Curcumin selectively induces apoptosis in deregulated cyclin D1 expressed cells at G2 phase of cell cycle in a p53-dependent manner

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Abbreviations: BrdU, 5-bromo-2-deoxyuridine; CCD, Charged coupled device; Cdk, cyclin-dependent kinase; DAPI, 4'6'-diamidino-2-phenylindole; NME, normal mammary epithelial; PI, propidium iodide; pRb, retinoblastoma protein

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Curcumin (diferuloylmethane) is known to induce apoptosis in tumor cells. In asynchronous cultures, with time-lapse video-microscopy in combination with quantitative fluorescence microscopy, we have demonstrated that curcumin induces apoptosis at G2 phase of cell cycle in deregulated cyclin D1 expressed mammary epithelial carcinoma cells leaving its normal counterpart unaffected. In our search towards delineating the molecular mechanisms behind such differential activities of curcumin, we found that it selectively increases p53 expression at G2 phase of carcinoma cells and releases cytochrome c from mitochondria, which is essential requirement for apoptosis. Further experiments using p53-null as well as dominant negative and wild-type p53-transfected cells have established that curcumin induces apoptosis in carcinoma cells via p53-dependent pathway. On the other hand, curcumin reversibly inhibits normal mammary epithelial cell cycle progression by down regulating cyclin D1 expression and blocking its association with Cdk4/ Cdk6 as well as by inhibiting phosphorylation and inactivation of retinoblastoma protein. In addition, curcumin significantly up-regulates cell cycle inhibitory protein (p21Waf-1) in normal cells and arrests them in G0 phase of cell cycle. Therefore, these cells escape from curcumin-induced apoptosis at G2 phase. Interestingly, these processes remain unaffected by curcumin in carcinoma cells where cyclin D1 expression is high. Similarly, in ectopically over-expressed system, curcumin cannot down-regulate cyclin D1 and thus block cell cycle progression. Hence these cells progress into G2 phase and undergo apoptosis. These observations together suggest that curcumin may have a possible therapeutic potential in breast cancer patients.

Cell proliferation is tightly regulated by multiple interactions between molecules in normal cells. Progression through several major checkpoints in the cell cycle is controlled by multiple protein kinases, each of which contains a regulatory cyclin and a catalytic cyclin-dependent kinase (Cdk) (1,2). The expression level of each component, its phosphorylation status, and the presence of specific Cdk inhibitory proteins regulate the activity of these kinases (2-5). In mammalian cells, the D cyclins with their catalytic partners Cdk4 and Cdk6 function as the cells leave G0 and progress through G1 into S phase (2-4). In majority of human breast cancers, deregulated over-expression of cyclin D1 is found, while in normal cells, its expression is tightly regulated by mitogenic signaling involving p21ras pathway (6). In many cell types, aberrant expression of cell cycle regulatory proteins can induce cell cycle progression under conditions which are normally growth suppressive, leading to apoptosis (cell death) (7,8). It is also reported that DNA damage response pathway causes cell cycle arrest by regulating cyclin/Cdk via checkpoint inhibitors, and thus provides time for DNA repair. However, when the damage is irreparable, the cells undergo apoptosis (9,10). Among these inhibitors, p21Waf-1 and p27Kip-1 belong to the family of broad-specificity inhibitors of cyclin/Cdk complexes (11-13) whereas each of the Ink4 family members binds directly to Cdk4 and Cdk6 and functions as specific inhibitor of cyclin D-dependent kinases (1,2). All these observations indicate the possibility of the candidacy of cyclin D1 as a therapeutic target in breast cancer and the importance of these checkpoint inhibitors in regulating this cyclin.
Besides cyclins, Cdk's and the inhibitors, as a tumor suppressor, p53 has a central role in cell cycle regulation. Researches conducted in the last two decades have firmly established the importance of p53 in mediating the cell cycle arrest that occurs following DNA damage. However, during the same time, the role of p53 in mediating apoptosis has become increasingly less clear, even as the number of putative pro-apoptotic proteins trans-activated by p53 has increased (14). Similarly unclear is how p53 makes a choice between cell-cycle arrest or apoptosis raising the possibility that p53 alone is not responsible for this crucial decision. An important function of p53 is to act as a transcription factor by binding to a p53-specific DNA consensus sequence in responsive genes (15), which would be expected to increase the synthesis of p21Waf-1 or Bax (11,16). Up-regulation of p21Waf-1 results in the inhibition of cell cycle progression from G1 to S phase of cell cycle (11). On the other hand, up-regulation of Bax alters Bcl-2/Bax ratio in cellular microenvironment and cause release of cytochrome c from mitochondria into the cytosol. Cytochrome c then binds to Apaf-1 and activates caspase-3 through caspase-9, which is responsible for the latter process of apoptosis (17). Therefore, any agent that can regulate these processes in cancer cells may have a role in tumor regression.

Recently, the use of phytochemicals as anti-cancer agent has gained high importance. Among various naturally occurring phytochemicals, curcumin is capturing the attention of cancer investigators worldwide because of its chemo-preventive properties against human malignancies (18-20). The anti-carcinogenic properties of curcumin in animals have been demonstrated by its inhibition of tumor initiation induced by various carcinogens (21,22). This yellow pigment interrupts cell cycle, disrupts mitotic spindle structure and induces micro-nucleation, thereby, acting as anti-proliferative agent in various cancer cells (23). We have already reported that curcumin induces apoptosis in human breast carcinoma cells (20). In contrast, curcumin has been found to protect normal cells from stress-induced apoptosis (24). Inhibition of both proliferation and apoptosis of T lymphocytes by this curry pigment led us to conclude that the inhibition of cell proliferation by curcumin was not always associated with programmed cell death (25). Interestingly, curcumin has been found to non-selectively inhibit proliferation of normal as well as malignant cell although its apoptogenic effect is more profound in malignant cells (26). Curcumin-induced suppression of cell proliferation correlates with down-regulation of cyclin D1 expression and Cdk4-mediated retinoblastoma protein phosphorylation (27). However, the molecular mechanisms underlying such differential effect of curcumin in normal and malignant cells are not yet clearly understood.

