

# Curcumin induces G2/M cell cycle arrest in a p53-dependent manner and upregulates ING4 expression in human glioma

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**Abstract** Gliomas are the most common and lethal primary tumors of the central nervous system (CNS). Despite current rigorous treatment protocols, effect of chemotherapy has failed to improve patient outcome significantly. Curcumin is a potent antioxidant that possesses both anti-inflammatory and anti-tumor activities, can suppress the initiation, promotion, and metastasis of different tumors. Its anti-tumor properties in various cancer models and negligible toxicity in normal cells make it a promising chemotherapeutic candidate. But the effect and the molecular mechanism of curcumin on gliomas are still recognized limitedly. The goal of the study is to elucidate the inhibitory effect and possible mechanisms of curcumin on glioma. After the treatment of curcumin, glioma cells U251 growth in vitro were significantly inhibited in a dose-dependent manner, and the low dose of curcumin induced G2/M cell cycle arrest. The high dose of curcumin not only enhanced G2/M cell cycle arrest, but also induced S phase of cell cycle arrest. But no obvious pre-G1 peak was observed at the different doses of curcumin. Genome DNA electrophoresis further confirmed that no DNA ladder was formed after the treatment of curcumin in U251 cells. Results of Western blot analysis demonstrated that ING4 expression was almost undetectable in U251 cells, but significantly up-regulated during cell cycle arrest induced

by curcumin, and p53 expression was up-regulated followed by induction of p21<sup>WAF-1/CIP-1</sup> and ING4. The results demonstrate that curcumin exerts inhibitory action on glioma cell growth and proliferation via induction of cell cycle arrest instead of induction of apoptosis in a p53-dependent manner, and ING4 possibly is in part involved in the signal pathways.

**Keywords** Glioma · Curcumin · Cell cycle arrest · ING (inhibitor of growth) family · Apoptosis

## Abbreviations

ING Inhibitor of growth  
CNS The central nervous system  
GBM Glioblastoma multiforme

## Introduction

Gliomas are the most common and lethal primary tumors of the central nervous system (CNS) with a median survival of less than 2 years. According to the malignancy, gliomas are divided into grade I, grade II (low grade), grade III (anaplastic) and grade IV (astrocytic phenotype or glioblastoma multiforme (GBM)) [1, 2]. Beginning in the 1980s chemotherapy has been incorporated into the treatment protocol of intractable gliomas, and remains part of the treatment triad that includes microneurosurgery and radiation therapy. Several anticancer drugs have resurfaced as potential agents for adjuvant brain tumor therapy. Unfortunately, for many intracranial neoplasms, effect of chemotherapy has failed to improve patient outcome significantly and the prognosis for the majority of patients

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harboring these tumors continues to be poor. The difficult clinical situation has fostered significant interest in defining novel therapeutic modalities for this heterogeneous group of neoplasms.

Curcumin is a diferuloylmethane derived from the plant *Curcuma longa*. It is a potent antioxidant that possesses both anti-inflammatory and antitumor activities [3]. Multiple lines of evidence from in vitro studies and animal models indicate that curcumin is a promising candidate for treating cancer by suppressing the initiation, promotion, and metastasis of various types of tumors [4–6]. Timiras et al. firstly reported that curcumin suppressed the proliferation and growth of glioma cells in vitro in a time-dose-dependent manner [7]. Kim et al. found that curcumin strongly repressed MMP-1, -3 and -9 gene expression via the inhibition of the PKC to MAP kinase signaling pathways and the repression of the DNA-binding and transcription activities of AP-1 in human astrogloma cells, and also significantly repressed the in vitro invasion of glioma cells [8]. In addition, curcumin is believed to be pharmacologically safe because it is a naturally occurring substance that is used in both the diet as well as in traditional oriental medicine to treat various diseases in East-Asian countries as well as in India. The anti-tumor efficacy and minimal toxicity indicate that curcumin can be used as an adjuvant agent with the traditional chemotherapeutic drugs for treating gliomas. The properties of curcumin have been attributed, at least in part, to its ability to inhibit COX-2 [9]. Recent studies indicate curcumin exerts its effect by down-regulating the transcription factors, NF- $\kappa$ B, AP-1 and Egr-1, and repressing the genes for cell adhesion molecules, chemokines, TNF, COX-2, and MMP-9 [10–12]. However, the molecular mechanism responsible for the anti-tumor action of curcumin on gliomas has not been entirely understood.

