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Curcuminoids Suppress the Growth and Induce Apoptosis through Caspase-3-Dependent Pathways in Glioblastoma Multiforme (GBM) 8401 Cells

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Curcuminoids, natural plant components, have been recently shown to display antioxidant and anti-inflammatory activities. They also produce potent chemo-preventive action against several types of cancer. In the present study, the anti-proliferative and induced apoptosis effects of curcuminoids have been investigated in human brain glioblastoma multiforme (GBM) 8401 cells. Results indicated that curcuminoids have produced an inhibition of cell proliferation in a dose-dependent manner as dosage increased from 12.5 to 100 μM (n = 6) via the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay as well as activation of apoptosis in GBM 8401 cells. Both effects were observed to increase in proportion with the dose of curcuminoids. We have studied the mitochondrial membrane potential ($\Delta\Psi$m), DNA fragmentation, caspase-3, caspase-8, and caspase-9 activation, and nuclear factor κB (NF-κB) transcriptional factor activity to analyze apoptosis in GBM 8401 cells. From these approaches, apoptosis was induced by curcuminoids in human brain GBM 8401 cells via mitochondria and a caspase-dependent pathway. The results observed with proliferation inhibition ($y = 94.694e^{-0.025x}$, $R^2 = 0.9901$, and $n = 6$) and apoptosis ($y = 0.9789e^{-0.0102x}$, $R^2 = 0.99854$, and $n = 3$) depend upon the amount of curcuminoid treatment in the cancer cells.

KEYWORDS: Curcuminoids; glioblastoma multiforme; apoptosis

INTRODUCTION

Curcuminoids [constitute curcumin (≥97%) and its related demethoxy compounds, demethoxycurcumin and bisdemethoxycurcumin] (**Figure 1**) are natural products from the rhizomatous herbaceous perennial plant (*Curcuma longa*) of the ginger family (*1*). It is contained in curry powder. It has been used extensively in Asian countries and also in traditional medicine. Interest in this herb has grown in recent years based on its putative beneficial pharmacological effects, which include antioxidant, anti-inflammatory (*2*, *3*), and cancer-preventive properties (*4*, *5*). It has been discovered that curcumin is also a potent scavenger of various reactive oxygen species (ROS), including superoxide anions and hydroxyl radicals (*6*, *7*). In addition to the actions of inhibiting cell proliferation and increasing apoptosis, other mechanisms have also been proposed to rationalize the anti-carcinogenic effect of curcuminoids, such as the induction of phase-II detoxification enzymes, the inhibition of cyclooxygenase 2 (COX-2), the effect on AP-1 and nuclear factor κB (NF-κB) transcription factors, the inhibition of matrix metalloproteinase, the effect on protein kinases, and others (*8*, *9*).

One report showed that brain cancer is the 18th most common malignancy cancer in Taiwan (*10*). Glioblastoma is the most common and lethal type of primary brain tumor (*11*). Glioblastoma multiforme (GBM) or astrocytoma grade 4 on the World Health Organization (WHO) classification is the most aggressive and most frequent of all primary brain tumors (*12*). They are lethal tumors, characterized by diffuse infiltration of the brain and a high resistance to conventional cancer therapies (*13*). Human brain GBM 8401 cells were established from a Chinese female patient with brain GBM. GBM is the highest grade glioma

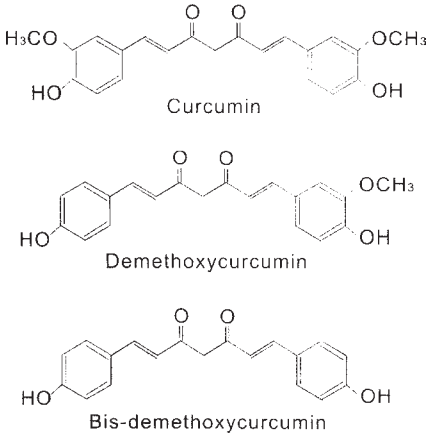

Figure 1. Curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin).

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(grade 4) tumor and is the most malignant form of astrocytomas (*14*). However, the chemotherapy for brain cancer is still unclear. One of the best strategies for tumor suppression is to induce an apoptosis pathway (caspase-dependent and -independent) in cancer cells. The caspase-dependent pathway involves the death receptor in the promotion and activation of caspase-3, caspase-8, and caspase-9 (*15*). The caspase-independent pathway is involved in mitochondria damage (*16*). Many studies have shown chemotherapy drugs derived from plants repress tumor growth through the induction of apoptosis (*17*, *18*).