Since a cell can undergo apoptosis at any phase of cell cycle and cell cycle regulatory proteins play vital role in deciding the fate of the cell, the possibility of these proteins to be the targets of curcumin cannot be overruled. We, therefore, hypothesized that curcumin may be regulating the cell cycle differentially in normal and malignant cells and thereby showing differential effects in those. To prove this hypothesis we aimed at mapping the molecular mechanisms of such differential effects of curcumin in normal and malignant cells.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—Human mammary epithelial carcinoma cell (MCF-7, T47-D, MDA-MB-468 and MDA-MB-231), prostate cancer cell lines (PC-3 and LNCap) and B cell lymphoma (Daudi) cells were obtained from National Center for Cell Sciences, India. The MDA-H0-41 post-crisis skin fibroblast cell line was derived from patient with Li-Fraumeni syndrome and TR9-7 cells, expressing wild-type p53 under the control of a tetracycline-regulated promoter were derived from this cell line (20). PC-3-wt-p53 cells were derived from p53-negative PC-3 cells through an adenoviral vectors expression system expressing wild-type p53 under the control of CMV-promoter and MCF-7-dn-p53 and T47-D-dn-p53 cells were derived from MCF-7 and T47-D cells through an adenoviral vectors expression system expressing dominant-negative p53 under the control of CMV-promoter (Clonetech). Mantle cell lymphoma, NIH 3T3 cells and NIH 3T3 cells expressing oncogenic p21ras (Val-16-ras) and Cyclin D1 knockout mouse embryonic fibroblast (MEF D1/-) cells were obtained from Dr. Dennis W. Stacey of The Cleveland Clinic Foundation, USA. Cyclin D1 over-expressed MEF-D1+ cells were derived from MEF-D1/- cells through CMV-promoter driven cyclin D1 expression system. Cells were routinely maintained in Dulbeco’s minimal essential medium (DMEM) or RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), insulin (0.1 units/ml), L-glutamine (2 mM),
sodium pyruvate (100 µg/ml), non-essential amino acids (100 µM), antibiotics (100 µg/ml streptomycin and 50 unit/ml penicillin) (Sigma) at 37°C in a humidified incubator containing 5% CO₂. Human normal mammary epithelial cells (NME; Clonetics) were maintained in mammary epithelial cell growth media supplemented with SingleQuots (Clonetics).

**Immunofluorescence and Measurement of Fluorescent Signal of Each Immunostained Cell—Cells**

Cells growing on a cover slip were incubated with 5 µM 5-bromo-2-deoxyuridine (BrdU; Boehringer Mannheim) for the last 30 min before fixation with 3% p-formaldehyde/ Triton x100. Incorporated BrdU or p53/cytochrome c content of individual cells was visualized by immunofluorescence using Cy2-/ Cy3-conjugated anti-BrdU (Boehringer Mannheim; 2 µg/ml), anti-p53 or anti-cytochrome c (Santa Cruz; 2 µg/ml) antibodies. For double-labeling experiments antibody treatments were performed consecutively. Nuclear DNA was stained with 46-diamidino-2-phenylindole (DAPI; Pharmingen). A Leica fluorescent microscope DM 900 was used to visualize the fluorescent images. Digital images were captured with a highly sensitive cool (-25°C) charged coupled device (CCD) camera (Princeton Instruments) controlled with the MetaMorph software (Universal Imaging). The exposure time were adjusted so that the brightest signal in the specimen gave less than 90% of the maximum linear range for the camera (a gray scale of 0 to 4096; 16 bit gray scale). Each captured image was corrected by subtracting the background image followed shading correction (to correct the uneven signal collection across the field). Nuclear region of each cell were defined by image analysis of DAPI pictures. Then the fluorescence signal from individual nuclear regions were integrated for DAPI, BrdU or p53 fluorescence using MetaMorph image analysis features as described earlier (for details see 13,28,29). Cells were separated into cell cycle phases based upon BrdU and DNA content. More than 400 cells were photographed for each set and the average fluorescence intensity of each fluorochrome in each cell cycle phase was determined.

**TUNEL Assay**—The fragmented DNA of apoptotic cells was labeled by catalytically incorporating FITC-dUTP at the 3'-hydroxyl ends of the fragmented DNA by enzyme terminal deoxynucleotidyl transferase (TdT) using Apo-direct kit (Pharmingen) following the principle of TUNEL (TdT-mediated dUTP Nick-End Labeling). The cells were then analyzed on flow cytometer (equipped with 488 nm Argon laser light source; 515 nm band pass filter, FL1-H, and 623 nm band pass filter, FL2-H) using CellQuest software (Becton Dickinson). Electronic compensation of the instrument was done to exclude overlapping of the emission spectra. Total 10,000 events were acquired and dual parameter dot plot of FL2-H (x-axis; PI-fluorescence, linear scale) versus FL1-H (y-axis; FITC-fluorescence, logarithmic scale) has been shown.

**Time-lapse Video-micrography for Determination of Cell Cycle Position—**Appropriate number of cells was seeded on cover slip in 35 mm petridish. For the determination of approximate cell cycle position of individual cells and the exact time required between two divisions, time-lapse video-micrography was applied. Cells were maintained at 37°C in a 95% air/5% CO₂ environment in a stage heater (20/20 Technology, Whitehouse Station) mounted on an inverted phase-contrast microscope. Digital images were obtained with a CCD camera attached to a frame grabber board controlled by the "NIH Image" program. Individual frames of 640 by 480 pixels were captured every 10 min. Up to 129 individual frames were captured in a single stack, which was then replayed by the “NIH Image” program at various speeds in the forward or backward direction to create a movie for analysis (for further references see 13,29 and 30). The area of the cover slip to be analyzed was marked with circle using a diamond object marker. This allowed realignment of the area of analysis and identification of individual cells following immunostaining and nuclear staining. When second time-lapse analysis was started following curcumin treatment, care was taken to ensure that the same area of the cover slip and same alignment was viewed in each movie. Individual cells were monitored and the time between two successive divisions were calculated by the difference between two individual frame numbers multiplied by 10 min.

To relate age with its cell cycle position, cells were followed in time-lapse with the final 30 min in the presence of BrdU. The cells were then fixed and stained with fluorescent anti-p53 and or anti-BrdU antibody. The age of each cell was determined individually by following that cell in the time-lapse movie beginning at the time of fixation and running the movie backwards until the cell was observed to pass through mitosis. Labeling with BrdU indicated that the cell had been in S.
phase during last 30 min. This approach allowed an analysis of cells in all cell cycle phases at the same time.

