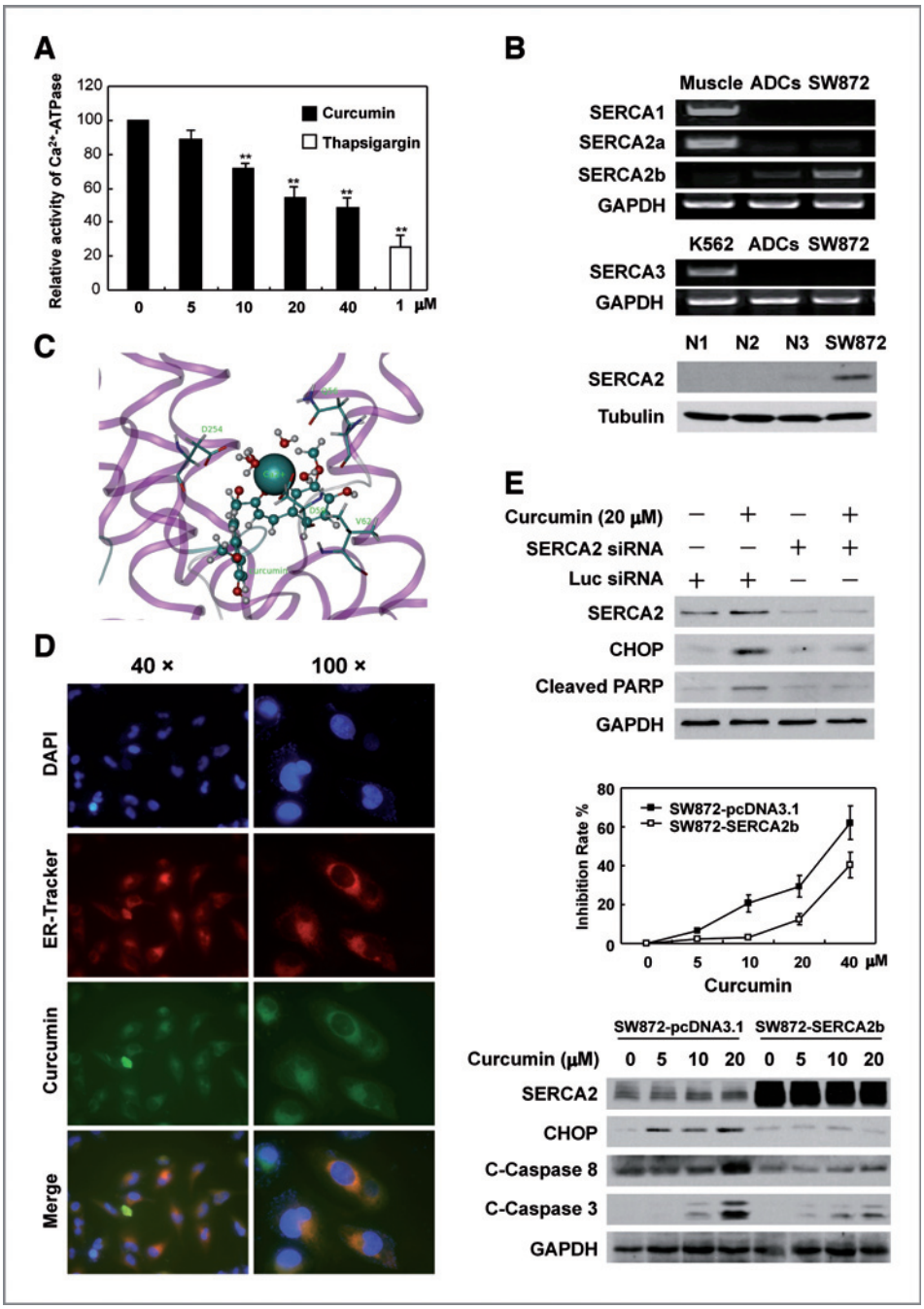

Figure 4. Curcumin binds to SERCA2 and inhibits its activity in SW872 cells. A, SW872 cells were incubated with various concentrations of curcumin or 1 μmol/L thapsigargin for 24 hours. Then the cells were harvested and the Ca^{2+}-ATPase activity was measured according to the instructions of the Ca^{2+}-ATPase kit provided by Nanjing Jiancheng Bioengineering Institute. B, the mRNA levels of SERCA1, SERCA2a, SERCA2b, SERCA3 in SW872 cells, and adipose-derived cells were measured by PCR. GAPDH was used as an invariant control; cDNA of human normal muscle tissue was used as a positive control for SERCA1 and SERCA2a (top panel); cDNA of K562 cells was used as a positive control for SERCA3 (middle panel). The protein levels of SERCA2 in SW872 cells and in human adipose-derived cells from 3 donors were determined at least 3 times and representative data are shown (bottom panel). C, docking analysis of curcumin, represented by the ball and stick structure. The colors are representative of atom type (cyan, carbon; red, oxygen; gray, hydrogen). D, SW872 cells were treated with 20 μmol/L curcumin for 24 hours and stained with DAPI and ER-tracker to show the position of karyon and endoplasmic reticulum, respectively. The micrographs were taken after 24 hours incubation. E, SW872 cells transfected with control siRNA or SERCA2-specific siRNA for 48 hours, respectively. Then, the cells were incubated with 20 μmol/L curcumin for another 24 hours. The protein level of SERCA2, CHOP, and cleaved PARP were determined by immunobloting (top panel). SW872 cells stably transfected with pcDNA3.1 and SERCA2 expression plasmids were seeded in 96-well or 6-well plate. After 24 hours, the cells were incubated with various concentrations of curcumin for additional 48 hours. The cell survival rate was determined by MTT assay (middle panel). The protein levels of SERCA2, CHOP, cleaved caspase 8, and cleaved caspase 3 were determined at least 3 times and representative data are shown (bottom panel).

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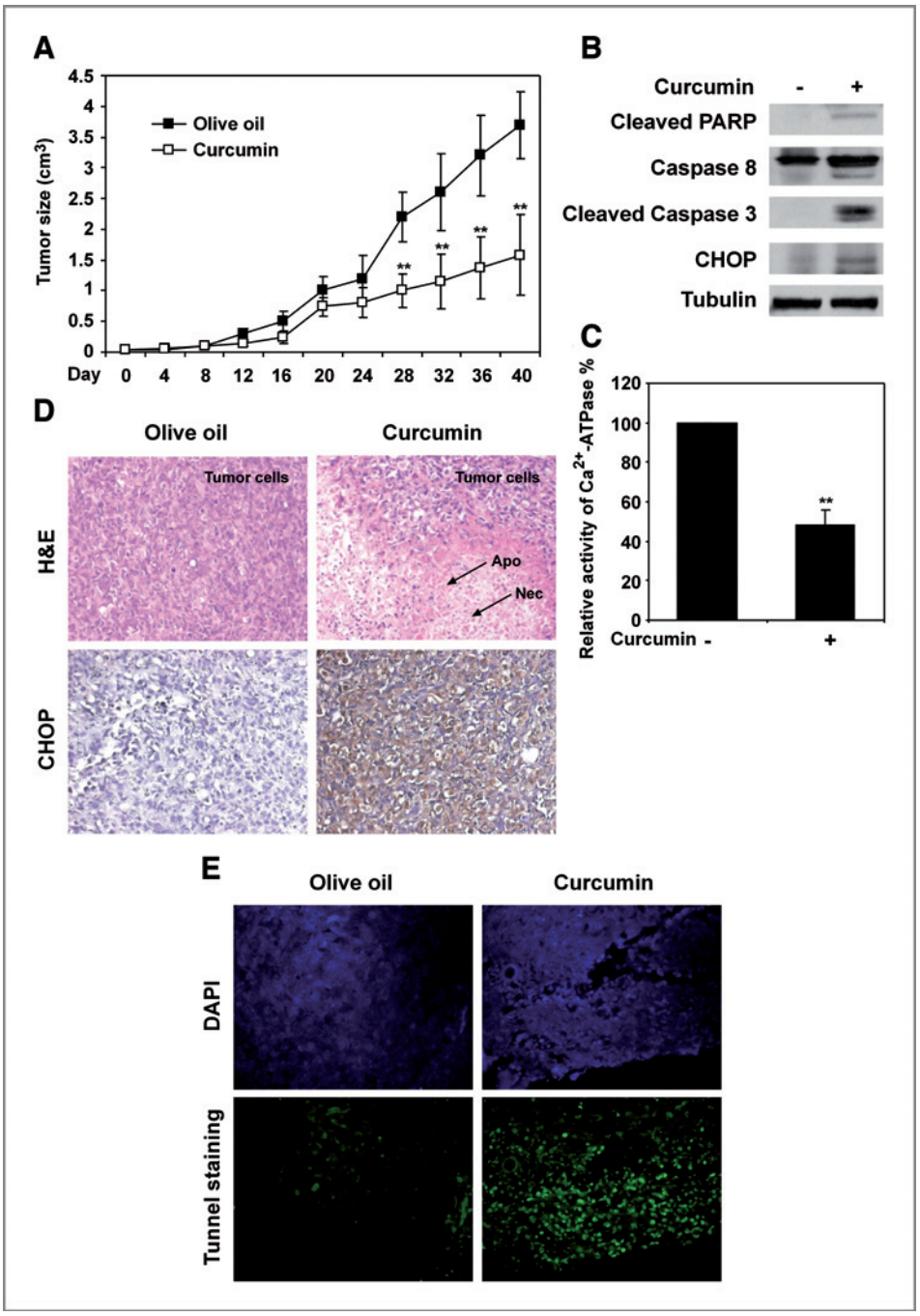