In this report, studies have been initiated to investigate whether curcuminoids could contribute to the antiproliferation and apoptosis of human brain GBM 8401 cells. We expect that all of these experiments could provide a scientific basis and technological support for brain glioblastoma therapy.

MATERIALS AND METHODS

Materials. Curcuminoids [curcumin >97%; 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], dimethyl sulfoxide (DMSO), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma (St. Louis, MO). Cell culture medium (RPMI 1640), fetal bovine serum (FBS), antibiotics, sodium pyruvate, trypsin, and phosphate-buffered saline (PBS) were purchased from Gibco, BRL (Grand Island, NY). Polyvinylidene fluoride (PVDF) membrane (Millipore, MA) and molecular-weight marker (Precision Plus Protein Dual Color standards) were purchased from Bio Rad (Hercules, CA). All other reagents and compounds were analytical-grade.

Cells. The human brain GBM 8401 cells were purchased from the Bioresource Collection and Research Center (BCRC), Food Industry Research and Development Institute (Hsinchu, Taiwan). The GBM 8401 cells were established from a Chinese female patient with brain GBM in the Triservice General Hospital (Taipei, Taiwan). The cells (passage numbers of $n + 23-34$) were maintained on 100 mm tissue culture dishes (TPP, Switzerland), in RPMI 1640 supplemented with 10% (v/v) FBS. The cells were cultured in an atmosphere containing 5% CO_2 in a 37 °C incubator.

Cell Proliferation Assay. The cells were seeded into a 96-well culture plate (TPP, Switzerland) at 5000 cells/well. The cells were treated with 0, 12.5, 25, 50, and 100 μM curcuminoids for 1 or 2 days. MTT dye (1 mg/mL) was added to each well for an additional 4 h of treatment. The reaction was stopped by the addition of dimethylsulfoxide (DMSO), and optical density was measured at 540 nm on a multi-well plate reader (Powerwave XS, BioTek, Winooski, VT). 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 the control, which was considered as 100%. Each assay was carried out in triplicate, and the results were expressed as the mean [±standard error of the mean (SEM)]. The change in cell morphology was determined microscopically by Olympus CKX41 (Olympus, Tokyo, Japan).

Mitochondrial Membrane Potential ($\Delta\Psi$m). The GBM 8401 cells were first seeded in the 24-well plates (Orange Scientific, Belgium) for 4 h. After the treatment with curcuminoids, rhodamine 123 (10 μg/mL, Sigma) was added to the culture medium (50 μL/well) and then incubated (37 °C, 20 min) for mitochondria staining. After the cells were washed twice with PBS, they were fixed with 2% paraformaldehyde, inspected by fluorescence microscopy (Olympus CKX41 and U-RFLT 50, Japan), and detected for the BioTek FLx800 TBI reader (BioTek, Winooski, VT) using the relative fluorescence unit (RFU). For rhodamine 123, the wavelength settings were 504 nm (excitation) and 534 nm (emission). 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 (0 μM curcuminoids).

DNA Fragmentation Assay. The DNA fragmentation was detected by the ApoAlert DNA fragmentation assay kit (Clontech, Mountain View, CA). 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 0, 12.5, 25, and 50 μM curcuminoids for 16 h, and the fluorescein-labeled DNA was detected via a confocal microscopy system (CARV II, BD Biosciences, San Jose, CA) and flow cytometry (Facscalibur, BD Biosciences, San Jose, CA). The data were analyzed by WinMDI 2.8 free software (BD Biosciences, San Jose, CA).

Western Blot Analysis of Caspase-3. A total of 30−50 μg of protein was separated by 10−12% sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) and transferred to PVDF membranes in a tank blotter (in 25 mM Tris/0.192 M glycine at pH 8.3/20% methanol) at 30 V or 200 mA overnight. The membranes were blocked with Odyssey blocking buffer (LI-COR Biotechnology, Lincoln, NE) overnight and incubated with anti-β-actin (Sigma, St. Louis, MO) and anti-caspase-3 (Santa Cruz Biotechnology, Santa Cruz, CA) antibody for 1.5−2 h. The blots were washed with Tris-HCl (pH 8.0/150 mM NaCl/0.05% Tween-20) for 3×10 min and incubated with a second antibody (anti-rabbit or anti-mouse immunoglobulins) (IRDye, LI-COR Biotechnology, Lincoln, NE) at $^1/_{20\,000}$ dilution for 30 min. The antigen was then visualized by the Odyssey infrared imaging system (LI-COR Biotechnology, Lincoln, NE), and the data were analyzed by Odyssey 2.1 software (LI-COR Biotechnology, Lincoln, NE).

