

Curcumin inhibits the proliferation of human hepatocellular carcinoma J5 cells by inducing endoplasmic reticulum stress and mitochondrial dysfunction

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Abstract. Curcumin (diferuloylmethane), which is obtained from turmeric, the rhizome of *Curcuma longa* (L.), inhibits many human cancer cells. However, the molecular mechanisms responsible for curcumin-induced endoplasmic reticulum stress in human hepatic cellular carcinoma J5 cells, are not yet clearly understood. J5 cells were treated with various concentrations of curcumin for different durations. The cell viability was detected by MTT assay. The protein expressions of caspase-12, ATF6, GADD153, Calnexin, Calreticulin, PDI and Ero1- $L\alpha$, which are associated with endoplasmic reticulum stress and the unfolding protein response pathway, were examined by Western blot analysis. The cell cycle was analyzed by flow cytometry. The protein expressions of TCTP, Mcl-1, Bcl-2 and Bax, which are related to mitochondrial dysfunction, were detected by Western blot analysis. We also detected the ATF6 protein location by immunocytochemistry. The results showed that curcumin inhibits the proliferation of J5 cells in a time- and dose-dependent manner. Curcumin induced the unfolding protein response by down-regulating the protein expressions of Calnexin, PDI and Ero1- $L\alpha$ and up-regulating the Calreticulin expression. Curcumin induces the GADD153 expression by cleaving caspase-12 and ATF6, and then by translocating ATF6 to the nucleus. Curcumin also down-regulates the protein expressions of TCTP, Mcl-1 and Bcl-2, in order to induce mitochondrial dysfunction. Curcumin induced cell cycle arrest at the G2/M phase by decreasing the Cdc2 expression. In conclusion, the present study showed that curcumin inhibits the proliferation of J5 cells by inducing endoplasmic reticulum stress and mitochondrial dysfunction.

Introduction

Curcumin [1, 7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], is extracted from the plant, *Curcuma longa*. It is well documented that curcumin induces apoptosis through the death receptor mediated pathway and mitochondrial dysfunction (1,2), and also induces DNA damage response by cleaving caspase-3 (3). In addition, curcumin induces cell cycle arrest by down-regulating the protein expression of cdc2 (4). The activation of endoplasmic reticulum (ER) stress in cancer therapy has been well documented. Upstream elements such as the protein kinase RNA (PKR)-like ER kinase (PERK), the inositol-requiring protein-1 (IRE1), the activating transcription factor-6 (ATF6) or caspase-12, were activated. Then the target proteins such as the C/EBP-homologous protein (CHOP; also known as GADD153), the ER chaperones, Bip and the glucose-regulated protein 94 (GRP94), were up-regulated (5). Curcumin induces significant endoplasmic reticulum stress in lung cancer cells (A549), as has been previously documented (6). The curcumin-induced ER stress was evident by the up-regulation of survival molecules such as phosphorylated PERK and eIF2 α , Bip, and apoptotic molecules such as caspase-4 and GADD153 (7). ER stress is one of the molecular mechanisms inducing cell death. The over-expression of GADD153 represents a new strategy for cancer therapy (8). The anti-cancer effects of curcumin have been documented in many cancers. However, the molecular mechanisms responsible for curcumin-induced endoplasmic reticulum stress and unfolding protein response, are not yet clearly understood. In the present study, we investigated the molecular mechanisms responsible for curcumin's ability to decrease the expression of the folding proteins and then induce ER stress and mitochondrial dysfunction in hepatocellular carcinoma J5 cells.

Materials and methods

Chemicals and reagents. Fetal bovine serum (FBS), sodium pyruvate, HEPES, dimethyl sulfoxide (DMSO), RPMI-1640, MTT, trypsin-EDTA, mouse anti- β -actin, Cdc25c and penicillin-streptomycin, were obtained from Sigma-Aldrich

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Key words: curcumin, J5 cells, endoplasmic reticulum stress, mitochondria dysfunction