Inhibitor of growth (ING) gene family is characterized by a highly conserved C-terminal plant homeodomain (PHD)-like zinc-finger domain, including five members to date identified [13]. Recent studies have demonstrated that some members of ING gene family are implicated in a variety of processes including oncogenesis, apoptosis, DNA repair, and cell cycle control [14–16]. The best-studied ING family members reside in the nucleus where they positively or negatively regulate gene expression through interactions between their N terminus and chromatin-remodeling complexes containing histone acetyltransferases, histone deacetyltransferases, or factor acetyltransferases [17]. ING1 can negatively regulate cell proliferation in a p53-dependent manner through induction of G1 phase arrest of cell cycle and apoptosis, where ING1 can physically associate with p53 and promote apoptosis [18–20]. ING2 increases the acetylation of p53 at Lys-382 and strongly enhances the transcriptional-transactivation

activity of p53 although there is no physical interaction between both the proteins [21]. ING3 can activate p53-transactivated promoters, including promoters of p21 and Bax; and meanwhile, the overexpression of ING3 induces a decreased population of cells in S phase and apoptosis [22]. The transient overexpression of ING5 could induce a decreased cell population in S phase of cell cycle and apoptosis in a p53-dependent manner, along with the increased p21 expression in RKO cells.

The ING4 was initially identified in a screen for novel tumor suppressors as a novel member of ING family [23]. Characterized as a candidate tumor suppressor gene, it has been reported that ING4 is frequently mutated in human cancer [24]. Recently Garkavtsev et al. reported that expression of ING4 is significantly reduced in gliomas as compared with normal human brain tissue, and the extent of reduction correlates with the progression from lower to higher grades of tumors, and ING4 physically interacted with p65 (RelA) subunit of nuclear factor NF- $\kappa$ B, and regulated brain tumor angiogenesis through transcriptional repression of NF- $\kappa$ B-responsive genes [25]. Hence, ING4 possibly has critical role in brain tumor pathogenesis. Moreover, ING4 can increase the acetylation of p53 via interaction with p300, negatively regulate the cell growth with significant G2/M arrest of cell cycle, and enhance the chemosensitivity of some tumor cells to DNA-damage drugs [26]. Downregulation of ING4 expression in brain tumor cells could significantly upregulate the expression of COX-2, indicating ING4 correlated with an increase in expression of COX-2 [25].

In current study, we first found that curcumin can significantly repress growth and proliferation of glioma cells via the induction of S phase and G2/M phase arrest of cell cycle, along with expression of ING4, p21 and p53 upregulated. It indicates that curcumin possibly exerts its inhibitory action on cell cycle in a p53-dependent manner, in which ING4 is also involved.

## Materials and methods

### Antibodies and reagents

Curcumin was purchased from Sigma-Aldrich, Inc (St. Louis, MO), proteinase inhibitors were purchased from Roche Diagnostics (Mannheim, Germany). Rabbit polyclonal antiserum to recombinant human ING4 was obtained from Rockland (Gilbertsville, PA). Mouse monoclonal antibodies against p21 and p53 and HRP-labeled secondary antibodies were obtained from Santa Cruz (Santa Cruz, CA). Enhanced chemiluminescence (ECL) system was obtained from Roche Molecular Biochemicals (Mannheim, Germany).

### Cell cultures and drug treatment

Glioma cells U251 were purchased from Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, and cultured at 37°C in an atmosphere of 5% CO<sub>2</sub> and 95% air in RPMI-1640 supplemented with 10% FBS, penicillin and streptomycin (Gibco BRL, Life Technologies, NY) and harvested for use as indicated. Curcumin was freshly prepared in dimethyl sulfoxide (DMSO) before use. Vehicle control of DMSO (<0.1%) was included in the studies. When cells reached 60–80% confluence, they were treated with various concentrations of Curcumin as indicated. They were then cultured for times ranging from 12 to 24 h. The presence of 0.5% DMSO did not affect cell growth in repeated experiments.

### MTT assay

Cell proliferation was measured by MTT assay. Exponentially growing cells were plated into 96 well plates containing 5,000 cells/well in 200 µl medium for 24 h. Then cells were treated with different concentrations of curcumin for 24 h. About 20 µl of MTT stock solution (5 mg/ml) was added into each well, and cells were further incubated at 37°C for 4 h. The supernatant was replaced with 200 µl isopropanol to dissolve formazan production. The absorbance at wavelength 595 nm was measured with a micro-ELISA reader (Bio-Rad, Hercules, CA). The negative control well, into which only the medium had been added, was used for zeroing the absorbance. Each assay was performed three times in triplicate. The ratio of the absorbance of treated cells relative to that of the control cells were calculated and expressed as percentage of growth inhibition.

### Fluorescence-activated cell sorter (FACS) analysis

The distribution of cells in the cell-cycle phases was determined using FACS analysis of DNA content. Exponentially growing cells were plated into 100 mm plates containing  $1 \times 10^6$  cells/plate in 5 ml medium overnight. Then cells were treated with 4 or 10 µM of curcumin for 24 h. Cells were collected and washed with ice-cold PBS twice. After centrifuge, 5 ml of 70% ethanol was added to fix cells at 4°C overnight. Prior to analysis, the cells were washed with ice-cold PBS for three times, and resuspended at  $1 \times 10^6$  cells/ml in PBS. Cells were incubated with 0.1 mg/ml RNase I and 40 mg/ml propidium iodide at 37°C for 30 min. Samples were analyzed using a FACS scanner (Coulter Epics XL, Luton, UK). The resultant histogram was analyzed using Multicycle AV software (Phoenix Flow System, San Diego, CA, USA). The apop-

otic cells can be observed on a DNA histogram as a subdiploid or pre-G1 peak.