Caspase Activity Assay. Caspase-2, caspase-3, caspase-8, and caspase-9 activities were assessed by the ApoAlert caspase assay plates (Clontech, Mountain View, CA). The cells were treated with 0, 12.5, 25, and 50 μM curcuminoids with or without a caspase-specific inhibitor for 8 h. The caspase activity was detected by ApoAlert caspase assay plates and inspected by the BioTek FLx800 TBI reader (BioTek, Winooski, VT). 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. AMC was normalized by total protein, and each assay was carried out in triplicate. The results were expressed as the mean (±SEM).

NF-κB Transcription Factor Assay. The NF-κB transcription factor was assessed by the NoShift II NF-κB transcription factor assay kit (Novagen, Merck, Germany). The cells were treated with 0, 12.5, 25, and 50 μM curcuminoids for 6 h. After treatment, the cell nuclear fraction was isolated by the NucBuster protein extraction kit (Novagen, Merck, Germany). The NF-κB transcription factor was measured for light intensity by a microplate luminometer (BioTek FLx800 TBI reader, BioTek, Winooski, VT). The relative light units (RLUs) were normalized by total protein, and each assay was carried out in triplicate. The results were expressed as the mean (±SEM).

Statistical Analysis. All data were reported as the mean (±SEM) of at least three separate experiments. A *t* test or one-way analysis of variation (ANOVA) with post-hoc test was employed for statistical analysis, with significant differences determined as $p < 0.05$.

RESULTS

Curcuminoids Inhibit the Proliferation of GBM 8401 Cells. It is hypothesized that curcuminoids could mediate the survival of human brain GBM 8401 cells and thus inhibit their proliferation. To explore this anti-tumor activity of curcuminoids against the GBM 8401 cells, an *in vitro* study was initiated by treating each of the GBM 8401 cells with increasing dosages of curcuminoids (0, 12.5, 25, 50, and 100 μM) for 24 or 48 h. The proliferation of these curcuminoid-treated cancer cells was then measured by the MTT method. The results summarized in **Figure 2A** indicate that the survival and proliferation of GBM 8401 cells were decreased by curcuminoid treatment and show a dose-dependent reduction ($y = 94.694e^{-0.025x}$, $R^2 = 0.9901$, 24 h, $n = 6$ and $y = 135.89e^{-0.0584x}$, $R^2 = 0.9754$, 48 h, $n = 6$). Moreover, curcuminoids were noted to induce a morphological change in the GBM 8401 cells. A microscopic examination showed that, following the exposure to curcuminoids (25 μM) for 0−24 h, the GBM 8401 cells have displayed a remarkable change in their morphology (**Figure 2B**). The curcuminoids induced the death of cancer cells, which formed a suspension in the medium.

Curcuminoids Reduce $\Delta\Psi$m in GBM 8401 Cells. To explore the possible effect of curcuminoids on $\Delta\Psi$m in the GBM 8401 cells,

