Demethoxycurcumin Retards Cell Growth andInduces Apoptosis in Human Brain Malignant Glioma GBM 8401 Cells

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Demethoxycurcumin (DMC; a curcumin-related demethoxy compound) has been recently shown to display antioxidant and antitumor activities. It has also produced a potent chemopreventive action against cancer. In the present study, the antiproliferation effects of DMC have been investigated in human brain malignant glioma GBM 8401 cells. We have studied the mitochondrial membrane potential (MMP), DNA fragmentation, caspase activation, and NF-κB transcriptional factor activity. By these approaches, our results indicated that DMC has produced an inhibition of cell proliferation as well as the activation of apoptosis in GBM 8401 cells. Both effects were observed to increase in proportion with the dosage of DMC treatment, and the apoptosis was induced by DMC in human brain malignant glioma GBM 8401 cells via mitochondria- and caspase-dependent pathways.

1. Introduction

Turmeric (curcuminoids) is contained in curry (powder). It has been used extensively in Asian countries and also in traditional medicine [1]. Interest in this herb has grown in recent years based on its reported putative beneficial pharmacological effects, which include antioxidant, anti-inflammatory [2, 3], and cancer-preventive properties [4, 5]. In addition to the actions of inhibiting cell proliferation and increasing apoptosis [6–8], other mechanisms have also been proposed to rationalize the anticarcinogenic effect of curcuminoids (curcumin and its related demethoxy compounds: demethoxycurcumin (MW: 338.35 g/mol, Figure 1(a)) and bisdemethoxycurcumin (bDMC)), such as the anti-inflammatory and antioxidant activities, the induction of phase-II detoxification enzymes, the inhibition of cyclooxygenase 2 (COX-2), the effect on AP-1 and NFXB transcription factors, the inhibition of matrix metalloproteinase (MMP) [9, 10], and the effect on protein kinases, others [11, 12].

A report showed that brain cancer is the eighteenth most common malignancy cancer in Taiwan [13]. However, the therapy for brain cancer is still not clear. One of the best strategies for tumor suppression is to induce the apoptosis pathway (caspase-dependent and -independent) in cancer cells [14]. The caspase-dependent pathway involves the death receptor and then promotes the activations of caspase 3, 8, and 9 [15]. The caspase-independent pathway is involved in mitochondria damage [16]. Many studies have demonstrated that chemotherapy drugs synthesized from plants repress tumor growth through the induction of apoptosis [17, 18]. Furthermore, other studies have linked chemoprevention and chemotherapy to the prevention of the downregulation of gap junctional intercellular communication by tumor
2. Materials and Methods

2.1. Cells Culture. The human brain malignant glioma GBM 8401 cells were purchased from the Food Industry Research and Development Institute (Hsinchu, Taiwan). These were maintained on culture dishes, in RPMI 1640 supplemented with 10% (v/v) FBS. The cells were cultured in an atmosphere containing 5% CO₂ at 37°C incubator.

2.2. Cell Proliferation Assay. The cells were seeded into 96-well culture plates at 5000 cells/well. Different cell wells were treated with 0, 12.5, 25, 50, and 100 μM DMC, respectively, for 1 or 2 days. MTT dye (1 mg/mL) was added to each well for the extra 4 hours after treatment. The reaction was stopped by the addition of DMSO, and optical density was measured at 540 nm on a multiwell plate reader (Powerwave XS, Biotek). Background absorbance of the medium in the absence of cells was subtracted. All samples were assayed in triplicate, and the mean for each experiment was calculated. Results were expressed as a percentage of control, which was considered as 100%. Each assay was carried out in triplicate.

We expect that all of these experiments could provide a scientific basis and technological support for brain glioblastoma therapy.
and the results were expressed as the mean (±SEM). The change in cell morphology was determined microscopically by Olympus CKX41.

2.3. Mitochondrial Membrane Potential (MMP). The GBM 8401 cells were first seeded in 24- or 6-well plates (Orange Scientific, E.U.). Following the treatment with DMC for 4 hours, Rhodamine 123 (10 μg/mL, Sigma-Aldrich, St. Louis, MO, USA) and JC-1 (25 μM) were added to the culture medium (500 μL/well) and then incubated (37°C, 20 min) for mitochondria staining. After washing twice with a warm PBS, the cells were fixed with 2% paraformaldehyde, inspected by a fluorescence microscopy (Olympus CKX41 and U-RFLT 50), and the RFU (relative fluorescence unit) was detected by the BioTek FLx800 TBI. For Rhodamine 123, the wavelength settings were 504 nm and 534 nm. Each assay was carried out in triplicate, and the results were expressed as the mean (±SEM) of RFU and reported as the percentage of the RFU for the control group (DMC 0 μM). For JC-1, the quantification by flow cytometry (BD FACS calibur, BD, USA) and mitochondria containing red JC-1 aggregates in healthy cells were detectable in the FL2 channel, and green JC-1 monomers in apoptotic cells were detectable in the FL1 channel.

2.4. Cell Cycle Analysis. The method for cell cycle analysis used propidium iodide (PI), that is, using the fluorescent nucleic acid dye PI to identify the proportion of cells that are in one of the three interphase stages of the cell cycle. The cells were treated with 0, 12.5, 25, and 50 μM DMC for 24 hours, then harvested and fixed in 1 mL cold 70% ethanol for at least 8 hours at −20°C. DNA was stained in PI/RNaseA solution, and the cell cycle (at least 10,000 single cells) was detected by flow cytometry (FACSCalibur, BD, USA). Data was analyzed by WinMDI 2.8 free software (BD, USA).