### Apoptosis assay

After the treatment of 10 µM of curcumin for 24 h, an equal number of cells was lysed with lysis buffer (10 mM Tris, 10 mM EDTA, 0.5% Triton X-100, pH 7.5) at 4°C for 10 min, and centrifuged at  $13,000g \times 15$  min at 4°C. The supernatant was collected and DNA was precipitated with 50% isopropylol/0.5 M NaCl at –20°C overnight. DNA pellet was dissolved in TE solution containing 1 mg/ml RNase and incubated at 37°C for 30 min, then 2 mg/ml proteinase K was added and digested at 37°C for 1 h. DNA fragment was electrophoresed on a 1% agarose gel, and examined after being stained with ethidium bromide.

### Western blotting analysis

Cells were treated with or without curcumin (10 µM) for 24 h, and then extracted with RIPA lysis buffer containing protease inhibitors (20 mM Tris-HCl, 1 mM EDTA, 1 mM EGTA, 1 mM sodium vanadate, 0.2 mM phenylmethylsulfonyl fluoride, 0.5% NP-40). The protein concentration was determined by bicinchoninic acid assay with bovine serum albumin (Sigma-Aldrich, Inc) as the standard. Equal aliquots of total cell lysates (50 µg) of each sample were solubilized in 2× sample buffer (100 mmol/l Tris, 200 mmol/l DTT, 4% SDS, 0.2% bromine blue, 20% glycerol) and electrophoresed on 12% SDS-PAGE gel. The proteins were then transferred to PVDF membrane (Millipore) using transfer buffer for 2 h. Non-specific binding was blocked with TTBS (10 mM pH 7.6 Tris-HCl buffer saline plus 0.05% Tween-20) containing 5% nonfat milk for 1 h at room temperature. The blots were probed with primary antibodies overnight at 4°C. The primary antibodies included rabbit anti-human ING4 polyclonal antibody (1:500), mouse anti-human p21 monoclonal antibody (1:500), mouse anti-human p53 monoclonal antibody (1:1,000) and mouse anti-human actin antibody (1:1,000). Antigen–antibody complexes were visualized by the ECL system.

### Statistical analyses

All experiments were performed in triplicate and were repeated at least three times. Mean values ± SEM were calculated to represent the three experiments. Statistical difference was determined by Student's *t*-test, or one-way ANOVA. A *P* value of <0.05 was considered significant.

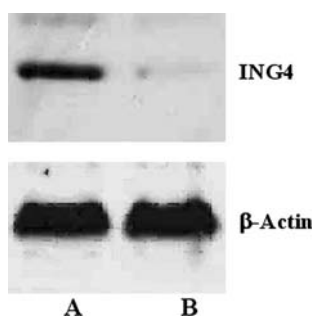
## Results

### The ING4 protein expression in glioma cells

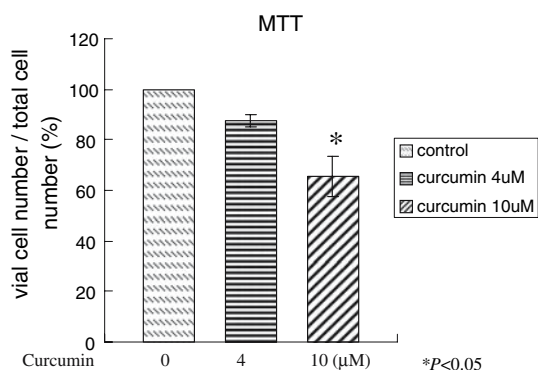
The ING4 protein expression in glioma cells was detected by Western blot. Normal brain tissue was used as the positive control. In U251 cells, ING4 protein was hardly detectable (Fig. 1).

### Inhibitory effect of curcumin on growth of U251

Different concentrations of curcumin were used to treated U251 cells for 24 h. MTT assay showed that curcumin repressed U251 cell growth in a dose-dependent manner (Fig. 2). At low dose (4  $\mu\text{M}$ ), curcumin did not significantly inhibit U251 cell growth, but at high dose (10  $\mu\text{M}$ ), curcumin suppressed cell growth to 35% ( $P < 0.05$ , one-way ANOVA) The concentration of curcumin for further experiments was chosen at 10  $\mu\text{M}$  based on the MTT assay results.