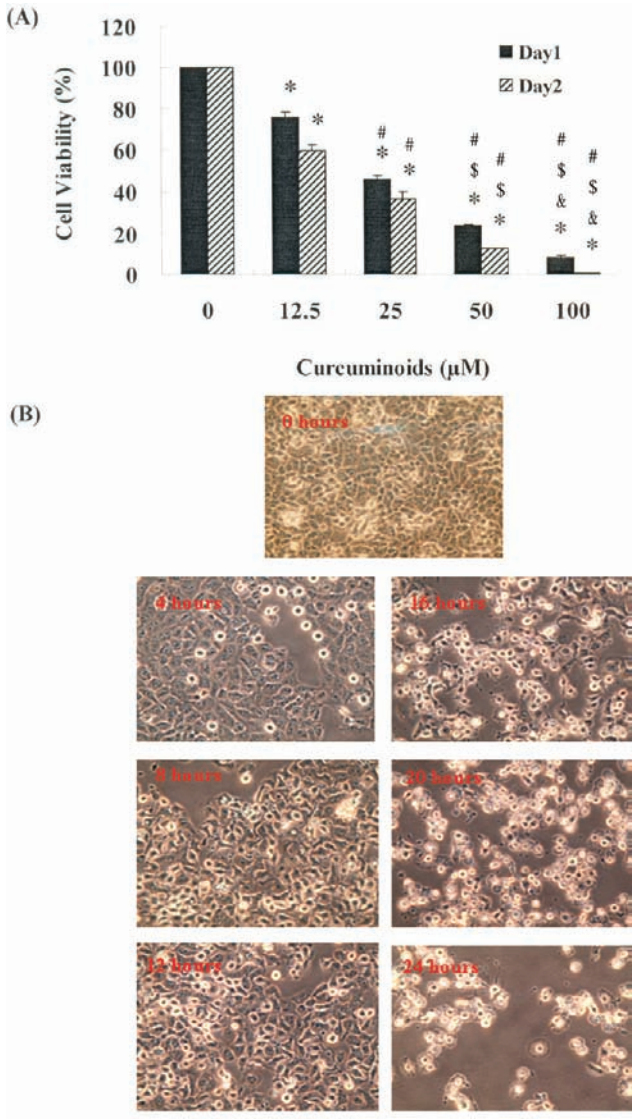

Figure 2. Curcuminoids mediate the survival of GBM 8401 cells and thus inhibit their proliferation. (A) Survival of these curcuminoid-treated (0, 12.5, 25, 50, and 100 μM) cells for 24 or 48 h were then measured by the MTT method. Results were expressed as a percentage of the control, which was considered as 100%. All data were reported as the mean (±SEM) of six separate experiments. Statistical analysis was obtained using one-way ANOVA, with the significant differences determined at the level of (∗) $p <$ 0.05 versus the control group (0 μM curcuminoids), (#) $p < 0.05$ versus 12.5 μM curcuminoids, ($) $p < 0.05$ versus 25 μM curcuminoids, and (&) $p < 0.05$ versus 50 μM curcuminoids. (B) Morphology of the human GBM 8401 cells after treatment with curcuminoids (100 μM) for 0−24 h. The cells were suspended in the medium.

rhodamine 123 was used to determine ΔΨm in the curcuminoid-treated cancer cells. ΔΨm is a key indicator of mitochondrial function, and the characterization of ΔΨm *in situ* allows for an accurate determination of mitochondrial bioenergetics and cellular metabolism and apoptosis. The results compared in **Figure 3A** indicate that ΔΨm of the GBM 8401 cells has been reduced after treatment with curcuminoids. The results summarized in **Figure 3B** indicate that the intensity of fluorescence, as determined by the BioTek FLx800 TBI fluorescence reader, decreases as the curcuminoid dosage increases. The observations imply that the reduction of ΔΨm in the GBM 8401 cells depends upon the dosage of curcuminoids used ($y = 100.24e^{-0.0129x}$, $R^2 = 0.9627$, and $n = 6$).