2.5. Measurement of Apoptosis. The GBM 8401 cells were first seeded in 6-well plates (Orange Scientific, E.U.). Following the treatment with DMC for 4 hours, the cells were harvested after the incubation period and washed in cold phosphate-buffered saline (PBS). A 1X annexin-binding buffer (BD Pharmingen, BD, USA) and 100 μg/mL working solution of propidium iodide (PI) (Sigma, USA) were prepared. The washed cells were recentrifuged (the supernatant discarded) and resuspended in 1X annexin-binding buffer. Five μL of FITC annexin V (BD Pharmingen, BD, USA) and 1 μL of the 100 μg/mL PI working solution were added to each 100 μL of cell suspension, and the cells were incubated at room temperature for 15 minutes. After the incubation period, the stained cells were analyzed by flow cytometry, and the fluorescence emission measurement showed only low levels, apoptotic cells showed green fluorescence and dead cells showed both red and green fluorescence.

2.6. DNA Fragmentation Assay. The DNA fragmentation was detected by ApoAlert DNA fragmentation assay kit (Clontech, USA). The assay is based on terminal-deoxynucleotidyl-transferase-(TdT-) mediated dUTP nick-end-labeling (TUNEL). TdT catalyzes incorporation of fluorescein-dUTP at the free 3′-hydroxyl ends of fragmented DNA. The cells were treated with DMC for 16 hours, and the fluorescein-labeled DNA was detected via confocal microscopy system (CARV II, BD, USA) and flow cytometry (FACSCalibur, BD, USA); data were analyzed by WinMDI 2.8 free software (BD, USA).

2.7. Western Blot Assay. A total of 30–50 μg proteins were separated by SDS-PAGE (10–12% SDS-polyacrylamide gel electrophoresis) and transferred to PVDF membranes (Millipore, USA) in a tank blotter (in 25 mM Tris/0.192 M glycine, pH 8.3/20% methanol) at 30 voltage overnight. The membranes were blocked with blocking buffer (Odyssey, USA) overnight and incubated with anti-β-actin (Sigma-Aldrich, St. Louis, MO, USA) and anti-caspase 3 (Santa Cruz BioTechnology, USA) antibody for 1.5–2 hours. The blots were washed with Tris-HCl (pH 8.0/150 mM NaCl/0.05% Tween-20) for 3 × 10 minutes and incubated with secondary antibody (anti-rabbit or anti-mouse immunoglobulins) (IRDye Li-COR, USA) at 1/20000 dilution for 30 minutes. The antigen (β-actin and caspase 3) was then visualized by Odyssey near-infrared imaging system (Odyssey LI-COR, USA) and data analyzed by Odyssey 2.1 software.

2.8. Caspase Activity Assay. The caspase (2, 3, 8, and 9) activity was assessed by the ApoAlert Caspase assay plates (Clontech, USA). The cells were treated with DMC of 0, 12.5, 25, and 50 μM with or without caspase-specific inhibitor for 8 hours. The caspase activity was detected by ApoAlert Caspase assay plates and inspected by the BioTek FLx800 TBI reader (BioTek, USA). The plates contained the fluorogenic substrates and inhibitors specific for different caspases. These substrates were covalently linked to their respective activated caspases. The substrates were covalently linked to the fluorogenic dye 7-amino-4-methyl coumarin (AMC). Peptide-bound AMC excites in the UV range (380 nm) and emits at 460 nm. The AMC was normalized by total protein; each assay was carried out in triplicate, and the results were expressed as the mean (±SEM).

2.9. NF-κB Transcription Factor Assay. The NF-κB transcription factor was assessed by the NoShift II NF-κB transcription factor assay kit (NOvagen, USA) and confocal microscopy. The cells were treated with 0, 12.5, 25, and 50 μM DMC for 6 hours. After treatment, the cell nuclear fraction was isolated by NucBuster Protein Extraction Kit (NOvagen, USA). The NF-κB transcription factor measures light intensity by microplate luminometer (BioTek FLx800 TBI reader BioTek, USA). The relative light units (RLUs) were normalized by total protein; each assay was carried out in triplicate, and the results were expressed as the mean (±SEM). Confocal microscopy was performed as described previously. Briefly, the GBM8401 cells (2 × 106 cells) were treated with 0 and 25 μM DMC for 6 hours and were fixed on coverslips. After treatment, samples were incubated with rabbit anti-human p50 antibody (SC-8414 PE, Santa Cruz Biotechnology) for 30 minutes then washed with PBS. The cells were mounted onto microscope slides using mounting medium containing DAPI.
2.10. Statistical Analysis. All data were reported as the means (±SEM) of at least three separate experiments. A t-test or one-way ANOVA with post hoc test was employed for statistical analysis, with significant differences determined as P < 0.05.

