*Journal of Biochemistry and Molecular Biology*, Vol. 38, No. 4, July 2005, pp. 474-480

JOURNAL OF Biochemistry and Molecular Biology

# Curcumin Derivatives Inhibit the Formation of Jun-Fos-DNA Complex Independently of their Conserved Cysteine Residues

**Chi Hoon Park, Ju Hyung Lee and Chul Hak Yang***

Division of Chemistry and Molecular Engineering, Seoul National University, Seoul 151-742, Korea

*Received 9 December 2004, Accepted 26 April 2005*

**Curcumin, a major active component of turmeric, has been identified as an inhibitor of the transcriptional activity of activator protein-1 (AP-1). Recently, it was also found that curcumin and synthetic curcumin derivatives can inhibit the binding of Jun-Fos, which are the members of the AP-1 family, to DNA. However, the mechanism of this inhibition by curcumin and its derivatives was not disclosed. Since the binding of Jun-Fos dimer to DNA can be modulated by redox control involving conserved cysteine residues, we studied whether curcumin and its derivatives inhibit Jun-Fos DNA binding activity via these residues. However, the inhibitory mechanism of curcumin and its derivatives, unlike that of other Jun-Fos inhibitors, was found to be independent of these conserved cysteine residues. In addition, we investigated whether curcumin derivatives can inhibit AP-1 transcriptional activity *in vivo* using a luciferase assay. We found that, among the curcumin derivatives examined, only inhibitors shown to inhibit the binding of Jun-Fos to DNA by Electrophoretic Mobility Shift Assay (EMSA) inhibited AP-1 transcriptional activity *in vivo*. Moreover, RT-PCR revealed that curcumin derivatives, like curcumin, downregulated *c-jun* mRNA in JB6 cells. These results suggest that the suppression of the formation of DNA-Jun-Fos complex is the main cause of reduced AP-1 transcriptional activity by curcuminoids, and that EMSA is a suitable tool for identifying inhibitors of transcriptional activation.**

**Keywords:** AP-1, Curcumin, Cysteine, EMSA, Jun-Fos

*To whom correspondence should be addressed.
Tel: +82-2-878-8545; Fax: +82-2-889-1568
E-mail: chulyang@plaza.snu.ac.kr

## Introduction

Curcumin, a dietary pigment responsible for the yellow color of curry, is used as a traditionalmedicine for the treatment of inflammatory conditions (Ammon *et al*., 1991). Further, curcumin has been reported to have anti-inflammatory and anti-oxidant activities, and chemopreventive effects (Srimal *et al*., 1973; Sharma, 1976; Toda *et al*., 1985; Satoskar *et al*., 1986). *In vivo*, the administration of curcumin to mice treated with skin and colon carcinogens was found to reduce the incidence and size of tumors produced (Conney *et al*., 1991; Huang *et al*., 1992; Huang *et al*., 1994; Rao *et al*., 1995). Many molecular investigations related to curcumin have been performed. Curcumin was found to inhibit cyclooxygenase-2 transcription (Zhang *et al*., 1999) and to mediate apoptosis in AK-5 tumor cells (Bhaumik *et al*., 1999). Moreover, curcumin was reported to inhibit proliferation and cell cycle progression and to inhibit the angiogenic response stimulated by Fibroblast Growth Factor-2 (Mohan *et al*., 2000). In addition, it is well known to inhibit the transcriptional activity of AP-1 (Huang *et al*., 1991; Han *et al*., 2002).

Jun and Fos proteins are members of the AP-1 family, a family of eukaryotic transcription factors that bind DNA in a sequence specific manner. The AP-1 binding site was first identified in the enhancer regions of simian virus 40, human metallothionein (Lee *et al*., 1987). And, it is now apparent that similar nucleotide sequence motifs are present in the negative and positive regulatory regions of several genes (Distel *et al*., 1987). The sequence involved is 5'-TGAG/CTCA-3', termed TRE (tetradecanoyl phorbol acetate[TPA] response elements). To form the Jun-Fos-DNA complex, two domains of Jun and Fos are important. One is a leucine zipper domain which is responsible for the dimerization of Jun and Fos (Kouzarides *et al*., 1988), whilst the other is a DNA binding domain containing clustered basic amino acids, which is located immediately upstream of the leucine zipper and is characterized by an abundance of positively charged residues (Bohmann *et al*., 1989).