**Fig. 1** Western blot analysis for ING4 protein expression in U251 cells. (A) Normal brain tissue was the positive control. (B) ING4 protein was hardly detectable in U251 cells



**Fig. 2** Inhibitory effects of curcumin on U251 cell growth. Curcumin inhibited U251 cell growth in dose-dependent manner, low dose of curcumin (4  $\mu\text{M}$ ) slightly inhibited U251 cell growth, but high dose of curcumin (10  $\mu\text{M}$ ) significantly inhibited U251 cell growth with the growth inhibitory rate to 35% (compared with the control,  $*P < 0.05$ )

### Effect of curcumin on cell cycle in glioma cells

Different doses of curcumin treated U251 cells for 24 h, FACS showed that after treatment with 4  $\mu\text{M}$  of curcumin, the percentage of U251 cells in G0/G1 phase was decreased, and in G2/M phase was increased; and at the high dose of curcumin (10  $\mu\text{M}$ ), the percentage of U251 cells in G0/G1 phase significantly decreased, in G2/M phase significantly increased (Fig. 3). And interestingly, the percentage in S phase was also significantly increased from 11.2 to 28.8% at the high dose of curcumin. But no significant increases in the percentage of apoptosis were induced at the different doses of curcumin, which suggested that curcumin played its inhibitory roles on glioma cells mainly via the induction of cell cycle arrest instead of apoptosis. The percentage of U251 cells distributed in the phases of cell cycle was shown in the Table 1.

### No apoptosis induced by curcumin in glioma cells

DNA ladder formation is the golden standard of apoptosis. After the treatment of curcumin (10  $\mu\text{M}$ ) for 24 h, no obvious DNA ladder was observed, which indicated no apoptosis was significantly induced (Fig. 4).

### Effect of curcumin on the expression of p21 and p53 in glioma cells

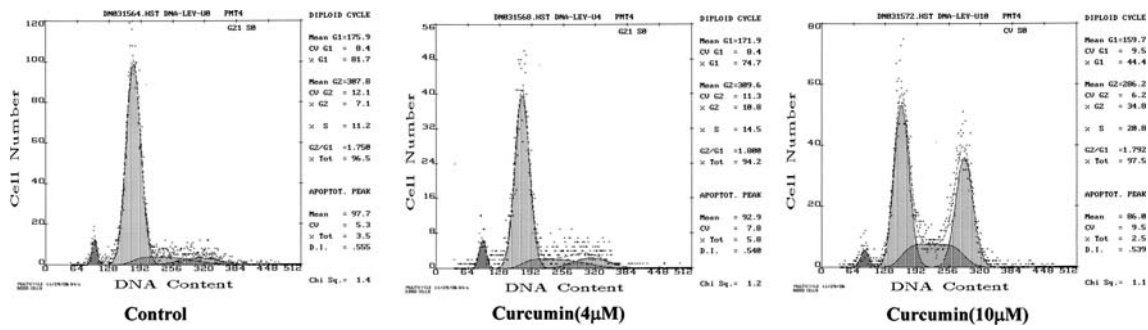
Western blot showed that after the treatment of curcumin (10  $\mu\text{M}$ ) for 16 h, the expression of p21 and p53 was up-regulated, and for 24 h the expression up-regulated significantly. It indicated that curcumin potentially induced the cell cycle arrest in a p53-dependent manner (Fig. 5).

### Inducement of ING4 expression by curcumin in glioma cells

Western blot showed that after the treatment of curcumin (10  $\mu\text{M}$ ) for 24 h, ING4 protein expression was induced significantly in glioma cells U251, indicating that ING4 possibly was involved in pathways in the cell cycle arrest induced by curcumin (Fig. 6).

## Discussion

Curcumin (diferuloylmethane), a major active component of turmeric (*Curcuma longa* Linn), is a crystalline compound that has been traditionally used in medicine and cooking in Eastern-Asia. Preclinical studies have revealed the chemopreventive potential of curcumin in several different animal tumor bioassay systems, including colon, duodenal, stomach, prostate, and breast carcinogenesis,

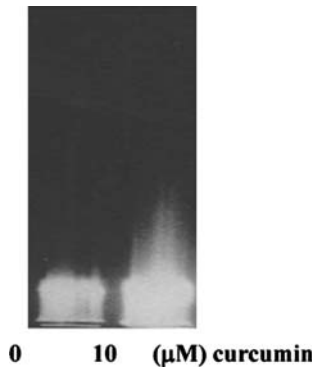


**Fig. 3** Effect of curcumin on cell cycle in glioma cells. After the treatment of curcumin (4 µM) for 24 h, the percentage of U251 cells in G<sub>0</sub>/G<sub>1</sub> phase decreased slightly and in G<sub>2</sub>/M phase increased. And at high dose of curcumin (10 µM), the percentage of U251 cells in

G<sub>0</sub>/G<sub>1</sub> phase was significantly decreased, and in S phase and G<sub>2</sub>/M phase increased obviously. But no significant increases in the percentage of apoptosis were observed at the different doses of curcumin