3. Results

3.1. DMC Inhibits the Proliferation of GBM 8401 Cells. It is hypothesized that DMC could mediate the survival of human brain malignant glioma GBM 8401 cells and thus inhibit their proliferation. To explore this antitumor activity of DMC against the GBM 8401 cells, an in vitro study was initiated by treating each sample of the GBM 8401 cells to increasing doses of DMC (0, 12.5, 25, 50, and 100 μM) for 24 or 48 hours. The proliferation of these DMC-treated cancer cells was then measured by MTT method. The results summarized in Figure 1(a) indicate that the survival and proliferation of GBM 8401 cells were decreased by DMC treatment in a dose-dependent reduction manner. The IC_{50} of DMC in the GBM 8401 cancer cells was determined, respectively, to be 22.71 μM; y = 88.413e^{-0.0251x}, R^2 = 0.921 (P < 0.05 versus DMC 0 μM). Moreover, DMC was noted to induce a morphological change in the GBM 8401 cells. In Figure 1(b), microscopic examination shows that, following the exposure to DMC (25 μM) for 4 to 24 hours, the cancer cells have displayed a remarkable change in their morphology. DMC induced the death of cancer cells, which formed a suspension in the medium.

3.2. DMC Reduces the MMP in GBM 8401 Cells. To explore the possible effect of DMC on the MMP in the GBM 8401 cells, Rhodamine 123 was used to determine the MMP in the DMC-treated cancer cells. The results compared in Figure 2(a) indicate that the MMP of the GBM 8401 cells has been reduced after treatment with DMC. The results summarized in Figure 2(b) indicate that the intensity of fluorescence, as determined by the BioTek FLx800 TBI fluorescence reader, decreases as the DMC dose increases. The observations imply that the reduction of MMP in the GBM 8401 cells depends on the dosage of DMC used (P < 0.05 versus DMC 0 μM). The loss of mitochondrial membrane potential is a hallmark for apoptosis. It is an early event coinciding with caspase activation. In non-apoptotic cells, JC-1 exists as a monomer in the cytosol (green) and accumulates as aggregates in the mitochondria, which appear red. In apoptotic and necrotic cells, JC-1 exists in monomeric form and stains the cytosol green. Figure 2(c) shows typical FL-1/FL-2 dot plots for JC-1 staining GBM8401 cells with and without apoptosis. DMC-free GBM8401 cells without apoptosis had red fluorescing J-aggregates. The green fluorescing monomers shown in the lower region indicate apoptotic cell lines (DMC 12.5, 25, and 50 μM treatment). By flow cytometry assays, we observed that the cells exposed to DMC exhibited a dose-dependent decrease in JC-1 staining compared to the untreated control cells. This indicated that there was a loss of mitochondrial membrane potential in DMC-treated cells, which approached the loss of potential observed after treating the cells. As clearly observed from the figure, DMC induced significant depolarization at 25 and 50 μM DMC concentrations wherein there was a 34- and 50-fold reduction in the ratio of red-green fluorescence intensity. Taken together, all these results suggest that DMC exhibited a potent antineoplastic effect in GBM 8401 cells through loss of mitochondrial membrane potential, ultimately leading to apoptosis.

3.3. DMC Treatment Induces Accumulated Sub-G0/G1 in GBM 8401 Cells. Cell-cycle distribution of DMC-treated GBM 8401 cells was analyzed by flow cytometry, aiming to determine whether the inhibitory effect was due to apoptosis. Before being processed and analyzed, the cells were exposed to DMC for a total of 24 hours. As shown in Figure 3(a), the cells exposed to DMC showed increase in the number of cells in the sub-G0/G1 phase, as compared with that of the untreated cells. The observations could imply that the GBM 8401 cells have undergone apoptosis. We found that treatment of DMC resulted in increase of cell populations in sub-G1 (Figure 3(b)) (P < 0.05 versus DMC 0 μM) and a concomitant decrease of cell numbers at other phases (Figure 3(a)).

3.4. Induction of Apoptosis-Dependent Cell Death by DMC in GBM 8401 Cells. To further elucidate the anticancer mechanism of DMC in GBM 8401 cells, we performed apoptosis studies. After treating the cells with different doses of DMC, the percentage of apoptotic cells were assessed by Annexin V-FITC and propidium iodide staining, followed by flow cytometric analysis (Figure 3(c)). The dot plot of Annexin V-FITC fluorescence versus PI fluorescence also indicated a significant increase of the percentage of apoptotic cells that were treated by DMC. It was observed that, at concentrations of 12.5 to 50 μM DMC, there was a significant increase in the percentage of cells undergoing apoptosis.