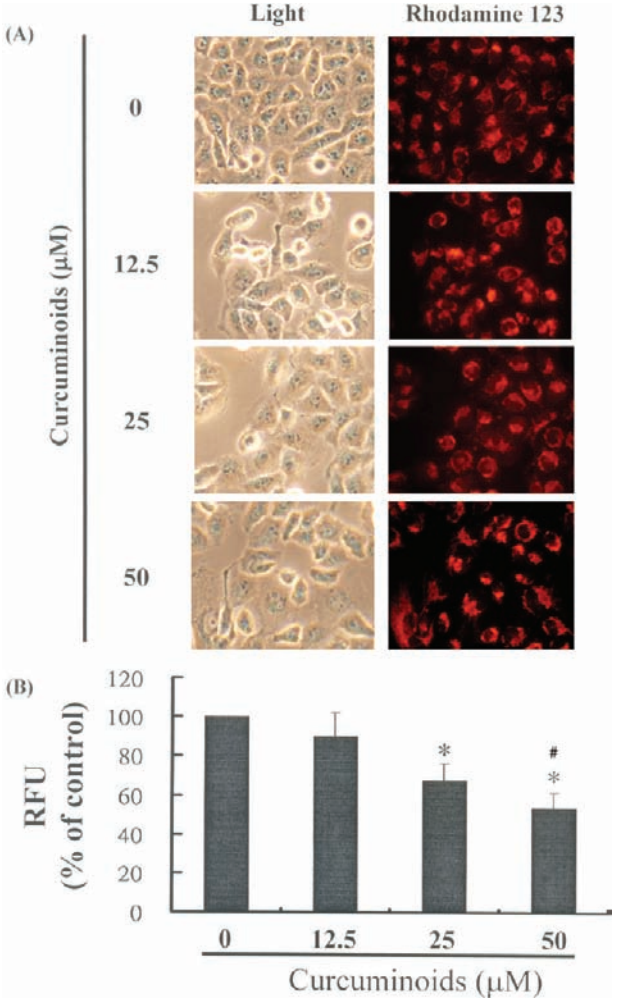

Figure 3. Reduction of ΔΨm in the GBM 8401 cells by curcuminoids, which was determined by rhodamine staining (red fluorescence). (A) ΔΨm is reduced in the cells treated with curcuminoids (0, 12.5, 25, and 50 μM). (B) Intensity of fluorescence was determined and found to decline (presented as the percentage of the controls) as the concentration of curcuminoids used to treat the cells increased. All of the data shown are the mean (±SEM) of six independent experiments. The symbols ∗ and # on each group of bars denote that a difference from the treatment with 0 and 12.5 μM curcuminoids is statistically significant at $p < 0.05$.

Curcuminoids Induced DNA Fragmentation in GBM 8401 Cells. It is hypothesized that curcuminoids could induce apoptosis of GBM 8401 cells via DNA fragmentation. To explore this effect of curcuminoids against the GBM 8401 cells, an *in vitro* study was initiated by treating each of the GBM 8401 cells with 25 μM curcuminoids for 16 h. After treatment, the DNA fragmentation was detected by fluorescein-labeled DNA via a confocal microscopy system and flow cytometry. The DNA fragmentation is illustrated in **Figure 4A**. Apoptotic cells exhibit nuclear green fluorescence. All cells stained with propidium iodide exhibit red cytoplasmic fluorescence. The results indicated that curcuminoids induced DNA fragmentation in GBM 8401 cells. The quantification of DNA fragmentation was measured by the fluorescence intensities by flow cytometry, showing that DNA fragmentation levels were significantly increased (110, 170, and 200% versus the 0 μM curcuminoid group) in cells incubated with curcuminoids (12.5, 25, and 50 μM) (**Figure 4B**). When these observations are taken together, they imply that curcuminoids have significantly induced the DNA fragmentation of GBM 8401 cells ($y = 8.5297x + 400.15$, $R^2 = 0.933$, and $n = 3$).

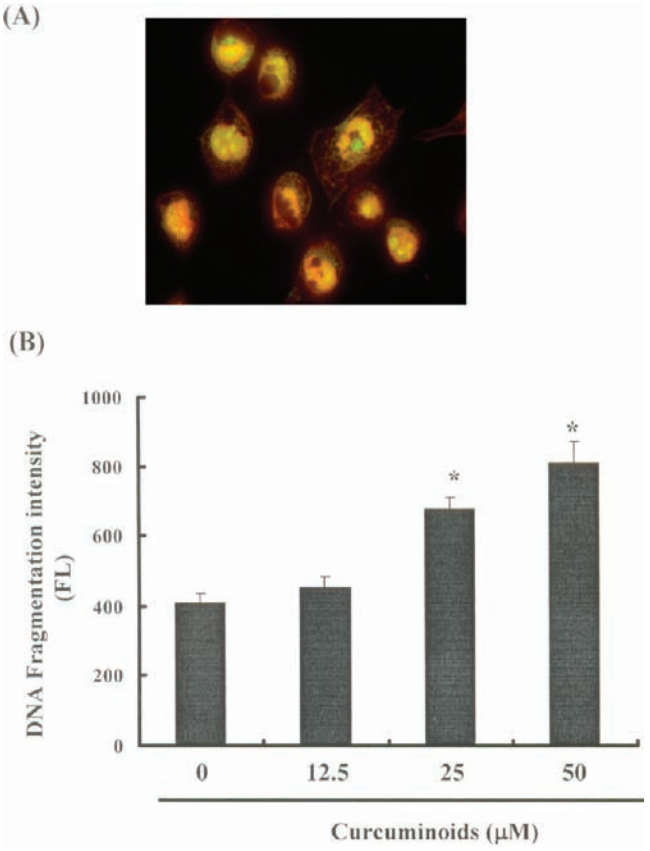

Figure 4. Curcuminoids induced DNA fragmentation in GBM 8401 cells. The cells were treated with curcuminoids for 16 h. The DNA fragmentation was detected by fluorescein-labeled DNA via a confocal microscopy system and flow cytometry. (A) Apoptotic cells exhibit nuclear green fluorescence (excitation, 490 mm; emission, 520 mm). All cells stained with propidium iodide exhibit red cytoplasmic fluorescence (excitation, 536 mm; emission, 617 mm). (B) Quantification of DNA fragmentation by measuring the fluorescence intensities. The data showed that DNA fragmentation levels were significantly elevated in cells incubated with curcuminoids incubated for 16 h. All data were reported as the mean (±SEM) of three separate experiments. Statistical analysis was obtained using the *t* test, with the significant differences determined at the level of (*) $p < 0.05$ versus the 0 μM control group.