**Co-immunoprecipitation and Western Blotting**—For direct Western blot analysis cells were lysed in lysis buffer (50 mM Hepes, pH 7.6, 200 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40 supplemented with protease (10 μg/ml each of benzamidine, trypsin inhibitor, bacitracin, 5 μg/ml each of leupeptin, pepstatin A, antipain, PMSF) and phosphatase (5 μM each of o-phosphoserine, o-phosphotyrosine, o-phosphothreonine, β-glycero-phosphate, p-nitrophenylphosphate, sodium vanadate) inhibitor cocktails). The lysate containing 50 μg protein was separated by SDS-polyacrylamide gel. After electrophoresis the gel was transferred to a nitrocellulose membrane. The protein of interest was visualized with chemiluminescence. The blots were re-probed with anti-α-actin antibody (Santa Cruz) to confirm equivalent protein loading (20).

For the determination of direct interaction between two or more proteins, co-immunoprecipitation technique was applied. For this purpose cells were lysed in lysis buffer. The lysate (200 μg protein) were incubated for 4 h at 4°C in rocking condition with 4 μg anti-cyclin D1 antibody and the immune-complexes were then incubated with protein A-sepharose beads (31). The immunopurified protein was then used to detect the presence of associated proteins (Cdk4 or Cdk6; Santa Cruz) by Western blot analysis using specific antibody. Portions of the cell lysates used for co-immunoprecipitation were blotted against anti-α-actin antibody (Santa Cruz) to confirm equivalent protein loading.

**Kinase Assay—In vitro** Rb phosphorylation was done using GST-Rb as substrates (32). In brief, Cdk4 from equal amount of cellular protein from untreated or curcumin-treated cells were immunopurified with specific antibody. The phosphorylation reaction of GST-Rb was initiated using 10 μl of immunoprecipitates in the kinase buffer (50 mM HEPES pH 7.5, 10 mM MgCl2, 2.5 mM EDTA, 1 mM DTT and phosphatase inhibitor cocktails) by adding ATP mix (1 μM of [γ-32P]ATP and 9 μM of unlabeled ATP). The reaction was incubated at 30°C for 30 min. Proteins were separated by 10% SDS-PAGE. Phosphorylation of Rb protein was quantitated by Phospho-Imager using ImageQuant software (Molecular Dynamics).

**RESULTS**

**Curcumin Altered Cell Cycle Progression in Mammary Epithelial Cells but not in Carcinoma Cells**—A quantitative digital image analysis approach was used to determine cell cycle position of individual cells. The utility of this approach was first demonstrated by pulsing asynchronous culture with BrdU for 30 min, followed by fixation and staining of DNA with DAPI and incorporated BrdU with a fluorescent antibody. The DAPI staining identified nuclear regions in which the level of both DAPI and anti-BrdU antibody fluorescence were quantitated as in previous studies (13,29). Cells were separated into cell cycle phases on the basis of BrdU incorporation and DNA content. When DAPI fluorescence was plotted against BrdU-associated fluorescence for individual cells, cells in G0/G1 and G2/M phases were localized in well-separated groups without BrdU staining. S phase cells displayed intermediate DNA level, and stained for BrdU, with highest levels of staining found in cells in mid-S phase (MCF-7 cells: Fig. 1A; NME cells: Fig. 1B).

Based upon BrdU incorporation and DNA content, when percent cells in each cell cycle phase were determined, MCF-7 cells showed typical cell cycle pattern with approximately 53% cells in G0/G1 phase, 28% cells in S and 16% cells in G2/M phases (Fig. 1C; 0h). Twenty-four hours of continuous exposure to different doses of curcumin resulted in significant MCF-7 cell death and LD50 was found to be 10 μM (data not shown). Treatment of these cells with 10 μM curcumin for various time intervals showed no difference in BrdU incorporation, indicating thereby that curcumin has no effect in normal cell cycle progression of these malignant cells. However, the number of cells in sub-G0/G1 populations (apoptotic cells) increased with time up to 24 hr of curcumin treatment with concomitant decrease in G0/G1 populations. Even though the cells were undergoing apoptosis, they passed from G1 to S and G2 phases with predictable kinetics and displayed the expected BrdU incorporation. On the other hand, when NME cells were treated with the same dose of curcumin for various time intervals, it interfered with BrdU incorporation (Fig. 1D). In fact, in the absence of curcumin these cells showed a typical cell cycle pattern (at 0h). When curcumin was added to the culture, transition of cells from G1 to S phase was blocked within 4 h and the inhibition persisted up to 24 h, the time
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Curcumin induced mammary epithelial carcinoma cell apoptosis at G2 phase of cell cycle—To find out the cell cycle phase at which curcumin induced mammary epithelial carcinoma cell apoptosis, we have adopted different methods. In the first approach we used the TUNEL method. The cell-cycle position of apoptotic cells was assessed by the terminal deoxynucleotidyl transferase assay of DNA strand breaks combined with DNA staining with PI. It was interesting to note that curcumin induced MCF-7 apoptosis only in G2/M phase (Fig. 2A).

In the second approach, a MCF-7 culture was followed by two sequential time-lapse movies of the same area of the cover slip (Figs. 2B and 2D). Curcumin was added between two movies. The age of cells (i.e., time since last mitosis) at the time of curcumin addition was determined from the first movie, while the fate of the cells after curcumin addition, was determined from the second (Fig. 2B). Almost all the cells that passed through the S phase during curcumin treatment period (4-6 hr since the addition of curcumin; curcumin is active in biological fluid for 5-6 h; 33) underwent apoptosis. Surprisingly, the cells which were in other cell cycle phases during active curcumin incubation period escaped from curcumin-induced apoptosis (Figs. 2B and 2D).

In Figure 2C, there is a cluster of cells with y-axis values between 6 h and 20 h and x-axis values between 4 h and 12 h. These cells died at various ages, and given the length of G1, were probably in S phase when curcumin was added. However, there is also a uniform line of cells, which traverses the graph above the cluster of apoptotic cells. This horizontal line of cells never died, but represented the cells that were tracked until the end of the experiment when nothing happened to them. To support these observations we performed a parallel experiment where BrdU was added during the curcumin incubation-period and then excess BrdU was washed off after 4 h. In that case almost all the cells which underwent apoptosis, were BrdU positive (data not shown). These data suggest that curcumin is effective only when added in S phase but could exert its apoptogenic effect in G2 phase of cell cycle.