3.5. DMC Induced DNA Fragmentation in GBM 8401 Cells. Cells undergoing apoptosis will lose part of their DNA (due to the DNA fragmentation in later apoptosis). The visibility of “sub-G1” peaks by flow cytometry might be an index of the formation of characteristic DNA ladders [30]. It is hypothesized that DMC could induce apoptosis of GBM 8401 cells via the DNA fragmentation. To explore this effect of DMC against the GBM 8401 cells, an in vitro study was initiated by treating each of the GBM 8401 cell samples with 25 μM DMC for 16 hours. After treatment, the DNA fragmentation was detected by fluorescein-labeled DNA via confocal microscopy system and flow cytometry. The DNA fragmentation is illustrated in Figure 4(a), with apoptotic cells exhibiting nuclear green fluorescence. All cells stained with propidium iodide exhibit red cytoplasmic fluorescence. The results indicated that DMC induced DNA fragmentation in GBM 8401 cells. The quantification of DNA fragmentation was measured by the fluorescence intensities by flow cytometry (Figure 4(b)), showing that DNA fragmentation levels were significantly increased in cells incubated with DMC. Taken together, the observations imply that DMC
Figure 2: Reduction of the mitochondrial membrane potential (MMP) in the GBM 8401 cells by DMC, which was determined by Rhodamine 123 and JC-1 staining and detected by fluorescence microscopy, fluorescence reader, and flow cytometry: (a) MMP is shown to be significantly reduced in the GBM 8401 cells treated with DMC (12.5, 25, and 50 μM) by Rhodamine 123 staining, and the same effect was also demonstrated by JC-1 staining (c). (b) Using BioTek FLx800 TBI fluorescence reader, the intensity of fluorescence was determined and found to decline (presented as the percentage of the controls) as the concentration of DMC used to treat the GBM 8401 cells increased. All the data shown are the mean (± SEM) of at least three independent experiments. The symbol (*) on each group of bars denotes that difference from the treatment with 0 μM DMC is statistically significant at \( P < 0.05 \).
3.6. Apoptosis Induction by DMC in GBM 8401 Cells via Caspase 3, 8, and 9 Activation. Immunoblotting of cellular proteins from GBM 8401 cells treated with DMC showed decrease of pro-caspase-3 after DMC incubation (Figure 5(a)). Quantification of pro-caspase-3, done by measuring the relative band intensities, showed that pro-caspase-3 levels were significantly lower in cells incubated with DMC (Figure 5(b)). The results indicated that DMC induced caspase 3 activity via cleaved pro-caspase-3 and apoptosis after DMC incubation. As shown in Figure 5(c), the DMC elevated the caspase 3, 8, and 9 activities in GBM 8401 cells that had been decreased with caspase-specific inhibitors. The results summarized in Figure 5 indicate that the increased levels of caspase activity may play an important role in DMC-induced apoptosis in GBM 8401 cells.

3.7. DMC Inhibits Nuclear NF-κB Transcription Factor Activity in GBM 8401 Cells. To explore the potential role where DMC inhibits nuclear NF-κB transcription factor activity of GBM 8401 cells, the NoShift II transcription factor assay kit has been used to identify the activity of NF-κB transcription...
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**Figure 4:** Demethoxycurcumin induced DNA fragmentation in GBM 8401 cells. (a) The cells were treated with DMC for 16 hours. The DNA fragmentation was detected by fluorescein-labeled DNA via confocal microscopy system. The apoptotic cells exhibit nuclear green fluorescence. All cells stained with propidium iodide exhibit red cytoplasmic fluorescence. (b) Quantification of DNA fragmentation by measuring the fluorescence intensities by flow cytometry. The data showed that DNA fragmentation levels were significantly elevated in cells incubated with DMC incubation for 16 hours. All data were reported as the means (±SEM) of at least three separate experiments. Statistical analysis used t-test, with the significant differences determined at the level of *P < 0.05 versus 0 μM control group.

factor in the GBM 8401 cells after the 6 hours of exposure to DMC followed by examination with microplate luminometer. The results summarized in Figure 6(a) indicate that the NF-κB transcription factor activity of GBM 8401 cells has been repressed through increasing the dose of DMC added into the cell cultures. The results in Figure 6(b) indicate that less NF-κB subunit p50/p52 was observed in the nuclei of GBM8401 cells treated with DMC 25 μM than in the nuclei of DMC-free GBM8401 cells. The results could imply that the GBM8401 cells have had their activity of NF-κB repressed in relation to increased dosage of DMC added into the cell cultures.

4. Discussion

Dietary constituents may display promising chemopreventive and chemotherapeutic potential and thus ameliorate the side effects associated with conventional chemotherapy [4]. Recently, more attention has been focused on complementary and alternative medicine (CAM) as an alternative therapeutic modality for treatment of cancer patients. Cell cycle progression and apoptosis are two pivotal signaling mechanisms used to maintain normal condition in healthy tissues. As a dietary supplement or spicing agent, curcuminooids are used worldwide and their many uses have led to studies aimed at elucidating the mechanism of their activities, in particular the anticancer activity [5]. Most anticancer agents and DNA damaging agents arrest the cell cycle at the G0/G1 or G2/M phase and then induce cell apoptosis. Our data from flow cytometry analysis showed that the cell cycle increased in the sub-G1 phase in GBM 8401 cells incubated with DMC. These results demonstrate an inhibitory role for DMC in GBM 8401 cells, which is associated with induction of apoptosis.

The results collected in this series of studies with the cell lines of human brain malignant glioma cells have provided experimental evidence to indicate that DMC could irreversibly induce apoptosis of these cancer cells. These culminate with several phase I human trials that have shown curcuminoids to be well tolerated [31]. The most common cell death mode on curcuminoids treatment seems to be apoptosis [32]. Apoptosis can be triggered by a large variety of different stimuli. To date, two major intracellular apoptosis signaling pathways have been identified: intrinsic and extrinsic. The intrinsic pathway involves an increase of outer mitochondrial membrane permeability. The extrinsic pathway involves ligation of death (Fas) receptor, resulting in the recruitment of the adaptor protein FADD through interaction between the death domains of both molecules [33]. In both pathways, the stress-mediated apoptosis is often triggered by mitochondrial function loss. Accordingly, this functional loss in DMC-mediated apoptosis was also explored in MTT viability and MMP assay. Therefore, the apoptosis induced by DMC was considered to be through the intrinsic pathway related to mitochondrial dysfunction.