Apoptosis Induction by Curcuminoids in GBM 8401 Cells via Caspase-3, Caspase-8, and Caspase-9 Activation. Immunoblotting of cellular proteins from GBM 8401 cells treated with curcuminoids showed a decrease of pro-caspase-3 after curcuminoid incubation (**Figure 5A**). Quantification of pro-caspase-3, performed by measuring the relative band intensities, showed that pro-caspase-3 levels were significantly lower in cells incubated with curcuminoids (**Figure 5B**). The results indicated that curcuminoids induced caspase-3 activity via cleaved pro-caspase-3 and apoptosis after curcuminoid incubation. As per the data shown in **Figure 5C**, the curcuminoid-enhanced caspase-3, caspase-8, and caspase-9 activities in GBM 8401 cells have been decreased with caspase-specific inhibitors. The results summarized in **Figure 5** indicate that the increased levels of caspase-3, caspase-8, and caspase-9 activities may play an important role in curcuminoid-induced apoptosis in GBM 8401 cells.

Curcuminoids Inhibit NF-κB Transcription Factor Activity in GBM 8401 Cells. To explore the potential role that curcuminoids play in the inhibition of NF-κB transcription factor activity of GBM 8401 cells, the NoShift II transcription factor assay kit has been used to identify the activity of the NF-κB transcription factor in the GBM 8401 cells after the 6 h of exposure to curcuminoids, which was then examined by a microplate luminometer. The results summarized in **Figure 6** indicate that the NF-κB transcription factor activity of GBM 8401 cells has been repressed as the dose of curcuminoids added into the cell cultures has increased ($y = 1570.6e^{-0.0177x}$, $R^2 = 0.9211$, and $n = 4$).

DISCUSSION

Because most populations worldwide use curcuminoids (or curcumin), its many uses have led to studies aimed at elucidating the mechanism of its activities, in particular the anticancer activity. In the Indian subcontinent and southeast Asia, curcuminoids have been used as a traditional therapy of inflammation, wounds, and tumors (*19*, *20*). In animal models, curcumin has been shown to have cancer chemopreventive, anti-neoplastic, and anti-inflammatory properties (*3*). In a colorectal cancer mice model, an affect was found on mice after either long-term ingestion of dietary curcumin or a single dose of [^{14}C]curcumin (100 mg/kg) via the intraperitoneal route. Whereas curcumin at 0.1% in the diet was without effect, at 0.2 and 0.5%, it reduced adenoma multiplicity by 39 and 40%, respectively, compared to untreated mice (*2*). Oral delivery of curcumin has been less effective because of its low solubility in water. Different factors contributing to the low bioavailability include low plasma level, tissue distribution, rapid metabolism, and elimination from the body. Although curcuminoids poor absorption and low systemic bioavailability limit curcuminoids translation into clinical uses (*21*), some of the methods, such as poly(lactic-co-glycolic acid) (PLGA) nanospheres (*22*), self-microemulsifying drug delivery systems (SMEDDSs) (*23*), and polycaprolactone (PCL) nanofibers, for its use can be approached to enhance the absorption and achieve a therapeutic level of curcuminoids (*24*). Purkayastha et al. show that a soluble formulation of curcumin 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 (*25*) or has a neuroprotective effect on focal cerebral ischemic rats (*26*).

It clearly appears from published data that the effect of curcumin is cell-type-specific (*27*). Indeed, Hsu et al. have previously shown that curcuminoids induce apoptosis in primary colon cancer (*28*) and pharynx and nasopharyngeal carcinoma (*29*). Curcuminoids were found to produce, in a number of cancer cell types, an anticancer activity and induce apoptosis (*27*).