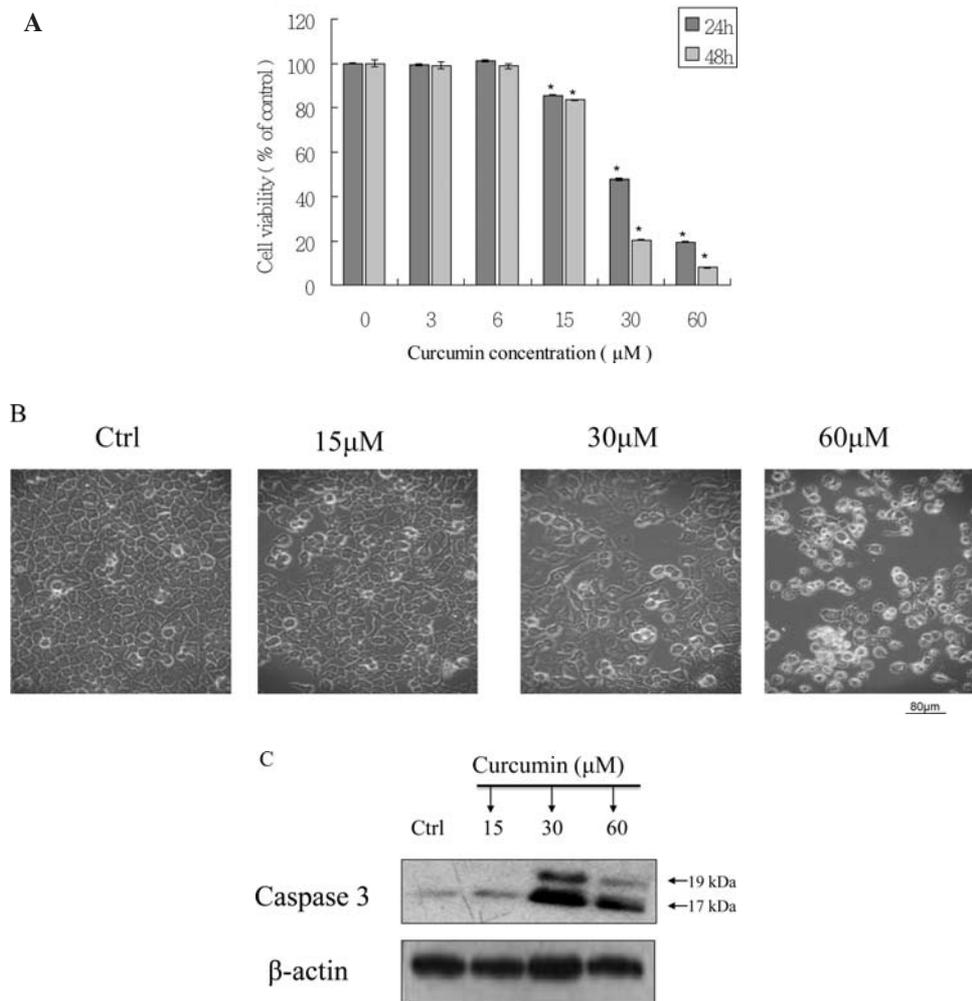


Figure 1. The cytotoxicity of curcumin in J5 cells. (A) The cytotoxicity of curcumin in J5 cells was determined using the MTT assay as described in Materials and methods. Curcumin significantly inhibited J5 cell growth in a time- and dose-dependent manner. Each point is the means \pm SD of 3 experiments. * $P < 0.05$, with respect to the control. (B) HCC J5 cells were treated with various concentrations of curcumin (0, 15, 30 and 60 μ M) for 24 h, and observed under a light microscope. The J5 cells morphology was changed to a rough surface with fragment chromosomes and the suspensions were identified as apoptotic cells. (C) J5 cells were treated with various concentrations of curcumin (0, 15, 30 and 60 μ M) for 24 h. Then the protein expressions were determined by Western blot analysis, as described in Materials and methods. The results showed that Curcumin increased the protein expression of caspase-3.

(St. Louis, MO, USA). Buffer (10X TG-SDS), Tris, Tween-20, SDS and glycine, were obtained from Amresco (St. Louis, MO, USA). BioMax Film was obtained from Kodak. Mouse anti-caspase-12, ATF6, Cdc2 and TCTP, were obtained from Abcam. Mouse anti-caspase-3 was obtained from R&D (Minneapolis, MN, USA). Rabbit anti-Bcl-2, Bip, Calreticulin, Calnexin, protein disulfide isomerase (PDI) and Ero1-L α , were obtained from Cell Signaling Technology. Mouse Mcl-1 was obtained from Millipore.

Cell culture. The J5 cells were kindly provided by Dr H.J. Harn (Graduate Institute of Cancer Biology and Center for Molecular Medicine, China Medical University and Hospital, Taichung, Taiwan, R.O.C.) (9). The J5 cells were maintained in RPMI-1640 medium containing 10% FBS, 1% penicillin-streptomycin (10,000 U/ml penicillin, 10 mg/ml streptomycin) at 37°C in a humidified atmosphere containing 5% CO₂ (10).