Curcumin Differentially Regulated Cyclin D1 Level—We have already shown that curcumin could not block mammary epithelial carcinoma cell cycle progression but arrest the same in normal mammary epithelial cells. So, we intend to test whether curcumin has any differential effects on cyclin D1 and D3 expression levels in these cells. Western blot data showed that curcumin could not alter the expression level of cyclin D1/D3 in MCF-7 cells, which showed normal cyclin D1 and D3 band intensities even during 48 h of curcumin treatment (Fig. 3A). In other breast (T47-D and MDA-MB-468) and prostate (PC-3 and LNCap) cancer cells as well as p53-knockout carcinoma cells (MDA-H0-41), curcumin could down-regulate cyclin D3 but not cyclin D1 level (Fig. 3B). On the other hand, in normal mammary epithelial cells, level of cyclin D1 again returned to its normal level (Fig. 3A). Interestingly, cyclin D3 started to diminish as early as 12 h of curcumin treatment and continued to be low up to 48 h in these cells. All these observations suggest that curcumin differentially regulates cyclin D1 and D3 levels in normal and malignant cells.

Curcumin Induced Apoptosis in Deregulated Cyclin D1 Over-expressed Cells—Our results demonstrated that curcumin could not down-regulate cyclin D1 expression in mammary carcinoma cells where it is over-expressed; instead it induced apoptosis in the same. These findings tempted us to hypothesize that curcumin cannot down-regulate cyclin D1 in deregulated system and thus cannot block cell cycle progression; hence these cells progress into G2 phase and undergo apoptosis. To test this hypothesis we exploited various cell lines, in which either cyclin D1 was over-expressed by genetic alterations or by natural chromosomal translocation or cyclin D1 was knocked out. In oncogenic p21ras transformed (NIH 3T3-V16ras) cells, deregulated cyclin D1 over-expression was observed (28). In these cells curcumin could not down-regulate cyclin D1 expression and hence these cells progressed into G2 phase and underwent apoptosis upon curcumin treatment (Figs. 3C and 3D). When cyclin D1 was over-expressed

point at which most of the cells were arrested in G0/G1 phases and were BrdU negative. However, there was no cell death. With longer treatment, the inhibition was released and the cells again started cycling (at 48 h). Together these data indicate that curcumin did not alter the cell cycle progression of mammary epithelial carcinoma cells although it induced apoptosis in the same. In contrast, it reversibly blocked normal mammary epithelial cell cycle progression without apoptosis.
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Curcumin selectively inhibited activities of Cdk4 and Cdk6 and blocked phosphorylation of retinoblastoma protein in normal cells. The activities of cyclin-dependent kinases (Cdk4 and Cdk6) are regulated by their association with the catalytic partner cyclin D1, which hyper-phosphorylates Rb protein (pRb), required to release transcription factor E2F from its inhibitory component, i.e., hypo-phosphorylated pRb. To test whether curcumin could interfere with the association of cyclin D1 with Cdk4 and Cdk6, we co-immunoprecipitated Cdk4 and Cdk6 with anti-cyclin D1 antibody from untreated or curcumin-treated cells. Immunopurified proteins were then Western blotted with either anti-Cdk4 or anti-Cdk6 antibody. Figure 4A shows that in carcinoma cells there was no change in Cdk4 or Cdk6 association with cyclin D1 as was observed from the chemiluminescence intensities of the immunoprecipitates after curcumin treatment even up to 48 h. However, in case of normal cells, since cyclin D1 was down regulated by curcumin, the amount of Cdk4 and Cdk6 as co-immunoprecipitated with cyclin D1 was reduced thereby indicating decrease in active complex formation. After 24 hours, the amount of the complex formation regained to the normal level (Fig. 4A).

In MCF-7 cells, levels of phosphorylated pRb are high. Curcumin treatment could not alter the phosphorylation status of pRb as was evident from the Western blot analysis of untreated and curcumin-treated cell lysate with phospho-specific antibody (Ser-780-Rb; Santa Cruz). However, in NME cells curcumin treatment inhibited phosphorylation of Rb protein (Fig. 4B). We also examined the effect of curcumin on Cdk4-mediated in vitro Rb phosphorylation using GST-Rb as substrates. As shown in Figure 4C, curcumin was unable to inhibit Rb phosphorylation in MCF-7 cells but was able to do the same in normal cells though the amount of Cdk4 remained same in untreated or treated cells. All these findings indicate that curcumin down-regulated cyclin D1 thereby decreasing the amount of active complex formation with Cdk4/Cdk6 in normal cells. As a result, phosphorylation of Rb protein was inhibited in these cells in a reversible manner but had no effect on carcinoma cells.

Curcumin up-regulated p53, p21Waf-1 and released cytochrome C. Since p53 plays an important role in cell cycle arrest and/or apoptosis; we employed time-lapse video-micrography and quantitative immunofluorescence technique to find out cell cycle expression characteristics of p53 in curcumin-treated cells. Cells were separated into cell cycle phases on the basis of BrdU incorporation and DNA content, and the average p53 content in each cell cycle phase was determined. In control MCF-7 cells p53 expression was low throughout the cell cycle, and upon curcumin treatment the expression increased dramatically only in G2 phase of cell cycle (Figs. 5A, 5B and 5J). On the other hand, curcumin could increase p53 expression to a lower extent throughout the cell cycle in normal cells (Figs. 5C, 5D and 5J). In the second approach, cells were synchronized in S phase with a single overnight treatment with thymidine. At various times following thymidine removal, cells were lysed for Western blot analysis of p53 expression level at different phases of cell cycle. Our quantitative immunofluorescence findings were reconfirmed with Western blot findings viz. curcumin in deed dramatically increased p53 expression at G2 phase of MCF-7 cell (Figs. 5K and 5L). On the other hand, in normal mammary epithelial cells, levels of p53 increased to a lower extent upon curcumin treatment. Interestingly, when normal cells were synchronized in S phase and were treated with curcumin immediately after the completion of DNA synthesis, it was found that curcumin failed to induce apoptosis in this cells, thereby indicating that these cells were refractory to curcumin-induced apoptosis at any phase of the cell cycle (Fig. 5L; lower panels). On the other hand, curcumin could induce apoptosis at...
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G2 phase-synchronized carcinoma cells (Fig. 5L; upper panels).