**Table 1** The percentage of U251 cells distributed in the phases of cell cycle

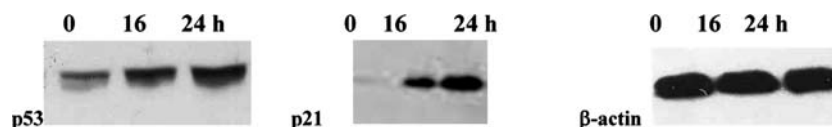
Cell line	Curcumin (µM)	Apoptotic	G <sub>0</sub> /G <sub>1</sub>	S	G <sub>2</sub> /M	G <sub>2</sub> /G <sub>1</sub>
U251	0	3.5	81.7	11.2	7.1	1.75
	4	5.8	74.7	14.5	18.8	1.888
	10	2.5	44.4	28.8	34.8	1.792



**Fig. 4** Curcumin did not induced the formation of DNA ladder in U251 cells. After the treatment of curcumin (10 µM) for 24 h, no obvious DNA ladder was observed

both in vitro and in vivo [27–30]. Curcumin can blocks tumor initiation, suppresses tumor proliferation and progression, and reduces the invasion and subsequently metastasis of various cancer cells [31, 32]. The absence of dose-limiting toxicity, even when curcumin is administered

up to 8 g/day in human clinical trials suggests it as a promising and safe anti-cancer drug [33]. Timiras et al. firstly reported that curcumin could inhibit glioma cells proliferation and growth in a dose-time-dependent manner [34]. Kim et al. found that curcumin could repress the expression of MMP-1, MMP-3 and MMP-9 in astroglia cells via the suppression of PKC and MAPK signal pathways and the inhibition of the DNA binding and transcription activities of AP-1, and in vitro assay also showed curcumin effectively reduced astroglia cells invasion [35]. It has been described in many studies the molecular targets of cancer that are modulated by curcumin as a consequence of its effect on NF-κB and other prominent transcription factors [36, 37]. In addition to suppressing various cell survival and cell proliferative genes, including Bcl-2, cyclin D1, IL-6, cyclooxygenase-2(COX-2), and MMP-9, curcumin induced apoptosis, as pointed out by caspase activation and poly(ADP-ribose) polymerase (PARP) cleavage. It has been proved that curcumin could induce apoptosis in human astrocytoma cells via the activation of procaspases and the release of cytochrome c from the mitochondrial, enhanced the sensitivity of malignant glioma cells to apoptosis mediated by TRAIL/Apo2L, enhance the caspase-3-dependent glioma cells death by suppressing the acetylation of histone, and activate the protein degradation pathways mediated by receptors and mitochondrial to induce glioblastoma cell apoptosis [38, 39]. Although a number of studies have described the potential use of curcumin in the prevention and treatment of brain tumor, its molecular mechanism has



**Fig. 5** Effect of curcumin on the expression of p21 and p53 in glioma cells. After the treatment of curcumin (10 µM) the expression of p21 and p53 was significantly up-regulated



**Fig. 6** Inducement of ING4 expression by curcumin in glioma cells. After the treatment of curcumin (10  $\mu$ M) for 24 h, ING4 expression was significantly induced in glioma cells

not been elucidated clearly. In our study, curcumin significantly inhibited cell proliferation and growth via the induction of S phase and G2/M phase of cell cycle arrest, instead of significant apoptosis in U251 glioma cells. At the low dose, curcumin mainly induced G2/M phase of cell cycle arrest, but at the high dose in addition to increase in G2/M cell cycle arrest, S phase of cell cycle arrest happened.

In order to elucidate the molecular mechanism, we tested p53 expression and function after the treatment of curcumin. The results showed curcumin up-regulated p53 expression in a time-dependent manner, followed by induction of p21<sup>WAF-1/CIP-1</sup> expression. Observations suggested that the cell cycle arrest induced by curcumin may be mediated via functionally activated p53 and merit further study. Most interesting, it was observed that ING4, a novel member of *ING* tumor suppressor family, was up-regulated along with cell cycle arrest induced by curcumin in U251 glioma cells.

To date, five members of the *ING* family have been identified, all containing a highly conserved plant homeodomain finger (PHF) motif in the C-terminal end of the proteins. The PHF is found in transcription factors that modulate chromatin structure, and the *ING* gene products interact with protein complexes containing histone acetyltransferase and deacetylase [15]. Thus, although the exact functions of *ING* genes have not been elucidated, the gene products are thought to participate in transcriptional regulation by means of chromatin remodeling [14]. The products of *ING* genes have been shown to interact with and enhance the function of p53 in gene transcription, apoptosis, and DNA repair [40]. It has been described that ING1 was involved in the regulation of apoptosis and cell cycle in a p53-dependent manner in several cancers and cell lines as a candidate tumor suppressor [41, 42]. ING2 could negatively regulate cell proliferation in a p53-dependent manner through induction of G1 phase arrest of cell cycle and apoptosis [21]. ING3 could activate p53-transactivated promoters, including promoters of p21 and Bax, and induce a decreased population of cells in S phase and apoptosis [22]. Shiseki et al. also described some characteristics of ING4 and ING5, which the transient overexpression of ING4 or ING5 could induce a decreased cell population in S phase of cell cycle and apoptosis in a p53-dependent manner, along with the increased p21