Cell proliferation assay. The J5 cells were plated in 96-well plates at a density of 2×10^4 cells/well and allowed to adhere

and grow for 24 h. The medium was then replaced with 100 μ l/well of fresh medium containing various concentrations of Curcumin (0, 3, 6, 15, 30 and 60 μ M) and the cells were cultured for different durations (24 and 48 h). Then, 100 μ l of 1 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were added, and the cells were incubated for 2 h at 37°C. Subsequently, the medium was removed and 100 μ l DMSO was added to the wells. The absorbance was measured using an ELISA plate reader at 590 nm. Data were calculated as the percentage of proliferation using the following formula: Proliferation (%) = $(OD_{\text{test}} - OD_{\text{blank}}) \times 100$, where OD_{test} and OD_{blank} are the optical densities of the test substances and the blank controls, respectively (11).

Protein preparation. Approximately 1×10^6 cells/10-cm dish were incubated with various concentrations of curcumin (0, 15, 30 and 60 μ M) for 24 h before the cells were harvested by centrifugation. The J5 cells were washed twice with ice-cold PBS and lysed in 100 μ l of lysis buffer (PRO-PREP

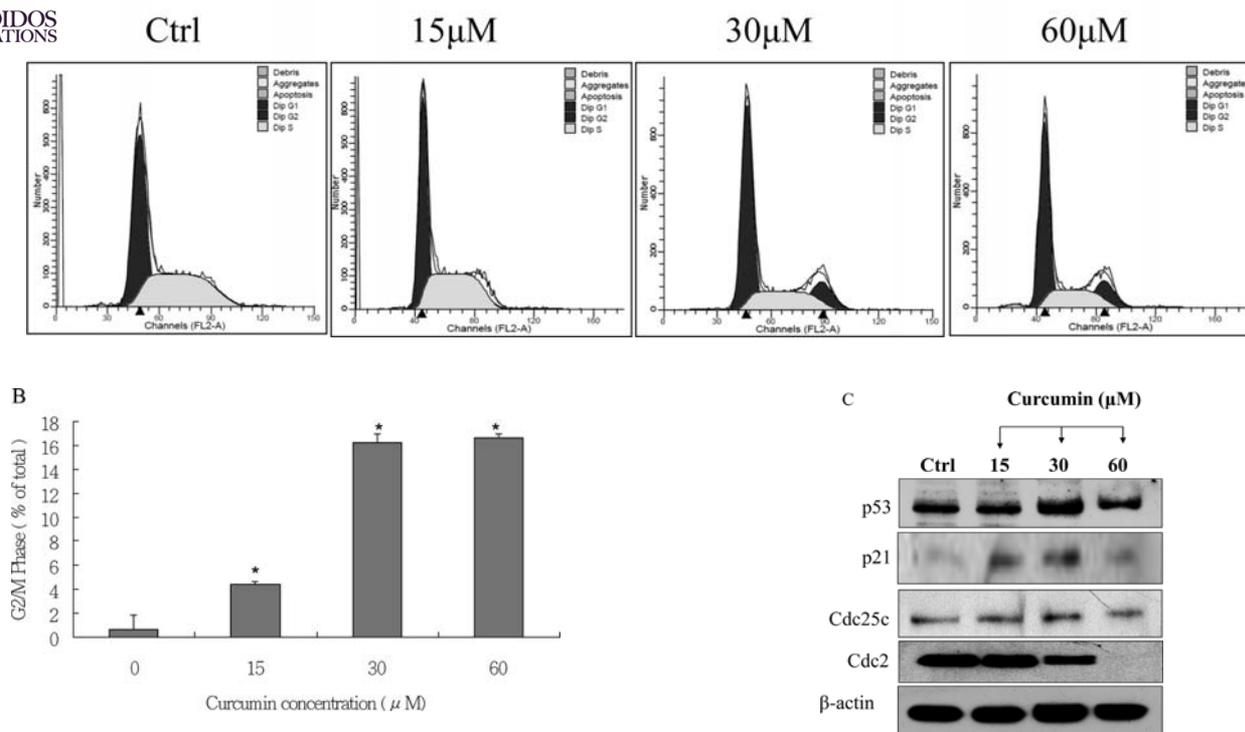


Figure 2. Curcumin induces cell cycle arrest at the G2/M phase. J5 cells were treated with various concentrations of curcumin (0, 15, 30 and 60 μM) for 24 h and then the cell cycle was determined by FACS analysis, as described in Materials and methods. The results showed that J5 cells induced G2/M phase arrest (A and B). J5 cells were treated with various concentrations of curcumin (0, 15, 30 and 60 μM) for 24 h. Then the protein expressions were determined by Western blot analysis, as described in Materials and methods. The results showed that curcumin decreased the Cdc2, but increased the P53 and P21 protein expressions (C).

buffer, Intron). After incubation on ice for 40 min, the cell lysates were centrifuged and the supernatants were collected. The protein concentration of the samples was determined by Bradford assay (Bio-Rad, Hercules, CA, USA) (12).