The results collected in this series of studies with the cell lines of human brain GBM cells have provided experimental evidence to indicate that curcuminoids could induce the apoptosis of GBM 8401 cells. These culminate with phase I human trials that have shown this compound to be well-tolerated (*30*). The clinical trials have not identified a maximum tolerated dose of curcumin in humans with clinical trials using doses up to 8000 mg/day (*31*). The most common cell death mode on curcumin treatment seems to be apoptosis (*32*), and there are two major apoptotic pathways of participation: the death (Fas) receptor and the mitochondrial pathways (*33*). Death receptors are activated by their cognate ligands, a group of complementary cytokines that belong to the tumor necrosis factor (TNF) protein family (*34*). The death receptors of the tumor necrosis factor receptor (TNFR) family are membrane receptors with a specific function of inducing cell death via both apoptotic and necrotic signaling pathways (*35*). The main representatives of pro-death signaling from this group, TNFR1, Fas/CD95, and DD-containing TRAIL receptors, in the initial stages of pro-apoptotic signaling form multi-protein complexes that serve as activating platforms for either efficient

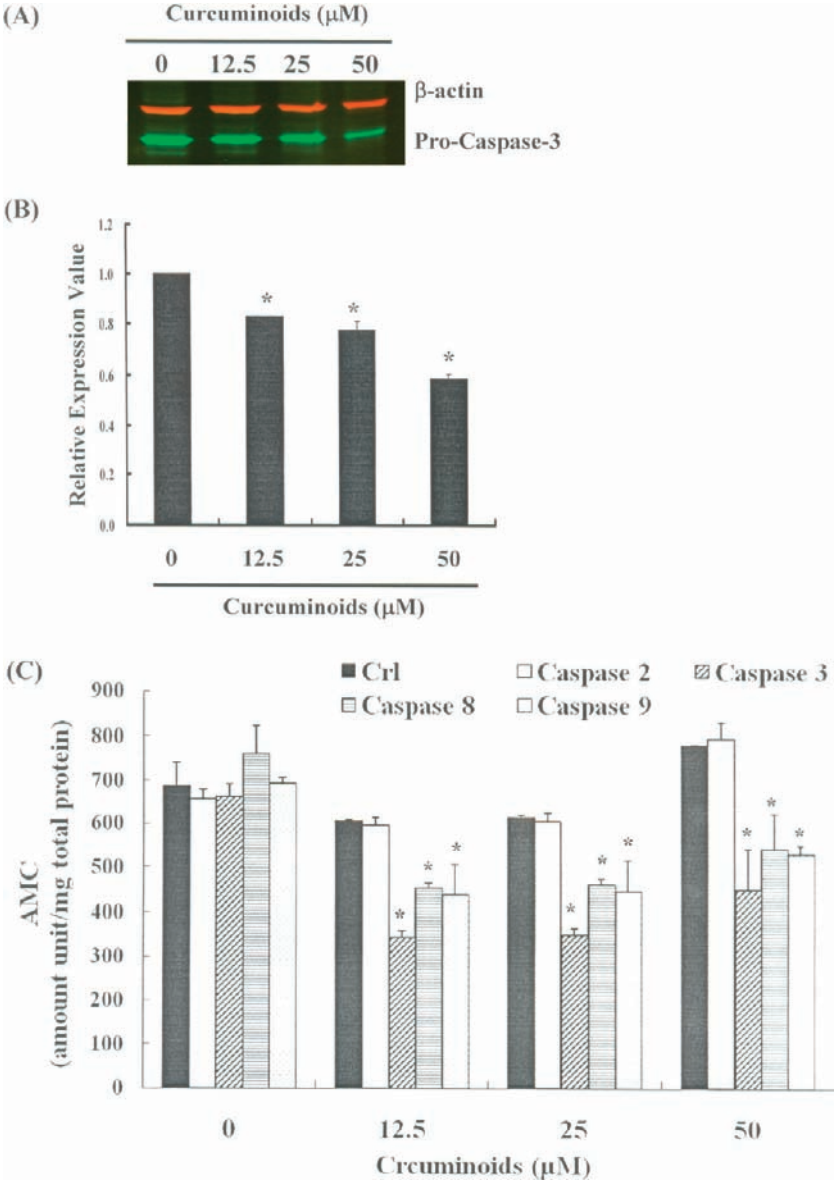

Figure 5. Apoptosis induction by curcuminoids in GBM 8401 cells via caspase-3, caspase-8, and caspase-9 activation. Curcuminoids activate pro-caspase-3 degradation in GBM 8401 cells. The cells were treated with curcuminoids (0, 12.5, 25, and 50 μM), and then western blot analysis was performed for pro-caspase-3. (A) Representative blot from three independent experiments. (B) Quantification of band intensities. (C) Caspase-2, caspase-3, caspase-8, and caspase-9 activities were analyzed by ApoAlert caspase assay plates. The curcuminoids induced the caspase activity of GBM 8401 cells. All data were reported as the mean (±SEM) of three separate experiments. Statistical analysis was obtained using the *t* test, with the significant differences determined at the level of (*) $p < 0.05$ versus the 0 μM control group.