Figure 5. Curcumin suppresses the growth of human liposarcoma SW872 cells in SCID mice with apoptosis induction. SW872 cells were injected subcutaneously into the right flank of SCID mice. Four weeks after injection, the tumor-bearing mice were distributed into 2 groups. Then, they were treated with 100 mg/kg curcumin, or olive oil (day 0) for further 40 days, respectively. A, time course of tumor growth and tumor volumes. Data are mean ± SEM of 6 mice in each group. *, $P < 0.05$, **, $P < 0.01$ versus control. B, after 40 days, tumors in each group were removed. The protein levels of caspase-8, cleaved-caspase 3, and cleaved-PARP were detected by immunoblotting. C, the Ca^{2+}-ATPase activity of tumor samples from each group was measured according to the instructions of the Ca^{2+}-ATPase kit provided by Nanjing Jiancheng Bioengineering Institute. D, tumor sample sections from each group were stained with H&E (top) or used for immunostaining with CHOP antibody (bottom). Necrotic tumor cells (Nec) and apoptotic-condensed nuclei (Apo) are shown by arrows. Original magnification, ×100. E, tunnel assay was done according to the manufacturer's instructions. The nuclei of tumor cells in each group were stained with DAPI. Original magnification, ×100.

tissues (3), in this study, no effect of MDM2 antagonist nutlin-3 on the SW872 cell growth was observed (Fig. 1B). Comparatively, the main chemotherapeutic drugs doxorubicin and cisplatin, as well as the multiple tyrosine kinases inhibitor sunitinib, showed a dose-dependent inhibition of SW872. However, these drugs also inhibited adipose-derived cells significantly (Fig. 1B), suggesting the predicable toxicity of normal tissue cells. Interestingly, we have for the first time highlighted a strong inhibition of SW872 cells by curcumin without any influence on the normal adipose-derived cells (Fig. 1B).

Curcumin has been reported to show wide-ranging anti-inflammatory (12) and anticancer properties (13). Several phase I and phase II clinical trials indicate that curcumin is quite safe (14) and exhibits therapeutic efficacy in patients with progressive advanced cancers. Curcumin upregulates the proapoptotic proteins of the Bcl-2 family Bax, Bim, Bak, Puma, and Noxa and downregulates the antiapoptotic proteins, Bcl-2 and Bcl-xl, in

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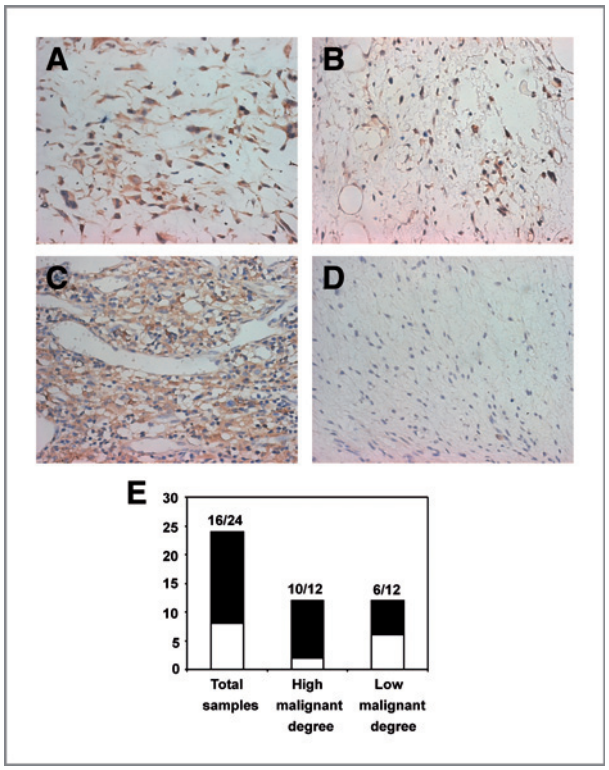

Figure 6. Elevated expression of SERCA2 is related to the malignant degree of different types of human liposarcoma. Immunohistochemical staining assay was carried out for SERCA2 by using human liposarcoma samples. Paraffin sections of human liposarcoma tissues were collected from 24 patients who had undergone surgical resection for liposarcoma between 2008 and 2009. A, pleomophic liposarcoma, B, round cell liposarcoma, C, dedifferentiated liposarcoma, D, well-differentiated liposarcoma. E, the percentages of SERCA2 positive samples in groups with different malignant degrees.

various cancer cells (15). Moreover, curcumin is able to decrease the number of aberrant crypt foci in an azoxymethane-induced rat colon cancer model through apoptosis induction via the mitochondrial pathway (16). Curcumin is also known for its antioxidant properties and acts as a free radical scavenger by inhibiting lipid peroxidation and oxidative DNA damage (17).