Caspases, a family of cysteinyl aspartate-specific proteases, play an essential role in the regulation and the execution of apoptotic cell death. Multiple apoptotic stimuli trigger the activation of proteases called caspases; caspases are constitutively expressed in almost all cell types as inactive zymogens that are processed and activated in response to a variety of proapoptotic stimuli [34]. All caspases are produced in cells as inactive zymogens and require a proteolytic cleavage and then convert to active form during apoptosis. Caspases are typically divided into three major groups, depending on the structure of their prodomain and their function [35]. The first is inflammatory caspases (1, 4, 5, 11, 12, and 14), the second group initiator-of-apoptosis caspases...
Figure 5: Apoptosis induction by DMC in GBM 8401 cells via caspase 3, 8, and 9 activations. DMC activated pro-caspase-3 degradation in GBM 8401 cells. The cells were treated with DMC (0, 12.5, 25, and 50 μM), and then (a) Western blot analysis was performed for pro- and cleaved- caspase-3. (b) Quantification of band intensities by Li-COR near-infrared imaging system. All data were reported as the means (±SEM) of at least three separate experiments. Statistical analysis used the t-test, with the significant differences determined at the level of *P < 0.05 versus 0 μM control group. (c) The caspase 2, 3, 8, and 9 activity was analyzed by ApoAlert Caspase assay plates. The DMC induced the caspase activity of GBM 8401 cells. All data were reported as the means (±SEM) of at least three separate experiments. Statistical analysis was as cited above.

Caspase 2 was initially described as a neuronally expressed caspase that was downregulated during the course of brain development [37]. Two forms of caspase 2 were found—a short antiapoptotic form and a longer proapoptotic form [38]. It can be demonstrated that other caspases can replace caspase 2 as the executioner if inhibitors and facilitators of apoptosis are appropriately regulated. It is also clear that there exist many cell death paradigms where the executioner is not caspase 2, but rather caspase 3 or 7 [39]. Caspase 3 is the most extensively studied apoptotic protein and is a key effector caspase in the apoptosis pathway, amplifying the signal from initiator caspases (such as caspase 8) and signifying full commitment to cellular disassembly [40]. Caspase 8 is a key enzyme in the apoptosis pathway. Caspase 8 contains two N-terminal region death effector domains which are removed to activate the enzyme along with cleavage into the two subunits [41]. These subunits then form the active protease which is capable of cleaving caspase 3, 6, and 7, which correspondingly initiate the death cascade and finally induce apoptosis. Therefore, the DMC-induced apoptosis was mediated through activation of caspase cascade (3, 8, and 9).
Many studies have led to the discovery of two major apoptotic nucleases, termed DNA fragmentation factor (DFF) [42] or caspase-activated DNase (CAD) [43] and endonuclease G. Both endonucleases attack chromatin to yield 3-hydroxyl and 5-phosphate termini, first creating 50 to 300 kb cleavage products and then oligonucleosomal fragmentation, but these nucleases show different cellular locations and are regulated in fundamentally different ways. Although activation of the executorial caspases seems to be indispensable for realization of the apoptotic program, several forms of cell demise have been shown to be caspase-independent or even accelerated by caspase inhibitors [44]. The observations of this study have implied that DMC has significantly induced the DNA fragmentation of GBM 8401 cells.

Nuclear factor-κB (NF-κB) plays an important role in cell proliferation and apoptosis by regulating the expression of genes involved in these processes [45]. Active NF-κB is most commonly composed of the heterodimer DNA-binding subunits p50 and p65. It has recently been shown that inactivation of p65 subunit of NF-κB leads to the death through apoptosis of liver cells [46]. Similarly, it has been shown in a wide range of cells that when NF-κB has been inactivated by Ik-Bα, cells were more sensitive to TNF-α—induced apoptosis. Evidence exists for NF-κB playing both anti- and proapoptosis roles [47]. The release of NF-κB facilitates its translocation to the nucleus, where it promotes cell survival by initiating transcription of genes encoding stress-response enzymes, cell-adhesion molecules, proinflammatory cytokines, and antiapoptotic proteins [48]. The reducing levels of NF-κB may be involved in curcuminoid-induced apoptosis of GBM 8401 cells.

Curcuminoids, well-established dietary antioxidants, are a safe natural food coloring additive with lipid-lowering potency in vivo [49, 50] and anticarcinogenic [51, 52], hepatoprotective [53], and neuroprotective properties [54], against heavy-metal-induced neurotoxicity and Alzheimer’s disease [55, 56]. Curcuminoids have shown a variety of biological activities for various human diseases in preclinical setting. Thier poor oral bioavailability poses significant pharmacological barriers to thier clinical application. Liposomal [57] or nanoemulsion curcuminoids (NECs) [58] may conducted the pharmacokinetics of curcuminoids in vivo [59]. Purkayastha et al. show that a soluble formulation of curcuminoids crosses the blood-brain barrier but does not suppress normal brain cell viability. Furthermore, tail vein injection, or more effectively intracerebral injection through a cannula, blocks brain tumor formation in mice [60] or has neuroprotective effect on focal cerebral ischemic rats [61].

Taken together, in this work, the bioactivities of DMC were studied. In particular, it could effectively induce the apoptosis and cell cycle sub-G1 and cell cycle arrest of GBM 8401 cells. The apoptosis induction was revealed to be through activation of the caspase cascade. These results indicate that DMC is deserving further study as a potential chemotherapeutic drug.