Among the normal cell cycle division inhibitors, p21Waf-1 belongs to a category of broad-specificity inhibitors of cyclin/Cdk complexes, whose level increases following anti-mitogenic signals or DNA damage (3). Our Western blot data revealed that in breast (MCF-7 and T47-D) and prostate (LNCap) cancer cells, p21Waf-1 level increased moderately upon curcumin treatment; however, in NME cells the levels increased dramatically (Fig. 5M). Interestingly, in MDA-H0-41 cells where p53 is absent, curcumin could not alter p21Waf-1 level. Since cytochrome c has been shown to release from mitochondria during chemotherapy-induced apoptosis, we next tested whether curcumin could release cytochrome c from mitochondria to cytosol or not. In control MCF-7 cells a typical mitochondrial pattern was obtained with immunofluorescence analysis of cytochrome c (Fig. 5E). In cells exposed to curcumin, translocation of cytochrome c from mitochondria to cytosol was observed (Fig. 5F). Interestingly, curcumin could not release cytochrome c from mitochondria in NME cells (Fig. 5G vs. 5H). The immunofluorescence results were reconfirmed with our indirect Western blot data, which showed that curcumin released cytochrome c in cytosol only in MCF-7 cells where p53 was over-expressed. Similar observations were found in cases of T47-D and LNCap cells, but the cells that lack p53 protein (MDA-H0-41) or expressed low p53 (NME) failed to release cytochrome c in cytosol (Fig. 5M) and hence to induce apoptosis (Fig. 5L) in response to curcumin. These results indicate that curcumin induces carcinoma cell apoptosis in G2 phase in a p53-mediated pathway where cytochrome c release plays an important role.

Curcumin Induced Apoptosis in p53-Dependent Manner—The results showing that curcumin (i) up-regulated p53 expression in G2 phase of cell cycle, (ii) released cytochrome c and (iii) induced apoptosis in the same phase, tempted us to carefully analyze the effect of curcumin in various cell lines expressing either wild-type or mutated p53. In wild-type p53-expressing cell lines, i.e., in MCF-7, T47-D and LNCap cells, curcumin asserted apoptotic insult in ~30-40% cells (Fig. 6). On the other hand, in p53-mutated breast cancer (MDA-MB-231), p53 knockout skin fibroblast (MDA-H0-41) or prostate cancer (PC-3) cells, curcumin induced apoptosis in only 12-18% cells. In contrast, when p53-knockout (MDA-H0-41 or PC-3) cells were transfected with highly expressing wild-type p53 gene, the cells became more susceptible to curcumin-induced apoptosis, i.e., from 12-15% in vector-transfected cells to 60-70% in p53-over-expressed cells. When p53 expression was regulated to a lower extent through tetracycline-regulated promoter (in the presence of tetracycline), curcumin could induce apoptosis in ~30% cells. Interestingly, when wild-type p53-expressing MCF-7 and T47-D cells were transfected with dominant-negative p53 gene, curcumin could only induce 18-22% apoptosis in comparison to 37-42% in vector-transfected cells. In all these cells p53 expression is well correlated with the percent of apoptotic cells (Fig. 6). All these results indicate that curcumin induced cancer cell apoptosis in p53-dependent manner although a basal p53-independent apoptosis was also there.

DISCUSSIONS

In neoplastic or pre-neoplastic cells, inhibition of proliferation and induction of apoptosis can be regarded as a therapeutic function aimed at eliminating damaged cells. On the basis of discoveries in signal transduction and cell cycle regulation, novel mechanism-based therapeutics have been developed (35). Although these cell cycle modulators are designed to target cancer cells, some of these can also be applied for a different purpose, i.e., to protect normal cells against the lethality of chemotherapy (36). In asynchronous cultures, with time-lapse video-micrography in combination with quantitative fluorescence microscopy we could demonstrate that curcumin, the yellow pigment of curry powder, induced apoptosis in mammary epithelial carcinoma cells at G2 phase of cell cycle. In contrast, it reversibly arrested normal mammary epithelial cells at G0 phases, which, therefore, could not enter into G2 phase and thus escaped from curcumin-induced apoptosis. These findings tempted us to search in detail the underlying mechanisms of such differential effects of curcumin in normal and malignant cells.

When mutations alter the interaction between the various cellular mechanisms required to control cell proliferation, serious problems can result for the cell. There is abundant evidence, for example, that if the system, which regulates cell division, is disconnected from the one that senses growth promoting conditions, the cell can be induced to divide in an inappropriate environment. This can result in cell death (7,8). Clearly, the cell normally has careful controls to ensure that cell
division induced by cyclin D1 never occurs without the protective action of mitogenic signaling; in deed, it is p21ras that normally induces cyclin D1 expression (29). In cancer cells, however, mutations commonly induce abnormally high levels of cyclin D1 expression. This raises the possibility that cancer cell division can be induced without the normally required growth promoting conditions. So, forced expression of cyclin D1 is reported to induce apoptosis (8,37), or sensitizes to DNA damage (38). Deregulated over-expression of cyclin D1 is frequently detected in primary breast cancers, while in normal tissues its expression is tightly regulated by mitogenic signaling (6,8). Consistent with the oncogenic role of cyclin D1 is the observation that transgenic mice engineered to over-express this cyclin in their breast tissue are prone to mammary adenocarcinomas (39).