Western blotting. Western blot analysis was conducted using antibodies (Abs) against caspase-3, Cdc25c, Cdc2, caspase-12, ATF6, GADD153, TCTP, Mcl-1, Bcl-2, Calreticulin, Calnexin, PDI and Ero1-L α . β -actin was used as the internal control to determine loading efficiency. Protein samples (containing 40 μg of protein) were separated on 10-15% SDS-polyacrylamide gels and transferred to immobilized polyvinylidene difluoride membranes (Millipore). The membranes were incubated in TBST buffer [0.1 M Tris-HCl (pH 7.4), 0.9% NaCl, 0.1% Tween-20] supplemented with 5% dry non-fat milk for 1 h to block non-specific binding. After incubation with the primary Abs, the membranes were washed thrice with TBST buffer followed by incubation with the appropriate streptavidin-HRP-conjugated secondary Abs. The immunoreactive bands were visualized using an enhanced chemiluminescence (Millipore) detection kit. Immunoreactive bands were scanned (GS-800; Bio-Rad) and analyzed by using a digital scanning densitometer (Quantity One, v4.4.0; Bio-Rad) (13).

Cell cycle analysis. The J5 cells were treated with various concentrations of curcumin (0, 15, 30 and 60 μM) for 24 h. The cells were harvested and washed with PBS and then re-suspended in 70% ethanol (at -20°C) overnight. The cells were washed thrice with PBS and then stained with 20 $\mu\text{g}/\text{ml}$ propidium iodide. DNA content was analyzed by fluorescence-

activated cell sorting analysis (FACScan, Becton-Dickinson, San Jose, CA) using ModFit software (Verity Software House, Turramurra, NSW, Australia) (13).

Immunocytochemical staining. The immunocytochemical analysis procedures have been previously described (6). Briefly, the J5 cells were treated with curcumin (30 μM) for 24 h, and were then fixed with 4% paraformaldehyde to allow the detection of ATF6, caspase-12, GADD153 and caspase-3 antibodies (1:200; Cell Signaling Technology). No treatment was used as the control, for the detection of the FITC-conjugated secondary antibody (1:1000; Chemicon). The cells were then observed under a fluorescent microscope (Zeiss Axioskop 2 plus).

Statistical analysis. Values are presented as the means \pm SD. The Student's t-test was used to analyze statistical significance. A p-value of <0.05 was considered statistically significant for all the tests.

Results

Cytotoxicity of curcumin in J5 cells. The results showed that curcumin inhibits the proliferation of human hepatocellular carcinoma J5 cells in a time- and dose-dependent manner (Fig. 1A). When cultured with various concentrations of curcumin (0, 3, 6, 15, 30 and 60 μM) for 24 and 48 h, the viable cell percentages relative to the control were 99.35 ± 0.51 ($p=0.15$), 101.25 ± 0.43 , 85.54 ± 0.24 , 47.67 ± 0.56 and 19.43 ± 0.26 ($p<0.01$) for 24 h, and 99.06 ± 1.52 ($p=0.49$), 98.68 ± 1.1 , 83.38 ± 0.36 , 20.43 ± 0.13 and 7.99 ± 0.28 ($p<0.01$)