ER participates in the initiation of apoptosis by at least 2 different mechanisms (18). The first is the unfolded protein response, which can be induced by many kinds of proteasome inhibitors (19). Proteins that are unable to fold properly in ER are ubiquitinated and degraded by the 26S proteasome (20), and inhibition of the activity of proteasome will then lead to induction of a terminal unfolded protein response in tumor cells, leading to ER stress (21). The other is Ca^{2+} signaling. Ca^{2+} is taken up from the cytosol by the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) and released through the inositol-1, 4, 5-trisphosphate receptor/Ca^{2+} channels or ryanodine receptor/Ca^{2+} channels. Disruption of the function of SERCA by thapsigargin will also lead to ER stress (22).

To find which mechanism is involved in the curcumin-induced ER stress, we detected the protein levels of GRP78 and PDI, the crucial markers of unfolded protein response (23), in curcumin-treated SW872 cells. However, there was no obvious change in both proteins (Supplementary Fig. S2). Then, we exposed SW872 cells and adipose-derived cells to various concentrations of thapsigargin, the inhibitor of SERCA (24), for 48 hours. As shown in Supplementary Fig. S3, thapsigargin showed the same specific inhibition effect as curcumin, where thapsigargin inhibited the growth of SW872 cells in a dose-dependent manner without affecting adipose-derived cells. Consistent with the results obtained by Wootton and Michelangeli (25) and Bilmen and colleagues (26), curcumin inhibited the activity of Ca^{2+}-ATPase in a dose-dependent manner like thapsigargin *in vitro* (Fig. 4A). These results revealed that curcumin-induced ER stress in SW872 cells through inhibiting the activity of SERCA but not inducing unfolded protein response. Although there could be a relationship between unfolded protein response and Ca^{2+} signaling, disruption of calcium homeostasis by thapsigargin may induce GRP expression. Curcumin, a well-known compound with multiple targets, may also have some inhibitory effect on the crosstalk between Ca^{2+} signaling and unfolded protein response.

In order to detect whether SERCA could be the most likely target of curcumin in SW872 cells, first, we compared the level of different isoforms of SERCA expression in SW872 cells and adipose-derived cells. As shown in Fig. 4B, there was a dramatic increase in SERCA2b but not the other SERCAs expression in SW872 cells. The docking study supported the interaction between curcumin and SERCA2 (Fig. 4C). On the other hand, we discovered for the first time a specific localization on the endoplasmic reticulum in SW872 cells (Fig. 4D), which is different from the membrane and nuclear localization of curcumin reported before (27). This finding also supported the interaction between curcumin and ER-located SERCA2 in SW872 cells. We then developed SW872 cells with SERCA2b stably overexpression. As expected, the SW872-SERCA2b cells became less sensitive to the curcumin treatment than SW872-pcDNA3.1 cells, both in the inhibition of cell survival and in the induction of apoptosis (Fig. 4E). This could be explained by the fact that overexpressed SERCA2b competed with endogenous SERCA2b when it interacted with curcumin.

SERCA has been reported to reside in the sarcoplasmic reticulum within muscle cells. It acts as a Ca^{2+}-ATPase that transfers Ca^{2+} from the cytosol to the lumen of the sarcoplasmic reticulum at the expense of ATP hydrolysis during muscle relaxation (28). As one of the family members, SERCA2 is known as an important regulator of normal calcium homeostasis and signaling (29, 30). Alterations in calcium-dependent signaling are involved in cell proliferation and differentiation, apoptosis, and disruption of calcium homeostasis, which may contribute to cancer development. Chung and colleagues have reported that elevated SERCA2 mRNA

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was detected in 90% of human colorectal cancer tissues, indicating the possible role of SERCA2 in the development and progression of human colorectal cancer (31). On the other hand, a null mutation in 1 copy of the SERCA2 has been shown to lead to squamous cell tumor in mice, suggesting that the proper amount of SERCA2 is crucial for the destiny of cells (32). Curcumin has been reported to be a compound with multiple targets, such as NF-κb, PI3K/Akt, Src/Stat3, and so on (33), in fact, many of which are crucial for normal cell survival and tissue development. The phase I and phase II clinical trials indicate that curcumin is quite safe and exhibits therapeutic efficacy in patients with progressive advanced cancers. The inhibition effect of curcumin on tumors but not normal tissue is still being studied, and one explanation is higher curcumin uptake in tumor cells than normal cells (27). By means of curcumin, a new role of SERCA2b in the formation and development of human liposarcoma has been found. Further investigation should be focused on the role of SERCA2b as the first target in the therapy of human liposarcoma.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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