expression in RKO cells [43]. Moreover, ING4 could physically interact with p300 and p53 in vivo, as well as enhance p53 acetylation at Lys-382 [44]. Most interestingly, Garkavtsev et al. reported that ING4 was involved in regulating brain tumor growth and angiogenesis by associating with p65(RelA) subunit of NF- $\kappa$ B [25]. Ze-Guang Han et al. found that ING4 could regulate negatively the cell growth with significant G2/M arrest of cell cycle and enhance chemosensitivity to certain DNA-damage agents in HepG2 cells [26]. Hence, we presumed that cell cycle arrest in U251 glioma cells induced by curcumin was in part attributed to up-regulation of ING4, which exert its function via a p53-dependent manner. But further evidences need to be clarified the signal pathways responsible for ING4 up-regulation induced by curcumin and whether physical interaction with p53 exists.

Barnett and Jain et al. found that the expression of ING4 at the level of mRNA was two to three times lower in the low-grade gliomas (grade II–III), and six times lower the ING4 mRNA in the glioblastomas than in the normal brain tissues [25]. Immunochemical staining showed that in the normal brain tissues ING4 was located in the nucleus [45]. In our previous immunostaining study, it was also proved that in gliomas the expression of ING4 at the level of protein was significantly lower than in the normal brain tissue, and the protein level of ING4 was reversely correlated with the progression from lower to higher grades of tumors (data not shown). The reverse correlation indicated that ING4 potentially plays critical roles on the development and progression of gliomas. In current study, we detected the expression of ING4 protein in glioma cells. In U251 cells the expression of ING4 at the level of protein was almost undetectable, however in another glioma cell line SGH the expression of ING4 protein was obviously detectable and located in the cytoplasm instead of the nucleus (data not shown). It suggested that in the initiation, promotion and progression of gliomas, the expression of ING4 is possibly repressed or only nominal repression because of gene deletion or mutation. Mutated ING4 protein was distributed in the cytoplasm and lost its tumor suppressor functions. Michael Bishop et al. found that mutations in *ING4* transcripts were prevalent in a number of tumor cells, such as the nuclear localization signal (KGKK) deletion, C-terminal truncation mutation, and an amino acid substitution mutation [23]. Such mutations

were functionally inactive as tumor suppressor and have a dominant-negative effect, which has the potential to create a null state in even those cells that are heterozygous for the wild-type *ING4* gene. Harris et al. recently identified three novel splice variants of *ING4* with differing activities in controlling cell proliferation, cell spreading, and cell migration [46].

Taken together, this study suggested that curcumin could induce S phase and G<sub>2</sub>/M phase of cell cycle arrest in a p53-dependent manner associated with increase expression of p21 and ING4. ING4 up-regulated possibly enhanced the binding to p53, and then increased p21 expression to negatively regulate cell proliferation by inducing cell cycle arrest. However the functionary mechanisms of ING4 have not revealed clearly and its potential use in the treatment of brain tumors need more deepen studies.