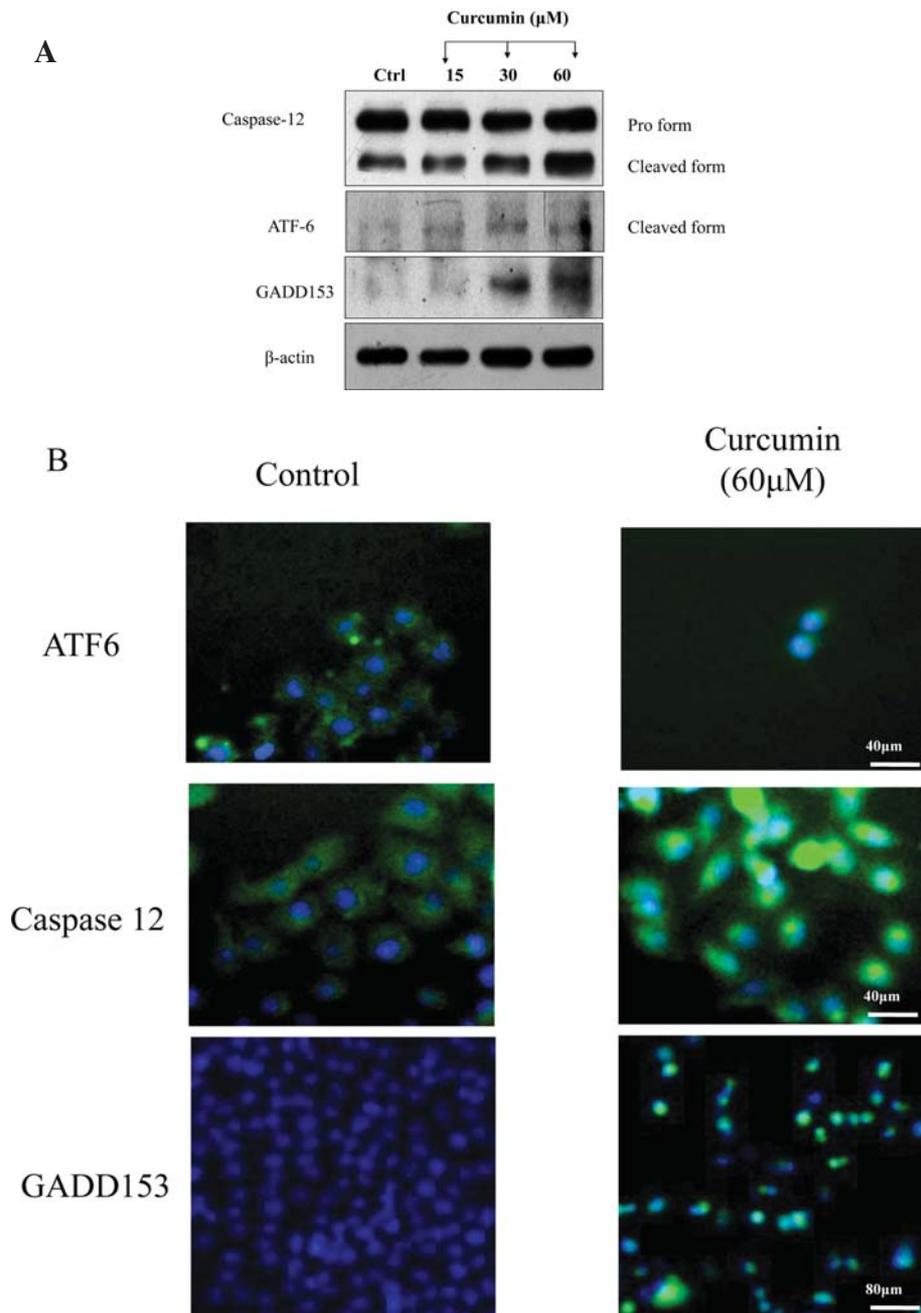


Figure 3. The effects of ER stress-associated protein in Hep-J5 cells treated with curcumin for 24 h. J5 cells were treated with various concentrations of curcumin (0, 15, 30 and 60 μM) for 24 h. The protein expressions were then determined by Western blot analysis, as described in Materials and methods. The results showed that curcumin cleaved pro-caspase-12 and up-regulated the ATF6 expression, and then stimulated the downstream GADD153 increase in the J5 cells (A). Hep-J5 cells were treated with curcumin for 24 h. The expressions and locations of ATF6, caspase-12 and GADD153 in the J5 cells, were observed by immunocytochemistry as described in Materials and methods. The results showed that curcumin induced high levels of ATF6, caspase-12 and GADD153 (B).

for 48 h, respectively. During curcumin treatment for 24 h, the half-maximum inhibitory concentration (IC_{50}) was 29.07 μM , and the maximum inhibition of cell growth (>75%) was obtained at 19.17 μM . During curcumin treatment for 48 h, the IC_{50} was 22.95 μM , and the maximum inhibition of cell growth (>75%) was obtained at 16.98 μM .

Curcumin induces morphological changes and increases caspase-3 expression in J5 cells. The morphological effects of curcumin in the J5 cells were first determined by morphological assay. The HCC J5 cells were treated with various concentrations of curcumin (0, 15, 30 and 60 μM) for 24 h,

and were then observed under a light microscope (Fig. 1B). The J5 cells morphology was changed to a rough surface with fragment chromosomes and the suspensions were identified as apoptotic cells. It is also evident from our results, that J5 cells treated with various concentrations of curcumin (0, 15, 30 and 60 μM) for 24 h, had an increased protein expression of caspase-3, as was shown by Western blot analysis (Fig. 1C). These results also showed that curcumin induced apoptosis.