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**References**

[1] S. K. Sandur, M. K. Pandey, B. Sung et al., “Curcumin, demethoxycurcumin, bisdemethoxycurcumin, tetrahydrocurcumin and turmerones differentially regulate anti-inflammatory and anti-proliferative responses through a ROS-independent mechanism,” *Carcinogenesis*, vol. 28, no. 8, pp. 1765–1773, 2007.

[2] S. Perkins, R. D. Verschoyle, K. Hill et al., “Chemopreventive efficacy and pharmacokinetics of curcumin in the min/+ mouse, a model of familial adenomatous polyposis,” *Cancer Epidemiology Biomarkers and Prevention*, vol. 11, no. 6, pp. 535–540, 2002.
[3] R. L. Eckert, J. F. Crish, T. Efimova, and S. Balasubramanian, "Antioxidants regulate normal human keratinocyte differentiation," Biochemical Pharmacology, vol. 68, no. 6, pp. 1125–1131, 2004.

[4] T. O. Khor, Y. S. Keum, W. Lin et al., "Combined inhibitory effects of curcumin and phenethyl isothiocyanate on the growth of human PC-3 prostate xenografts in immunodeficient mice," Cancer Research, vol. 66, no. 2, pp. 613–621, 2006.

[5] A. Ray, "Cancer preventive role of selected dietary factors," Indian Journal of Cancer, vol. 42, no. 1, pp. 15–24, 2005.

[6] B. B. Aggarwal, C. Sundaram, N. Malani, and H. Ichikawa, "Curcumin: the Indian solid gold," Advances in Experimental Medicine and Biology, vol. 595, pp. 1–75, 2007.

[7] Y. C. Hsu, H. C. Weng, S. Lin, and Y. W. Chien, "Curcuminoids—cellular uptake by human primary colon cancer cells as quantitated by a sensitive Hplc assay and its relation with the inhibition of proliferation and apoptosis," Journal of Agricultural and Food Chemistry, vol. 55, no. 20, pp. 8213–8222, 2007.

[8] Y. T. Lin, L. F. Wang, and Y. C. Hsu, "Curcuminoids suppress the growth of pharynx and nasopharyngeal carcinoma cells through induced apoptosis," Journal of Agricultural and Food Chemistry, vol. 57, no. 9, pp. 3765–3770, 2009.

[9] R. L. Thangapazham, A. Sharma, and R. K. Maheshwari, "Beneficial role of curcumin in skin diseases," Advances in Experimental Medicine and Biology, vol. 595, pp. 343–357, 2007.

[10] J. Jiang, W. Wang, Y. J. Sun, M. Hu, F. Li, and D. Y. Zhu, "Neuroprotective effect of curcumin on focal cerebral ischemic rats by preventing blood-brain barrier damage," European Journal of Pharmacology, vol. 561, no. 1–3, pp. 54–62, 2007.

[11] S. Shishodia, T. Singh, and M. M. Chaturvedi, "Modulation of transcription factors by curcumin," Advances in Experimental Medicine and Biology, vol. 595, pp. 127–148, 2007.

[12] S. K. Sandur, H. Ichikawa, M. K. Pandey et al., "Role of pro-oxidants and antioxidants in the anti-inflammatory and apoptotic effects of curcumin (diferuloylmethane)," Free Radical Biology and Medicine, vol. 43, no. 4, pp. 568–580, 2007.

[13] C. C. Lin, J. T. Chen, J. S. Yang et al., "Danthron inhibits the migration and invasion of human brain glioblastoma multiforme cells through the inhibition of mRNA expression of focal adhesion kinase, rho kinases-1 and metalloproteinase-9," Oncology Reports, vol. 22, no. 5, pp. 1033–1037, 2009.

[14] T. Y. Huang, T. H. Tsai, C. W. Hsu, and Y. C. Hsu, "Curcuminoids suppress the growth and induce apoptosis through caspase-3-dependent pathways in glioblastoma multiforme (GBM) 8401 cells," Journal of Agricultural and Food Chemistry, vol. 58, no. 19, pp. 10639–10645, 2010.

[15] L. A. Pradelli, M. Bénetteau, and J. E. Ricci, "Mitochondrial control of caspase-dependent and-independent cell death," Cellular and Molecular Life Sciences, vol. 67, no. 10, pp. 1589–1597, 2010.

[16] K. Schrader, J. Huai, L. Jöckel, C. Oberle, and C. Borner, "Non-caspase proteases: triggers or amplifiers of apoptosis?" Cellular and Molecular Life Sciences, vol. 67, no. 10, pp. 1607–1618, 2010.

[17] A. Rasul, B. Yu, M. Khan et al., "Magnolol, a natural compound, induces apoptosis of SGC-7901 human gastric adenocarcinoma cells via the mitochondrial and PI3K/Akt signaling pathways," International Journal of Oncology, vol. 40, no. 4, pp. 1153–1161, 2012.

[18] A. Parihar, T. D. Eubank, and A. I. Doseff, "Monocytes and macrophages regulate immunity through dynamic networks of survival and cell death," Journal of Innate Immunity, vol. 2, no. 3, pp. 204–215, 2010.

[19] J. E. Trosko and R. J. Ruch, "Gap junctions as therapeutic agents," Current Drug Targets, vol. 3, pp. 465–482, 2002.

[20] J. Y. Lin, L. F. Wang, and Y. C. Hsu, "Curcuminoids suppress the growth and induce apoptosis through caspase-3-dependent pathways in glioblastoma multiforme in an MAPK-dependent manner," Anticancer Research, vol. 30, no. 12, pp. 4915–4920, 2010.