## References

- Maher EA, Furnari FB, Bachoo RM, Rowitch DH, Louis DN, Cavenee WK, DePinho RA (2001) Malignant glioma: genetics and biology of a grave matter. *Genes Dev* 15:1311–1333
- Norden AD, Wen PY (2006) Glioma therapy in adults. *Neurologist* 12(6):279–292
- Ahsan H, Parveen N, Khan NU, Hadi SM (1999) Pro-oxidant, antioxidant and cleavage activities on DNA of curcumin and derivatives demethoxycurcumin and isodemetoxycurcumin. *Chem-Biol Inter* 121:161–175
- Aggarwal BB, Kumar A, Bharti AC (2003) Anticancer potential of curcumin: preclinical and clinical studies. *Anticancer Res* 23:363–398
- Han SS, Keum YS, Seo HJ, Surh YJ (2002) Curcumin suppresses activation of NF- $\kappa$ B and AP-1 induced by phorbol ester in cultured human promyelocytic leukemia cells. *J Biochem Mol Biol* 31:337–342
- Jobin C, Bradham CA, Russo MP, Juma B, Narula AS, Brenner DA, Sartor RB (1999) Curcumin blocks cytokine-mediated NF- $\kappa$ B activation and proinflammatory gene expression by inhibiting inhibitory factor I- $\kappa$ B kinase activity. *J Immunol* 163:3474–3483
- Ambegaokar SS, Wu L, Alamshahi K, Lau J, Jazayeri L, Chan S, Khanna P, Hsieh E, Timiras PS (2003) Curcumin inhibits dose-dependently and time-dependently neuroglial cell proliferation and growth. *Neuro Endocrinol Lett*. 24(6):469–473
- Woo MS, Jung SH, Kim SY, Hyun JW, Ko KH, Kim WK, Kim HS (2005) Curcumin suppresses phorbol ester-induced matrix metalloproteinase-9 expression by inhibiting the PKC to MAPK signaling pathways in human astrogloma cells. *Biochem Biophys Res Commun* 335(4):1017–1025
- Jiang H, Deng CS, Zhang M, Xia J (2006) Curcumin-attenuated trinitrobenzene sulphonic acid induces chronic colitis by inhibiting expression of cyclooxygenase-2. *World J Gastroenterol* 12(24):3848–3853
- Kim SY, Jung SH, Kim HS (2005) Curcumin is a potent broad spectrum inhibitor of matrix metalloproteinase gene expression in human astrogloma cells. *Biochem Biophys Res Commun* 337(2):510–516
- Su CC, Chen GW, Lin JG, Wu LT, Chung JG (2006) Curcumin inhibits cell migration of human colon cancer colo 205 cells through the inhibition of nuclear factor  $\kappa$ B/p65 and down-regulates cyclooxygenase-2 and matrix metalloproteinase-2 expressions. *Anticancer Res* 26(2A):1281–1288
- Lee KW, Kim JH, Lee HJ, Surh YJ (2005) Curcumin inhibits phorbol ester-induced up-regulation of cyclooxygenase-2 and matrix metalloproteinase-9 by blocking ERK1/2 phosphorylation and NF- $\kappa$ B transcriptional activity in MCF10A human breast epithelial cells. *Antioxid Redox Signal*. 7(11–12):1612–1620
- Shi X, Gozani O (2005) The fellowships of the INGs. *J Cell Biochem* 96:1127–1136
- Gong W, Suzuki K, Russell M, Riabowol K (2005) Function of the ING family of PHD proteins in cancer. *Int J Biochem Cell Biol* 37(5):1054–1065
- Campos EI, Chin MY, Kuo WH, Li G (2004). Biological functions of the ING family tumor suppressors. *Cell Mol Life Sci* 61(19–20):2597–2613
- Nagashima M, Shiseki M, Miura K, Hagiwara K, Linke SP, Pedoux R, Wang XW, Yokota J, Riabowol K, Harris CC (2001) DNA damage-inducible gene p33ING2 negatively regulates cell proliferation through acetylation of p53. *Proc Natl Acad Sci USA* 98(17):9671–9676
- Feng X, Hara Y, Riabowol K (2002) Different HATS of the ING1 gene family. *Trends Cell Biol* 12(11):532–538
- Zhu Z, Lin J, Qu JH, Feitelson MA, Ni CR, Li FM, Zhu MH (2005) Inhibitory effect of tumor suppressor p33(ING1b) and its synergy with p53 gene in hepatocellular carcinoma. *World J Gastroenterol* 11(13):1903–1909
- Goeman F, Thormeyer D, Abad M, Serrano M, Schmidt O, Palmero I, Banihmad A (2005) Growth inhibition by the tumor suppressor p33ING1 in immortalized and primary cells: involvement of two silencing domains and effect of Ras. *Mol Cell Biol* 25(1):422–431
- Nouman GS, Anderson JJ, Lunec J, Angus B (2003) The role of the tumour suppressor p33 ING1b in human neoplasia. *J Clin Pathol* 56(7):491–496
- Wang Y, Wang J, Li G (2006) Leucine zipper like domain is required for tumor suppressor ING2-mediated nucleotide excision repair and apoptosis. *FEBS Lett* 580(16):3787–3793
- Nagashima M, Shiseki M, Pedoux RM, Okamura S, Kitahama Shiseki M, Miura K, Yokota J, Harris CC (2003) A novel PHD-finger motif protein, p47ING3, modulates p53-mediated transcription, cell cycle control, and apoptosis. *Oncogene* 22(3):343–350
- Kim S, Chin K, Gray JW, Bishop JM (2004) A screen for genes that suppress loss of contact inhibition: identification of ING4 as a candidate tumor suppressor gene in human cancer. *Proc Natl Acad Sci USA* 101(46):16251–16256
- Gunduz M, Nagatsuka H, Demircan K, Gunduz E, Cengiz B, Ouchida M, Tsujigiwa H, Yamachika E, Fukushima K, Beder L, Hirohata S, Ninomiya Y, Nishizaki K, Shimizu K, Nagai N (2005) Frequent deletion and down-regulation of ING4, a candidate tumor suppressor gene at 12p13, in head and neck squamous cell carcinomas. *Gene* 356:109–117
- Garkavtsev I, Kozin SV, Chernova O, Xu L, Winkler F, Brown E, Barnett GH, Jain RK (2004) The candidate tumour suppressor protein ING4 regulates brain tumour growth and angiogenesis. *Nature* 428(6980):328–332
- Zhang X, Xu LS, Wang ZQ, Wang KS, Li N, Cheng ZH, Huang SZ, Wei DZ, Han ZG (2004) ING4 induces G<sub>2</sub>/M cell cycle arrest and enhances the chemosensitivity to DNA-damage agents in HepG2 cells. *FEBS Lett* 570(1–3):7–12
- Duvoix A, Blasius R, Delhalle S, Schnekenburger M, Morceau F, Henry E, Dicato M, Diederich M (2005) Chemopreventive and therapeutic effects of curcumin. *Cancer Lett* 223(2):181–190
- Moragoda L, Jaszewski R, Majumdar AP (2001) Curcumin induced modulation of cell cycle and apoptosis in gastric and colon cancer cells. *Anticancer Res* 21(2A):873–878