Curcumin induces cell cycle arrest at the G2/M phase. Our results showed that J5 cells treated with various concentra-

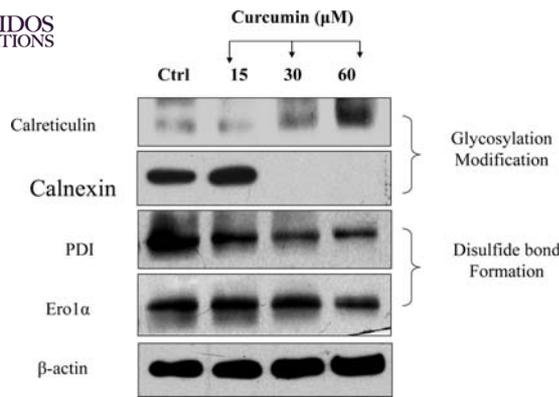


Figure 4. The effects of UPR-associated proteins in J5 cells treated with curcumin for 24 h. The J5 cells were treated with various concentrations of curcumin (0, 15, 30 and 60 μM) for 24 h. The protein expressions were then determined by Western blot analysis, as described in Materials and methods. The expressions of the folding-associated proteins, Calnexin, Ero1-L α and PDI, were decreased, but the protein expression of Calreticulin was increased.

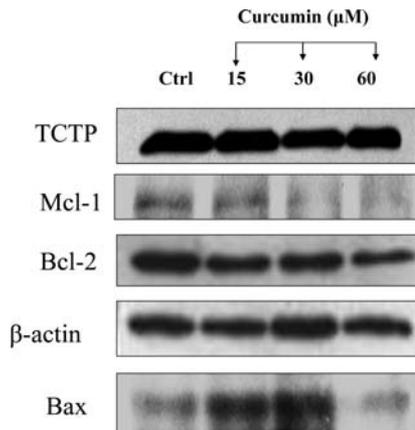


Figure 5. Curcumin induced apoptosis through mitochondrial dysfunction. J5 cells were treated with various concentrations of curcumin (0, 15, 30 and 60 μM) for 24 h. The protein expressions were then determined by Western blot analysis, as described in Materials and methods. Our results showed that J5 cells treated with curcumin had decreased protein expressions of TCTP, Mcl-1 and Bcl-2, but an increased Bax expression.

tions of curcumin (0, 15, 30 and 60 μM), induced G2/M phase arrest (Fig. 2A and B). We also detected the cell cycle associated protein expression. It is also evident from our results, that J5 cells treated with various concentrations of curcumin (0, 15, 30 and 60 μM) for 24 h, had a decreased Cdc2 but increased P53 and P21 protein expression, as was shown by Western blot analysis (Fig 2C).

Curcumin induces ER stress through caspase-12 and ATF6 in J5 cells. Our results showed that J5 cells treated with various concentrations of Curcumin (0, 15, 30 and 60 μM) for 24 h, had a cleaved pro-caspase-12 and an up-regulated ATF6 expression. Curcumin then stimulated the downstream GADD153 increase in the J5 cells (Fig. 3A). Curcumin induced high levels of GADD153 and then translocated ATF6 to the nucleus (Fig. 3B). Our results also showed that when the J5 cells were treated with various concentrations of Curcumin (0, 15, 30 and 60 μM) for 24 h, the expression of

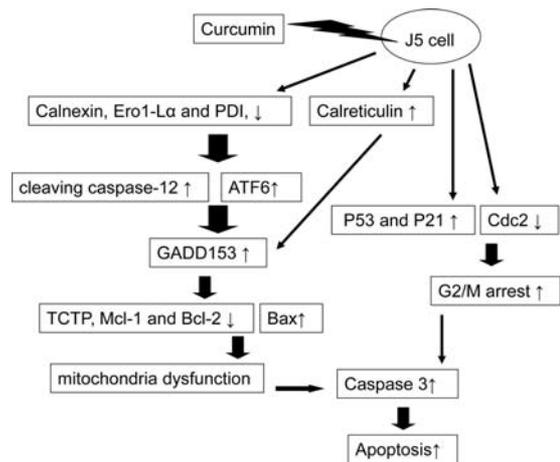


Figure 6. Proposed molecular mechanisms responsible for the curcumin-inhibited proliferation in J5 cells. Curcumin induced UPR by decreasing the protein expressions of Calnexin, Ero1-L α and PDI, and increasing the Calreticulin expression. Curcumin also cleaved caspase-12 and increased the ATF6 expression and stimulated ER stress and the downstream GADD153 over-expression. In addition, GADD153 decreased the TCTP, Mcl-1 and Bcl-2 expressions and induced mitochondrial dysfunction. Curcumin also induced cell cycle arrest at the G2/M phase by decreasing the Cdc2 expression.

the folding-associated proteins, Calnexin, Ero1-L α and PDI, was decreased, but the protein expression of Calreticulin was increased (Fig. 4). These results suggest that Curcumin inhibits the folding-associated proteins and induces ER stress in J5 cells.

Curcumin induces apoptosis through mitochondrial dysfunction. Our results showed that J5 cells treated with various concentration of curcumin (0, 15, 30 and 60 μM), had a decreased protein expression of TCTP, Mcl-1 and Bcl-2, and an increased Bax expression (Fig. 5). Bcl-2 played a key role in regulating the function of mitochondria (14,15). These results suggest that curcumin inhibits TCTP and destroys the function of mitochondria in J5 cells.