[21] C. F. Chen, J. M. Hwang, S. W. Jao, F. J. Leu, and K. Y. Chen, "Microencapsulation of tumor cells and assay for selecting anticancer drugs," Proceedings of the National Science Council, Republic of China. Part B, vol. 12, no. 4, pp. 252–261, 1988.

[22] J. M. Hwang, C. F. Chen, W. L. Hsu, and K. Y. Chen, "A new in vivo assay of the reactions of microencapsulated human tumor cells to chemotherapeutic drugs," Chinese Medical Journal, vol. 51, no. 3, pp. 166–175, 1993.

[23] H. F. Chang, W. T. Huang, H. J. Chen, and L. L. Yang, "Apoptotic effects of γ-mangostin from the fruit hull of garcinia mangostana on human malignant glioma cells," Molecules, vol. 15, no. 12, pp. 8953–8966, 2010.

[24] H. J. Harn, H. S. Lee, L. I. Ho, W. H. Lee, and J. H. Ding, "Selective expression of CD44 messenger RNA splice variants in four high grade human brain tumour cell lines," Biochemistry and Molecular Biology International, vol. 33, no. 4, pp. 743–749, 1994.

[25] H. F. Juan, J. H. Chen, W. T. Hsu et al., "Identification of tumor-associated plasma biomarkers using proteomic techniques: from mouse to human," Proteomics, vol. 4, no. 9, pp. 2766–2775, 2004.

[26] S. K. Lim, S. R. A. Llaguno, R. M. McKay, and L. F. Parada, "Glioblastoma multiforme: a perspective on recent findings in human cancer and mouse models," BMB Reports, vol. 44, no. 3, pp. 158–164, 2011.

[27] D. Kessel and Y. Luo, "Cells in cryptophycin-induced cell-cycle arrest are susceptible to apoptosis," Cancer Letters, vol. 151, no. 1, pp. 25–29, 2000.

[28] J. J. Johnson and H. Mukhtar, "Curcumin for chemoprevention of colon cancer," Cancer Letters, vol. 255, no. 2, pp. 170–181, 2007.

[29] J. Cao, Y. Liu, L. Jia et al., "Curcumin induces apoptosis through mitochondrial hyperpolarization and mtDNA damage in human hepatoma G2 cells," Free Radical Biology and Medicine, vol. 43, no. 6, pp. 968–975, 2007.

[30] P. Limtrakul, "Curcumin as a chemosensitizer," Advances in Experimental Medicine and Biology, vol. 595, pp. 269–300, 2007.

[31] C. Park, D. O. Moon, I. W. Choi et al., "Curcumin induces apoptosis and inhibits prostaglandin E2 production in synovial fibroblasts of patients with rheumatoid arthritis," International Journal of Molecular Medicine, vol. 20, no. 3, pp. 365–372, 2007.

[32] D. Moquin and F. K. M. Chan, "The molecular regulation of programmed necrotic cell injury," Trends in Biochemical Sciences, vol. 35, no. 8, pp. 434–441, 2010.
[36] S. Orrenius, P. Nicotera, and B. Zhivotovsky, “Cell death mechanisms and their implications in toxicology,” *Toxicological Sciences*, vol. 119, no. 1, pp. 3–19, 2011.

[37] S. Kumar, M. Kinoshita, M. Noda, N. G. Copeland, and N. A. Jenkins, “Induction of apoptosis by the mouse Nedd2 gene, which encodes a protein similar to the product of the Caenorhabditis elegans cell death gene ced-3 and the mammalian IL-1p-converting enzyme,” *Genes and Development*, vol. 8, no. 14, pp. 1613–1626, 1994.

[38] L. Wang, M. Miura, L. Bergeron, H. Zhu, and J. Yuan, “Ich-1, an ice/ced-3–related gene, encodes both positive and negative regulators of programmed cell death,” *Cell*, vol. 78, no. 5, pp. 739–750, 1994.

[39] C. M. Troy and M. L. Shelanski, “Caspase-2 redux,” *Cell Death and Differentiation*, vol. 10, no. 1, pp. 101–107, 2003.

[40] A. Abdi, H. Sadraie, L. Dargahi, L. Khalaj, and A. Ahmadiani, “Apoptosis inhibition can be threatening in Aβ–induced neuroinflammation, through promoting cell proliferation,” *Neurochemical Research*, vol. 36, no. 1, pp. 39–48, 2010.

[41] Y. Zhao, X. Sui, and R. Hong, “From procaspase-8 to caspase-8: revisiting structural functions of caspase-8,” *Journal of Cellular Physiology*, vol. 225, no. 2, pp. 316–320, 2010.

[42] J. S. Dempe, E. Pfieffer, A. S. Grimm, and M. Metzler, “Metabolism of curcumin and induction of mitotic catastrophe in human cancer cells,” *Molecular Nutrition and Food Research*, vol. 52, no. 9, pp. 1074–1081, 2008.

[43] E. Sikora, A. Bielak-Zmijewska, A. Magalska et al., “Curcumin induces caspase-3–dependent apoptotic pathway but inhibits DNA fragmentation factor 40/caspase-activated DNase expression in human Jurkat cells,” *Molecular Cancer Therapeutics*, vol. 5, no. 4, pp. 927–934, 2006.