No direct clue explains the relationship between AP-1 transcriptional activator and cancer promotion. However,

some investigators have identified a variety of different tumor promoters, including phorbol esters, UV irradiation, and trace ion like $As^{3+}$ that stimulate AP-1 transcriptional activity (Angel *et al*., 1991; Devary *et al*., 1991; Devary *et al*., 1992). Therefore, chemicals capable of inhibiting the transcriptional activity of AP-1 are important from the viewpoint of tumor suppression. Recently, we showed that curcumin derivatives can inhibit the formation of Jun-Fos-DNA complex as efficiently as curcumin (Hahm *et al*., 2002); however, the inhibitory mechanism has not been elucidated. To disclose this mechanism we searched for residues of Jun-Fos dimer that interact with curcumin and curcuminoids. It is believe that reduced cysteine residues in Jun and Fos proteins enhance DNA-binding whereas oxidized residues inhibit binding (Abate *et al*., 1990b). We noticed cysteine residues (Fos-$Cys^{154}$ and Jun-$Cys^{272}$ ) in the DNA-binding domains of the two proteins, and presumed that these curcumin and curcuminoids could inhibit Jun-Fos-DNA formation by affecting the redox states of these cysteine residues. After performing *in vitro* experiments, we examined whether the inhibition of Jun-Fos-DNA complex formation by curcuminoids is related to the intracellular suppression of AP-1 transcriptional activity by curcumin and curcuminoids. Through these studies, it was possible to uncover the inhibitory mechanism by which curcuminoids inhibit AP-1 in cells, and to demonstrate that EMSA is a suitable tool for identifying inhibitors of transcription activators.

## Materials and Methods

**Materials** Curcumin was purchased from Acros Organics (2440 Geel, Belgium). Wild and mutant wbjun (224-334), and wild and mutant wbfos (116-211) genes were kindly provided by Dr. T. Curran (St Jude's Children's Research Hospital, USA). JB6 cells, stably transfected with the AP-1 luciferase gene (Ding *et al*., 2001), were kindly provided by Dr. V. Vallyathan (National Institute for Occupational Safety and Health, USA).

**Cell culture** JB6 cells were cultured in MEM supplemented with penicillin, streptomycin, neomycin and 10% FBS in a humidified 5% $CO_2$ atmosphere. AGS (gastric adenocarcinoma), HCT116, or SW480 (colon cancer) cells were grown in RPMI1640 supplemented with penicillin, streptomycin, and 10% FBS. To verify the effect of curcuminoids on AP-1, JB6 cells were treated with curcuminoids and harvested for luciferase assays and RT-PCR. SW480, HCT116, and AGS cells were harvested for MTT assays and EMSA.

**Isolation of AGS nuclear extracts** Nuclear pellets were prepared by resuspending cells in 400 μl of lysis buffer [10 mM HEPES, pH 7.8, 10 mM KCl, 2 mM $MgCl_2$, 0.1 mM EDTA], placing them on ice for 15 min, and then by vigorous mixing after adding 25 μl of 10% Nonidet P-40. After 30-s centrifugation (16,000 × *g*, 4°C), the pelleted nuclei were resuspended in 50il of extraction buffer [50 mM HEPES, pH 7.8, 50 mM KCl, 300 mM NaCl, 0.1 mM EDTA, 10% glycerol] and incubated on ice for 20 min. Nuclear extracts were stored at −70°C.

**Expression of wild and mutant truncated Jun and Fos proteins** Truncated polypeptides corresponding to amino acid residues 116-211 of Fos and 224-334 of Jun, were expressed as hexahistidine fusion proteins in E. coli and purified by nickel chelate affinity chromatography (Park *et al*., 2002). $Cysteine^{154}$ was replaced by serine in the Fos mutant (FosM), and $cysteine^{272}$ was replaced by serine in the Jun mutant (JunM) (Abate *et al*., 1990a).