29. Somasundaram S, Edmund NA, Moore DT, Small GW, Shi YY, Orłowski RZ. (2002) Dietary curcumin inhibits chemotherapy-induced apoptosis in models of human breast cancer. *Cancer Res* 62(13):3868–3875
30. Manson MM, Farmer PB, Gescher A, Steward WP (2005) Innovative agents in cancer prevention. *Recent-Results-Cancer-Res* 166:257–275
31. Karunagaran D, Rashmi R, Kumar TR (2005) Induction of apoptosis by curcumin and its implications for cancer therapy. *Curr Cancer Drug Targets* 5(2):117–129
32. Hong JH, Ahn KS, Bae E, Jeon SS, Choi HY (2006) The effects of curcumin on the invasiveness of prostate cancer in vitro and in vivo. *Prostate Cancer Prostatic Dis* 9(2):147–152
33. Aggarwal BB, Kumar A, Bharti AC (2003) Anticancer potential of curcumin: preclinical and clinical studies. *Anticancer Res* 23(1A):363–398
34. Ambegaokar SS, Wu L, Alamshahi K, Lau J, Jazayeri L, Chan S, Khanna P, Hsieh E, Timiras PS (2003) Curcumin inhibits dose-dependently and time-dependently neuroglial cell proliferation and growth. *Neuro Endocrinol Lett* 24(6):469–473
35. Kim SY, Jung SH, Kim HS (2005) Curcumin is a potent broad spectrum inhibitor of matrix metalloproteinase gene expression in human astrogloma cells. *Biochem Biophys Res Commun* 337(2):510–516
36. Kim GY, Kim KH, Lee SH, Yoon MS, Lee HJ, Moon DO, Lee CM, Ahn SC, Park YC, Park YM (2005) Curcumin inhibits immunostimulatory function of dendritic cells: MAPKs and translocation of NF-kappa B as potential targets. *J Immunol* 174(12):8116–8124
37. Bharti AC, Takada Y, Aggarwal BB (2004) Curcumin (diferuloylmethane) inhibits receptor activator of NF-kappa B ligand-induced NF-kappa B activation in osteoclast precursors and suppresses osteoclastogenesis. *J Immunol* 172(10):5940–5947
38. Nagai S, Kurimoto M, Washiyama K, Hirashima Y, Kumanishi T, Endo S (2005) Inhibition of cellular proliferation and induction of apoptosis by curcumin in human malignant astrocytoma cell lines. *J Neurooncol* 74(2):105–111
39. Kang SK, Cha SH, Jeon HG (2006) Curcumin induced histone hypoacetylation enhances caspase-3 dependent glioma cell death and neurogenesis of neural progenitor cells. *Stem Cells Dev* 15(2):165–174
40. Nourani A, Howe L, Pray Grant MG, Workman JL, Grant PA, Cote J (2003) Opposite role of yeast ING family members in p53 dependent transcriptional activation. *J Biol Chem* 278(21):19171–19175
41. Garkavtsev I, Boucher Y (2005) An intact ING1-P53 pathway can potentiate the cytotoxic effects of taxol. *Cancer Biol Ther* 4(1):48–49
42. Tachibana M, Kawamata H, Fujimori T, Omotehara F, Horiuchi H, Ohkura Y, Igarashi S, Kotake K, Kubota K (2004) Dysfunction of p53 pathway in human colorectal cancer: analysis of p53 gene mutation and the expression of the p53-associated factors p14ARF, p33ING1, p21WAF1 and MDM2. *Int J Oncol* 25(4):913–920
43. Shiseki M, Nagashima M, Pedeux RM, Kitahama Shiseki M, Miura K, Okamura S, Onogi H, Higashimoto Y, Appella E, Yokota J, Harris CC (2003) p29ING4 and p28ING5 bind to p53 and p300, and enhance p53 activity. *Cancer Res* 63(10):2373–2378
44. Kim S (2005) HuntING4 new tumor suppressors. *Cell Cycle* 4(4):516–517
45. Zhang X, Wang KS, Wang ZQ, Xu LS, Wang QW, Chen F, Wei DZ, Han ZG (2005) Nuclear localization signal of ING4 plays a key role in its binding to p53. *Biochem-Biophys Res Commun* 331(4):1032–1038
46. Motoko U, Jiang CS, Zhi-Ming Z, Curtis CH (2006) Novel splice variants of ING4 and their possible roles in the regulation of cell growth and motility. *J Biol Chem* 281(45):34677–34686