processing of the initiator caspase-8 and caspase-10 or later on the transduction of necrotic RIP kinase-mediated signaling (*36*). The intrinsic apoptosis pathway is also referred to as the mitochondrial pathway because it requires the release of cytochrome *c* from the mitochondrial intermembrane space. Cytochrome *c* is the major inducer of caspase activation downstream of mitochondria. The proapoptotic activity of cytochrome *c* requires interaction with apoptosis protease activating factor-1 (Apaf-1) (*37*). This promotes assembly of the apoptosome, a protein complex that serves as a platform for activation of the initiator caspase pro-caspase-9. Subsequently, caspase-9 cleaves and activates executioner caspases, such as caspase-3, leading to the death of the cell (*38*).

Multiple apoptotic stimuli trigger the activation of proteases called caspases, which in turn initiate and execute the apoptotic program (*39*). Many studies have led to the discovery of two major apoptotic nucleases, termed DNA fragmentation factor (DFF) (*40*) or caspase-activated DNase (CAD) (*41*) and endonuclease G (*42*). Both endonucleases attack chromatin to yield 3-hydroxyl and 5-phosphate termini, first creating 50−300 kb cleavage products and then oligonucleosomal fragmentation, but these nucleases show different cellular locations (*43*) and are regulated in fundamentally different ways (*44*). 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 (*45*). The observations of this study have implied that curcuminoids have significantly induced the DNA fragmentation of GBM 8401 cells. We assume that the DFF or CAD may play an important role in curcuminoid-induced DNA fragmentation.

NF-κB plays an important role in cell proliferation and apoptosis by regulating the expression of genes involved in these processes (*46*). Active NF-κB is most commonly composed of the

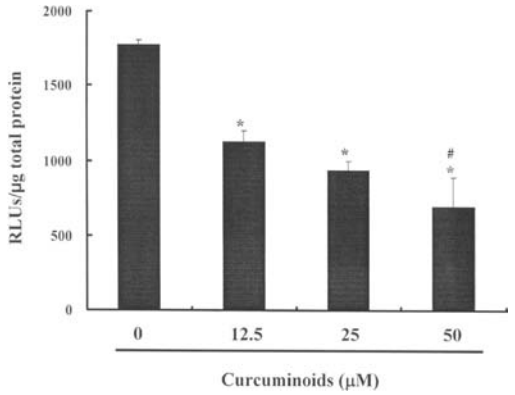

Figure 6. Curcuminoids inhibit NF-κB transcription factor activity in GBM 8401 cells. Identification of the activity of the NF-κB transcription factor in the cells was made after 16 h of exposure to curcuminoids, which was then examined by a microplate luminometer. The RLUs were normalized by total protein, and all data were reported as the mean (±SEM) of four separate experiments. Statistical analysis was obtained using one-way ANOVA with post-hoc tests. The significant differences were determined at the level of (*) $p < 0.05$ versus the 0 μM control group and (#) $p < 0.05$ versus 12.5 μM curcuminoids.

heterodimer DNA-binding subunits p50 and p65. It has recently been shown that inactivation of the p65 subunit of NF-κB leads to death through apoptosis of liver cells (*47*). Similarly, it has been shown in a wide range of cells that, when NF-κB has been inactivated by Iκ-Bα, cells were more sensitive to TNF-α-induced apoptosis. Evidence exists for NF-κB playing both anti- and pro-apoptosis roles (*48*). The reducing levels of NF-κB may be involved in curcuminoid-induced apoptosis of GBM 8401 cells. The results of mechanistic analysis have led us to conclude that both the inhibition of proliferation and the induction of apoptosis are highly dependent upon the curcuminoids and different cell types would be affected differently on cellular uptake of curcuminoids (*27*).

Moreover, the fact that humans have been reported to be capable of consuming curcuma extract of up to 2.2 g daily (equivalent to 180 mg of curcumin) appears safe in the framework of the phase-I study without a toxic effect (*49*), making curcumin a very interesting chemopreventive agent. Studying the inhibition of cell proliferation and the induction of apoptosis by curcumin (*50*−*52*) could achieve better insight into the mechanisms underlying cancer chemoprevention by curcumin or its derivatives (*53*−*55*). We expect that all of these experiments could provide a scientific basis and technological support for cancer therapy.

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