We also found high cyclin D1 expression in mammary carcinoma cells. Curcumin treatment neither altered cyclin D1 expression level nor interfered with its association with Cdk4 or Cdk6 as well as phosphorylation of pRb in these cells. Therefore, in these cells curcumin could not block cell cycle progression although it induced apoptosis at G2 phases of cell cycle. In other cells where cyclin D1 was over-expressed by genetic alterations or by natural chromosomal translocation, curcumin could not down-regulate cyclin D1 expression and hence these cells progressed into G2 phase and underwent apoptosis. In fact, it is recognized that anti-tumor activity of various agent can be exerted through either inhibition of proliferation or induction of apoptosis. It is known that, in some cancer cells there was no change in cyclin D1 or Cdk5 level as a result of curcumin treatment, and these cells were susceptible to apoptosis (40,41), whereas, in the others, curcumin down-regulated cyclin D1 and inhibited Cdk4-mediated phosphorylation of retinoblastoma protein (27). Interestingly, with mitogenic signal-regulated cyclin D1 expressed cells (e.g., normal mammary epithelial cells), curcumin reversibly down-regulated cyclin D1 expression, thereby decreasing the levels of cyclin D1/Cdk4 and cyclin D1/Cdk6 complexes. As a result phosphorylation of pRb was inhibited leading to growth arrest of these cells. Cyclin D3 also showed similar pattern in these cell lines. In cyclin D1 knockout system, curcumin down-regulated cyclin D3 and failed to induce apoptosis. On the basis of these findings it is hypothesized that the breast cancer cells with deregulated cyclin D1 might be able to continue cell division even if the mitogenic signals were completely inhibited. It is further predicted that this deregulated cyclin D1 activity might induce cell cycle progression without normal protective influence of mitogenic signaling and thereby lead to cell death. In normal cells, on the other hand, the connection between mitogenic signaling and cyclin D1 action was unaltered. Blockage of this signaling in these cells by curcumin resulted in the blockage of cell division, and the cells remained healthy. Up to this point, our results could explain the mechanisms as to why curcumin induced cell cycle arrest but not apoptosis in normal cells. However, the mechanisms of curcumin-induced carcinoma cell death have not yet been understood.

Apart from the cell cycle regulatory factors, various oncoproteins play determining role in deciding cellular fates. As a tumor suppressor, p53 has a central role in onco genesis; it inhibits the growth of abnormal cells and thus prevents cancer development. The frequent occurrence of p53 mutations or its ablation in human cancer and its role as "guardian of the genome" have led to numerous investigations evaluating its role as a potential therapeutic target in terms of restoring wild-type p53 and thereby either reverting the malignant phenotype or enhancing drug sensitivity. An important function of p53 is to act as a trans-activator for p21Waf-1 or Bax (11,16). Up-regulation of p21Waf-1 results in the inhibition of cell cycle progression, whereas increase in Bax expression causes release of cytochrome c from mitochondria into the cytosol, which is responsible for the latter process of apoptosis (11,17).

We have already shown that in mammary carcinoma cells, curcumin induced Bax expression through a p53-dependent pathway (20). Here we report that in deregulated cyclin D1 over-expressing cells curcumin induced p53 elevation at G2 phase, which led to cytochrome c release from mitochondria to cytosol to finally activate downstream apoptotic cascade. In these cells, in spite of little increase in p21Waf-1 level, there was no cell cycle arrest, which may be due to the fact that the level of cyclin D1 was very high in these cells and remained unchanged upon curcumin treatment. Thus, the amount of p21Waf-1 as up-regulated by this yellow pigment was not sufficient to overpower cyclin D1 and stop cell cycle progression. On the other hand, in normal curcumin-treated cells, the level of p21Waf-1 increased dramatically with parallel down regulation of cyclin D1,
Curcumin induces cancer cell apoptosis at G2 phase thereby making the ratio of p21Waf-1 to cyclin D1 >1 in these cells. This might be one of the causes of cell cycle arrest without apoptosis in non-malignant cells. Considering the importance of p53 in curcumin-induced apoptosis at G2 phase of cell cycle, we further tested the possibility whether curcumin induced apoptosis in a p53-dependent manner or not. For the same, we carefully analyzed the effect of curcumin in various cells expressing wild type or mutated p53 as well as cells transfected with dominant negative p53. The cells expressing high level of wild-type p53 were more sensitive to curcumin-toxicity. On the other hand, p53-knockout as well as p53 mutated cells also showed some toxicity though the apoptotic-index was very low. It may be that p53-independent apoptotic pathways were operative in these cells. This can also well-explain the data of dominant negative p53-transfected cells, where even 100 fold more dominant negative p53-transfection could not bring down the apoptotic index to zero level.

On the basis of our data analysis, we have reasons to conclude that the effect of curcumin in inducing apoptosis in cancer cells sparing normal cells is dependent upon the differential regulation of various cell cycle regulatory proteins in these cells. As a concerted effect of all the above factors, curcumin failed to arrest carcinoma cell cycle; instead, it allowed them to pass into the G2 phase in a deregulated manner where they were finally led into the apoptotic cascade as initiated by the up-regulation of p53. On the other hand, in mitogenic signal-regulated cyclin D1 expressed normal cells, the cells were arrested at G0 phase and the cells remained healthy due to the blockage of mitogenic signaling which caused down-regulation of cyclin D1. The knowledge gathered from this study may be significant in the area of cancer therapy.