Discussion

Our results showed that curcumin inhibits the proliferation of J5 cells in a time- and dose-dependent manner. In addition, Curcumin increased the protein expression of caspase-3. These results are in agreement with those from other studies, showing that curcumin induces apoptosis and inhibits the proliferation in hepatocellular carcinoma cell lines, such as Hep G2, Hep 3B and HL-7702 (16). Our results also showed that curcumin induced cell cycle arrest at the G2/M phase by decreasing the Cdc2 expression. ER stress is one of the molecular mechanisms responsible for curcumin-induced apoptosis (6). The unfolding protein response (UPR) is regulated by Bip and GADD153 (17). When UPR exceeds the threshold, damaged cells are committed to apoptosis through the ATF6 mediated GADD153 signaling pathway. Our results showed that curcumin induced ER stress in the J5 cells, by increasing the protein expressions of caspase-12, ATF6 and GADD153, and then GADD153 translocated to the nucleus (Fig. 6). It has been well documented that GADD153 suppresses Bcl-2 expression (17,18). Mcl-1 regulated by

TCTP, inhibits ER stress-induced apoptosis (19,20). In the present study, curcumin induced mitochondrial dysfunction by decreasing the expressions of TCTP, Mcl-1 and Bcl-2.

UPR is one of the cellular stress responses. It is activated in response to an accumulation of unfolding or misfolding proteins in the endoplasmic reticulum. J5 cells treated with curcumin had a decreased Calnexin protein expression and an increased Calreticulin expression. It has been well documented that Calreticulin exposure induces cancer cell death (21-23). DNA damage decreases the protein expression of PDI and Ero1- α (the human homolog of Ero1p) in the endoplasmic reticulum (24-26). Our results also showed that curcumin decreased the protein expression of PDI and Ero1- α in J5 cells. In conclusion, our study showed that curcumin induced UPR through the down-regulation of the chaperones and folding proteins (Bip, Calnexin, PDI and Ero1- α). Curcumin also increased the GADD153 expression by up-regulating the protein expressions of caspase-12 and ATF6. GADD153 induced mitochondrial dysfunction by down-regulating the protein expressions of TCTP, Mcl-1 and Bcl-2. In addition, curcumin caused DNA damage response, and thus cell cycle arrest at the G2/M phase by down-regulating the Cdc2 expression.