[44] A. Magalska, A. Brzezinska, A. Bielak-Zmijewska, K. Piwocka, G. Mosieniak, and E. Sikora, “Curcumin induces cell death without oligonucleosomal DNA fragmentation in quiescent and proliferating human CD8+ cells,” *Acta Biochimica Polonica*, vol. 53, no. 3, pp. 531–538, 2006.

[45] G. Kuttan, K. B. Hari Kumar, C. Guruvayoorappan, and R. Kuttan, “Antitumor, anti-invasion, and antimetastatic effects of curcumin,” *Advances in Experimental Medicine and Biology*, vol. 595, pp. 173–184, 2007.

[46] A. B. Kunnunakkara, S. Guha, S. Krishnan, P. Diagaradjane, J. Gelovani, and B. B. Aggarwal, “Curcumin potentiates antitumor activity of gemcitabine in an orthotopic model of pancreatic cancer through suppression of proliferation, angiogenesis, and inhibition of nuclear factor-κB–regulated gene products,” *Cancer Research*, vol. 67, no. 8, pp. 3853–3861, 2007.

[47] H. Yoon and H. L. Rui, “Effect of selected phytochemicals and apple extracts on NF-κB activation in human breast cancer MCF-7 Cells,” *Journal of Agricultural and Food Chemistry*, vol. 55, no. 8, pp. 3167–3173, 2007.

[48] M. Breccia and G. Alimena, “NF-κB as a potential therapeutic target in myelodysplastic syndromes and acute myeloid leukemia,” *Expert Opinion on Therapeutic Targets*, vol. 14, no. 11, pp. 1157–1176, 2010.

[49] A. Asai and T. Mayazawa, “Dietary curcuminoids prevent high-fat diet–induced lipid accumulation in rat liver and epididymal adipose tissue,” *Journal of Nutrition*, vol. 131, no. 11, pp. 2932–2935, 2001.

[50] J. L. Quiles, M. D. Mesa, C. L. Ramirez-Tortosa et al., “Curcuma longa extract supplementation reduces oxidative stress and attenuates aortic fatty streak development in rabbits,” *Atherosclerosis, Thrombosis, and Vascular Biology*, vol. 22, no. 7, pp. 1225–1231, 2002.

[51] Y. Zeng, F. Qiu, Y. Liu, G. Qu, and X. Yao, “Isolation and identification of phase I metabolites of demethoxycurcumin in rats,” *Drug Metabolism and Disposition*, vol. 35, no. 9, pp. 1564–1573, 2007.

[52] Shishu, A. K. Singla, and I. P. Kaur, “Inhibitory effect of curcumin and its natural analogues on genotoxicity of heterocyclic amines from cooked food,” *Indian Journal of Experimental Biology*, vol. 40, no. 4, pp. 1365–1372, 2002.

[53] E. K. Song, H. Cho, J. S. Kim et al., “Diarylheptanoids with free radical scavenging and hepatoprotective activity in vitro from Curcuma longa,” *Planta Medica*, vol. 67, no. 9, pp. 876–877, 2001.

[54] A. Dairam, J. L. Limson, G. M. Watkins, E. Antunes, and S. Daya, “Curcuminoids, curcumin, and demethoxycurcumin reduce lead-induced memory deficits in male wistar rats,” *Journal of Agricultural and Food Chemistry*, vol. 55, no. 3, pp. 1039–1044, 2007.

[55] T. Ahmed, S. A. Enam, and A. H. Gilani, “Curcuminoids enhance memory in an amyloid-infused rat model of Alzheimer’s disease,” *Neuroscience*, vol. 169, no. 3, pp. 1296–1306, 2010.

[56] T. Ahmed and A.–H. Gilani, “A comparative study of curcuminoids to measure their effect on inflammatory and apoptotic gene expression in an Aβ plus ibotenic acid-infused rat model of Alzheimer’s disease,” *Brain Research*, vol. 1400, pp. 1–18, 2011.

[57] M.-J. Chen, Y.-Y. Chu, P.-H. Lai, Y.-M. Cheng, and Y.-C. Hsu, “Experimental results of colorectal cancer chemoprevention by curcuminoids loaded nano-carrier drug delivery system increased in vitro biocompatibility,” *Digest Journal of Nanomaterials and Biostuctures*, vol. 6, no. 3, pp. 1187–1197, 2011.

[58] M.-J. Chen, Y.-W. Lin, Y.-M. Cheng, J.-F. Wu, and Y.-C. Hsu, “Early experimental results of colorectal carcinoma chemotherapeutics by liposomal curcuminoids,” *Digest Journal of Nanomaterials and Biostuctures*, vol. 6, no. 3, pp. 1445–1456, 2011.

[59] L. Zhongfa, M. Chiu, J. Wang et al., “Enhancement of curcumin oral absorption and pharmacokinetics of curcuminoids and curcumin metabolites in mice,” *Cancer Chemotherapy and Pharmacology*. In press.

[60] S. Purkayastha, A. Berliner, S. S. Fernando et al., “Curcumin blocks brain tumor formation,” *Brain Research*, vol. 1266, pp. 130–138, 2009.

[61] J. Zhao, S. Yu, W. Zheng et al., “Curcumin improves outcomes and attenuates focal cerebral ischemic injury via antiapoptotic mechanisms in rats,” *Neurochemical Research*, vol. 35, no. 3, pp. 174–179, 2010.