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REFERENCES

1. Sherr, C. J. (1996) *Science* **274**, 1672-1677
2. Hall, M., and Peters, G. (1996) *Adv. Cancer Res.* **68**, 67-108
3. Peter, M., and Herskowitz, I. (1994) *Cell* **79**, 181-184
4. Cheng, M., Olivier, P., Diehl, J. A., Fero, M., Roussel, M. F., Roberts, J. M., and Sherr, C. J. (1999) *EMBO J.* **18**, 1571-1583
5. Dynlacht, B. D., Moberg, K., Lees, J. A., Harlow, E., and Zhu, L. (1997) *Mol. Cell. Biol.* **17**, 3867-3875
6. Yu, Q., Geng, Y., and Sicinski, P. (2001) *Nature* **411**, 1017-1021
7. Evan, G. I., Wyllie, A. H., Gilbert, C. S., Littlewood, T. D., Land, H., Brooks, M., Waters, C. M., Penn, L. Z., and Hancock, D. C. (1992) *Cell* **69**, 119-128.
8. Sofer, L. Y., and Resnitzky, D. (1996) *Oncogene* **13**, 2431-2437.
9. Hartwell, L. H., and Kastan, M. B. (1994) *Science* **266**, 1821-1828
10. Elledge, S. J. (1996) *Science* **274**, 1664-1672
11. El-Diery, W. S., Harper, J. W., O’Connor, P. M., Velculescu, V. E., Canman, C. E., Jackman, J., Pietenpol, J. A., Burrell, M., Hill, D. E., Wang, Y., et al. (1994) *Cancer Res.* **54**, 1169-1174
12. Toyoshima, H., and Hunter, T. (1994) *Cell* **78**, 67-74
13. Sa, G., and Stacey D. W. (2004) *Exp. Cell Res.* **300**, 427-439
14. Agarwal, M. L., Taylor, W. R., Chernov, M. V., Chernov, O. B., and Stark, G. R. (1998) *J. Biol. Chem.* **273**, 1-4
15. Zambetti, G. P., and Levine, A. J. (1993) *FASEB J.* **7**, 855-865
16. Miyashita, T., and Reed, J. C. (1995) *Cell* **80**, 293-299
17. Liu, X., Kim, C. N., Yang, J., Jemmerson, R., and Wang X. (1996) *Cell* **86**, 147-157
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18. Pal, S., Choudhuri, T., Chattopadhyay, S., Bhattacharyya, A., Datta, G. K., Das, T., and Sa, G. (2001) *Biochem. Biophys. Res. Commun.* **288**, 658-665
19. Rinaldi, A. L., Morse, M. A., Fields, H. W., Rohtas, D. A., Pei, P., Rodrigo, K. A., Renner, R. J., and Mallery, S. R. (2002) *Cancer Res.* **62**, 5451-5456
20. Choudhuri, T., Pal, S., Agwarwal, M. L., Das, T., and Sa, G. (2002) *FEBS Lett.* **512**, 334-340
21. Liu, J. Y., Lin, S. J., and Lin, S. K (1993) *Carcinogenesis* **14**, 857-861
22. Kawamori, T., Lubet, R., Steele, V. E., Kelloff, G. J., Kaskey, R. B., Rao, C.V., and Reddy, B. S. (1999) *Cancer Res.* **59**, 597-601
23. Holy, J. M. (2002) *Mutat Res.* **518**, 71-84
24. Oyama, Y., Masuda, T., Nakata, M., Chikahisa, L., Yamazaki, Y., Miura, K., and Okagawa, M. (1998) *Eur. J. Pharmacol.* **360**, 65-71
25. Sikora, E., Bielak-Zmijewska, A., Piwocka, K., Skierski, J., and Radziszewska, E. (1997) *Biochem. Pharmacol.* **54**, 899-907
26. Ramchandran, C., and You, Y. (1999) *Breast Cancer Res. Treat.* **54**, 269-278.
27. Mukhopadhyay, A., Banerjee, S., Stafford, L. J., Xia, C., Liu, M., and Aggarwal, B. B. (2002) *Oncogene* **21**, 8852-8861
28. Hitomi, M., and Stacey, D. W. (1999) *Mol. Cell Biol.* **19**, 4623-4632
29. Sa, G., Hitomi, M., Harwalkar, J., Stacey, A. W., Chen, G. C., and Stacey, D. W. (2002) *Cell Cycle* **1**, 50-58
30. Fox, P. L., Sa, G., Dobrowolski, S. F., and Stacey, D. W. (1994) *Oncogene* **9**, 3519-3526
31. Sa, G., Murugesan, G., Jaye, M., Ivashchenko, Y., and Fox, P. L. (1995) *J. Biol. Chem.* **270**, 2360-2366
32. Matsushima H., Quelle D. E., Shurtleff S. A., Shibuya M., Sherr C. J., and Kato J.Y. (1994). *Mol. Cell. Biol.*, 14, 2066-2076.
33. Wang, Y. J., Pan, M. H., Cheng, A. L., Lin, L. I., Ho, Y. S., Hsieh, C. Y., and Lin, J. K. (1997) *J. Pharma. Biomed. Anal.* **15**, 1867-1876.
34. Taya, Y. (1997) *Trends Biochem. Sci.* **22**, 14-17
35. Senderowicz, A. M., and Sausville, E. A. (2000) *J. Natl. Cancer Inst.* **92**, 376–387
36. Blagosklonny, M. V., and Pardee, A. B. (2001) *Cancer Res.* **61**, 4301–4305
37. Kranenburg, O., van der Eb, A., and Aantema, A. (1996) *EMBO J.* **15**, 46-54
38. Coco, M. J., Balkenende, A., Verschoor, T., Lallemand, F., and Michalides, R. (1999) *Cancer Res.* **59**, 1134-1140
39. Wang, T. C., Cardiff, R. D., Zukerberg, L., Lees, E., Arnold, A., and Schmidt, E. V. (1994) *Nature* **369**, 669-671.
40. Chuang, S. E., Cheng, A. L., Lin, J. K., and Kuo, M. L. (2000) *Food Chem. Toxicol.* **38**, 991-995
41. Park, M. J., Kim, E. H., Park, I. C., Lee, H. C., Woo, S. H., Lee, J.Y., Hong, Y. J., Rhee, C. H., Choi, S. H., Shim, B. S., Lee, S. H., and Hong, S. I. (2002) *Int. J. Oncol.* **21**, 379-383
FIGURE LEGENDS

Fig. 1. **Role of curcumin on mammary epithelial carcinoma as well as normal cell cycle progression.** Mammary epithelial carcinoma (MCF-7) and normal (NME) cells were treated with 10 µM curcumin for different time intervals and then pulse labeled with BrdU for 30 min before fixation. DNA was stained with DAPI and incorporated BrdU was detected with indirect immunofluorescence. The nuclear fluorescence for BrdU and DAPI staining was determined by analysis of digital images. Dot plot analyses of DNA content vs. BrdU level were displayed for (A) MCF-7 cells and (B) NME cells. (C) MCF-7 cells and (D) NME cells were separated into cell cycle phases based upon BrdU and DNA content, and percent cells in each cell cycle phase were determined. More than 400 cells were photographed for each set and the average fluorochrome intensity was determined. Values are mean ± SEM of five independent sets of experiments.

Fig. 2. **Time-lapse determination of approximate cell cycle position of curcumin-induced apoptosis.** (A) TUNEL method was employed to detect strand breaks within the DNA. In a double label system, nuclear DNA from untreated (-Cur) and 10 µM curcumin-treated (+Cur) MCF-7 cells was labeled with FITC-conjugated dUTP and PI. Cells were analyzed flow cytometrically and dot plot display of PI-fluorescence (x-axis; linear scale) versus FITC-fluorescence (y-axis; logarithmic scale) has been displayed. FITC-dUTP positive cells were regarded as apoptotic cells. (B) The diagram illustrates the cycling characteristics of four typical cells. At the beginning of the first time-lapse, these cells were at M, G1, S and G2 phases, respectively. The ages of cells at the time of curcumin addition were determined from first time-lapse movie. Soon after curcumin addition, the second time-lapse movie was made for 16 hrs to determine the position of cell cycle where curcumin induced apoptosis (dark shades). (C) Age of each cell was analyzed from a time-lapse analysis before curcumin addition. The occurrence and the time of apoptosis after curcumin addition were determined from a time-lapse analysis after addition. (D) Time-lapse video-micrography was employed to monitor curcumin-induced apoptosis of MCF-7 cells. Curcumin was added during S phase and at different time intervals a typical event was pictured (some of the relevant time points have been given here; arrow head indicates apoptotic nucleus).