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References

- Mukherjee N, Chakraborty S, Ghosh U, Bhattacharyya NP, Bhattacharya RK, Dey S and Roy M: Curcumin-induced apoptosis in human leukemia cell HL-60 is associated with inhibition of telomerase activity. *Mol Cell Biochem* 297: 31-39, 2007.
- Lu HF, Lai KC, Hsu SC, Lin HJ, Yang MD, Chen YL, Fan MJ, Yang JS, Cheng PY, Kuo CL and Chung JG: Curcumin induces apoptosis through FAS and FADD, in caspase-3-dependent and -independent pathways in the N18 mouse-rat hybrid retina ganglion cells. *Oncol Rep* 22: 97-104, 2009.
- Sahu RP, Batra S and Srivastava SK: Activation of ATM/Chk1 by curcumin causes cell cycle arrest and apoptosis in human pancreatic cancer cells. *Br J Cancer* 100: 1425-1433, 2009.
- Lee DS, Lee MK and Kim JH: Curcumin induces cell cycle arrest and apoptosis in human osteosarcoma (HOS) cells. *Anticancer Res* 29: 5039-5044, 2009.
- Ma Y and Hendershot LM: The role of the unfolded protein response in tumour development: friend or foe? *Nat Rev Cancer* 4: 966-977, 2004.
- Lin SS, Huang HP, Yang JS, Wu JY, Hsia TC, Lin CC, Lin CW, Kuo CL, Gibson Wood W and Chung JG: DNA damage and endoplasmic reticulum stress mediated curcumin-induced cell cycle arrest and apoptosis in human lung carcinoma A-549 cells through the activation caspases cascade- and mitochondrial-dependent pathway. *Cancer Lett* 272: 77-90, 2008.
- Pae HO, Jeong SO, Jeong GS, Kim KM, Kim HS, Kim SA, Kim YC, Kang SD, Kim BN and Chung HT: Curcumin induces pro-apoptotic endoplasmic reticulum stress in human leukemia HL-60 cells. *Biochem Biophys Res Commun* 353: 1040-1045, 2007.
- Oyadomari S and Mori M: Roles of CHOP/GADD153 in endoplasmic reticulum stress. *Cell Death Differ* 11: 381-389, 2004.
- Yu YL, Yu SL, Su KJ, Wei CW, Jian MH, Lin PC, Tseng IH, Lin CC, Su CC, Chan DC, Lin SZ, Harn HJ and Chen YL: Extended O(6)-methylguanine methyltransferase promoter hypermethylation following n-butylidenephthalide combined with 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) on inhibition of human hepatocellular carcinoma cell growth. *J Agric Food Chem* 58: 1630-1638, 2010.
- Chen YL, Jian MH, Lin CC, Kang JC, Chen SP, Lin PC, Hung PJ, Chen JR, Chang WL, Lin SZ and Harn HJ: The induction of orphan nuclear receptor Nur77 expression by n-butylidenephthalide as pharmaceuticals on hepatocellular carcinoma cell therapy. *Mol Pharmacol* 74: 1046-1058, 2008.
- Su CC and Lin YH: Tanshinone IIA inhibits human breast cancer cells through increased Bax to Bcl-xL ratios. *Int J Mol Med* 22: 357-361, 2008.
- Su CC and Lin YH: Tanshinone IIA down-regulates the protein expression of ErbB-2 and up-regulates TNF-alpha in colon cancer cells *in vitro* and *in vivo*. *Int J Mol Med* 22: 847-851, 2008.
- Su CC, Chen GW and Lin JG: Growth inhibition and apoptosis induction by tanshinone I in human colon cancer Colo 205 cells. *Int J Mol Med* 22: 613-618, 2008.
- Chiu TL and Su CC: Curcumin inhibits proliferation and migration by increasing the Bax to Bcl-2 ratio and decreasing NF- κ Bp65 expression in breast cancer MDA-MB-231 cells. *Int J Mol Med* 23: 469-475, 2009.
- Kuo HM, Tsai HC, Lin YL, Yang JS, Huang AC, Yang MD, Hsu SC, Chung MC, Gibson Wood W and Chung JG: Mitochondrial-dependent caspase activation pathway is involved in baicalein-induced apoptosis in human hepatoma J5 cells. *Int J Oncol* 35: 717-724, 2009.
- Anand P, Sundaram C, Jhurani S, Kunnumakkara AB and Aggarwal BB: Curcumin and cancer: an 'old-age' disease with an 'age-old' solution. *Cancer Lett* 267: 133-164, 2008.
- Kim R, Emi M, Tanabe K and Murakami S: Role of the unfolded protein response in cell death. *Apoptosis* 11: 5-13, 2006.
- Rasheva VI and Domingos PM: Cellular responses to endoplasmic reticulum stress and apoptosis. *Apoptosis* 14: 996-1007, 2009.
- Jiang CC, Lucas K, Avery-Kiejda KA, Wade M, deBock CE, Thorne RF, Allen J, Hersey P and Zhang XD: Up-regulation of Mcl-1 is critical for survival of human melanoma cells upon endoplasmic reticulum stress. *Cancer Res* 68: 6708-6717, 2008.
- Liu H, Peng HW, Cheng YS, Yuan HS and Yang-Yen HF: Stabilization and enhancement of the antiapoptotic activity of mcl-1 by TCTP. *Mol Cell Biol* 25: 3117-3126, 2005.
- Obeid M, Tesniere A, Ghiringhelli F, Fimia GM, Apetoh L, Perfettini JL, Castedo M, Mignot G, Panaretakis T, Casares N, Metivier D, Larochette N, van Endert P, Ciccosanti F, Piacentini M, Zitvogel L and Kroemer G: Calreticulin exposure dictates the immunogenicity of cancer cell death. *Nat Med* 13: 54-61, 2007.
- Clarke C and Smyth MJ: Calreticulin exposure increases cancer immunogenicity. *Nat Biotechnol* 25: 192-193, 2007.
- Apetoh L, Ghiringhelli F and Zitvogel L: Calreticulin dictates the immunogenicity of anti-cancer chemotherapy and radiotherapy. *Med Sci (Paris)* 23: 257-258, 2007.
- Krynetskaia NF, Phadke MS, Jadhav SH and Krynetskiy EY: Chromatin-associated proteins HMGB1/2 and PDIA3 trigger cellular response to chemotherapy-induced DNA damage. *Mol Cancer Ther* 8: 864-872, 2009.
- Benham AM, Cabibbo A, Fassio A, Bulleid N, Sitia R and Braakman I: The CXXCXXC motif determines the folding, structure and stability of human Ero1-Lalpha. *EMBO J* 19: 4493-4502, 2000.
- Frandsen AR and Kaiser CA: Ero1p oxidizes protein disulfide isomerase in a pathway for disulfide bond formation in the endoplasmic reticulum. *Mol Cell* 4: 469-477, 1999.