Fig. 3. **Role of cyclin D1 in curcumin-induced apoptosis.** (A) MCF-7 and NME cells were treated with curcumin for different time intervals. (B) T47-D, MDA-MB-468, MDA-HO-41, LNCap and PC-3 cells were treated with 10 µM curcumin for 24 hours. Same amount of protein from cell lysates were subjected to Western blot analysis for the determination of the expression levels of cyclin D1 and D3 using specific antibody. The blots were re-probed with anti-α-actin antibody to confirm equal protein loading. (C and D) Oncogenic Ras (V16ras) transformed or control vector transformed NIH 3T3, cyclin D1 knockout mouse embryonic fibroblast (MEF-D1+/−) or cyclin D1 over-expressed MEFD1+/+, Daudi and Mantle cells were treated with curcumin for 24 hours. (C) Same amount of protein from cell lysates were subjected to Western blot analysis for the determination of the expression levels of cyclin D1 and D3 using specific antibody. (D) In parallel experiments cells were pulse labeled with BrdU at the end of experiment and were separated in cell cycle phases as described in Fig. 1. Sub-G0/G1 population was regarded as apoptotic cells. Values are mean ± SEM of five independent sets of experiments.

Fig. 4. **Role of curcumin on the association of cyclin D1 with Cdk5 and pRb-phosphorylation.** MCF-7 and NME cells were treated with curcumin for various time intervals. (A) Cyclin D1/Cdk complexes were immunopurified with anti-cyclin D1 antibody from cell lysates. The immuno-purified proteins were subjected to Western blot analysis to identify the associated cyclin D1; Cdk4 and Cdk6. Same amount of protein from cell lysate used for immunoprecipitation was confirmed by Western blot analysis of cell lysates with α-actin antibody. (B) Cell lysates were analyzed by Western blotting with anti-phospho (Thr-780)-pRb antibody. To get detectable phospho-pRb signals MCF-7 blot was exposed for 45 seconds and NME blot was exposed for 3 minutes in chemiluminescence. The blots were re-probed with anti-α-actin antibody to confirm equal protein loading. (C) In parallel experiment Cdk4 was immunopurified with specific antibody from 200 µg cell lysates and its in-gel kinase activity was determined using GST-Rb as a substrate. The same experiment was performed at least three times and values are mean ±SEM. The amount of Cdk4 immunopurified in each set was estimated by Western blotting with specific Cdk4 antibody.
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Fig. 5. Curcumin differentially up-regulates p53, p21Waf-1 expression and releases cytochrome c. Time-lapse video-micrography and immunofluorescence techniques were employed to find out cell cycle expression characteristics of p53 in (A) untreated MCF-7 cells, (B) MCF-7 cells treated with 10 µM curcumin, (C) control NME cells and (D) NME cells treated with curcumin (arrowhead: G2 phase cell). Quantitative immunofluorescence technique was employed to find out cell cycle expression characteristics of p53 in curcumin treated (I) carcinoma and (J) normal cells. Cells were separated into cell cycle phases based upon BrdU and DNA content, and the average p53 level in each cell cycle phase was determined. More than 400 cells were photographed for each set and the average fluorochrome intensity was determined. Values are mean ±SEM of three independent sets of experiments. Immunofluorescence detection of cytochrome c in (E) untreated MCF-7 cells, (F) MCF-7 cells exposed to curcumin, (G) control NME cells and (H) NME cells treated with curcumin. (K) MCF-7 and NME cells were synchronized in S phase and at various times following thymidine removal cells were lysed for Western blot analysis of p53 expression level at different phases of cell cycle in the presence or absence of curcumin. (L) MCF-7 and NME cells were synchronized at S (2h), G2/M (6h) and G0/G1 (10h) phases following thymidine removal and DNA content in each cell cycle phase was determined from DAPI-fluorescence. Curcumin was added 2h after thymidine removal and data represent a typical experiment conducted three times with similar results (sub-G0/G1 phase cells were regarded as apoptotic cells). (M) Same amount of protein from cell lysates (for p53 or p21Waf-1) or the cytosolic fractions (for cytochrome c) from untreated or curcumin-treated MCF-7, NME, MDA-H0-41, T47-D and LNCap cells were subjected to Western blot analysis for the determination of the expression levels of p53, p21Waf-1 or release of cytochrome c using specific antibody. The blots were re-probed with anti-α-actin antibody to confirm equal protein loading.

Fig. 6. Involvement of p53 in curcumin-induced apoptosis. Wild-type p53-expressing (MCF-7, T47-D and LNCap) cells; p53-mutated (MDA-MB-231) cell; p53-knockout (MDA-H0-41 and PC-3) cells; tetracycline-regulated low p53-expressing cells (TR9-7 +Tet; in the presence of tetracycline) or high p53-expressing (TR9-7 –Tet; in the absence of tetracycline and PC-3-wt-p53) cells; dominant negative p53-transfected (MCF-7-dn-p53 and T47-D-dn-p53) cells were incubated with or without 10 µM curcumin for 24 hours. TUNEL method was used to determine percent of apoptotic cells. Values are mean ±SEM of triplicate experiments. In parallel experiment same amount of protein from cell lysates were subjected to Western blot analysis for the determination of the expression levels of p53 using specific antibody.
Curcumin induces cancer cell apoptosis at G2 phase

Figure 1
Figure 2
Curcumin induces cancer cell apoptosis at G2 phase.

Figure 3
Curcumin induces cancer cell apoptosis at G2 phase

Figure 4
Curcumin induces cancer cell apoptosis at G2 phase

Figure 5
Curcumin induces cancer cell apoptosis at G2 phase

Figure 6
Curcumin selectively induces apoptosis in deregulated cyclin D1 expressed cells at G2 phase of cell cycle in a p53-dependent manner
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