**Electrophoretic mobility-shift assay (EMSA)** Nuclear extract (5 μg), prepared as described above, or truncated Jun and Fos proteins were incubated for 30min at room temperature in binding buffer [50 mM sodium phosphate (pH 7.3), 5% glycerol, 5 mM $MgCl_2$, 0.01% BSA] with curcumin or curcuminoids and a $^{32}$P-labeled probe. The inhibitory effect of curcumin and some curcuminoids were analyzed by was analyzed by nondenaturing 5% polyacrylamide gel electrophoresis in 0.5 × TBE buffer at 100 V for 40 min, and gels were visualized by autoradiography. For quantitative study, the Fos-Jun-DNA complex band on autoradiographed film was scanned and interpreted quantitatively using the *Total*Lab™ program from Phoretix (v. 1.0, NonLinear Dynamics Ltd., UK).

**Luciferase assay** A confluent monolayer of JB6 cells was trypsinized and $3 \times 10^5$ cells were seeded in 60 mm cell-culture plates, which were incubated at 37°C in a humidified atmosphere of 5% $CO_2$ for 2 days. Cells were then starved for 24 h and treated for another 5 h by culturing them in 0.5% FBS MEM containing the indicated curcuminoids (Table 1). The cells were then exposed to TPA (20 ng/ml). After 24 h in culture, the cells were extracted with 300 μl of lysis buffer and luciferase activity was measured using a luminometer. Luciferase assays were performed using the Luciferase reporter assay system as per the manufacturer's instructions (Promega, Madison, USA). For normalization, the Bradford assay was used.

**Synthesis and identification of curcumin derivatives** Curcumin derivatives were synthesized according to the method of Pabon (Pabon, 1964), and identified by $^1$H NMR, melting points, Mass Spectrometry, IR and by elemental analysis (Hahm *et al*., 2002).

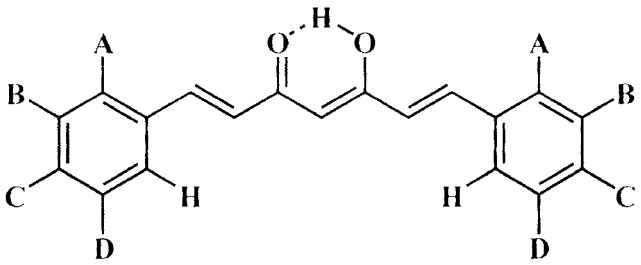
**RNA-isolation and RT-PCR** Total RNA was isolated from cells using the easy-BLUE™ RNA Extraction Kit (iNtRON, Korea), according to the manufacturer's instructions. For cDNA synthesis, 4 μg of total RNA was placed in a 25 μl reaction volume containing oligo (dT) primers and reverse transcriptase (Promega, USA), following the manufacturer's instructions. For PCR, 1/25 of the reverse transcription reaction mixture was amplified using 35 cycles for *c-jun*, 30 cycles for *c-fos* or 17 cycles for â-actin. To amplify *c-jun*, *c-fos*, and β-actin fragments 15, 20 and 30 cycles were used to determine whether the DNA amplification was linear. The PCR conditions were as follows: at 94°C for 30 s, at 55°C for 1 min, at 72°C for 1 min. All PCR products were analyzed by electrophoresis on 1.5% agarose gels and photographed. The sequences of the primers used from RT-PCR were as follows: β-actin: 5'-TCATGAAGTGTGACGTTGACATCCGT-3' (sense) and 5'-CCTAGAAGCATTTGCGGTGCACGATG-3' (antisense); *c-jun*: 5'-GCATGAGGAACCGCATCGCTGCCTCCAAGT-3' (sense) and 5'-GCGACCAAGTCCTTCCCACTCGTGCACACT-3' (antisense); *c-fos*: 5'-AAGGAGAATCCGAAGGGAAAGGAATAAGATGGCT (sense) and 5'-CCTAGAAGCATTTGCGGTGCACGATG (antisense)

**MTT assay** The MTT assay is based on the ability of mitochondria in live cells to oxidize thiazolyl blue, a tetrazolium salt (MTT; Sigma, St. Louis, USA), to an insoluble blue formazan product (Shim *et al*., 2002). To determine cell growth rates, cells were counted, and $7 \times 10^3$ were plated in 96-well culture plates (in octuplicate) in RPMI-1640 medium 24 h prior to being treated with curcumin or curcuminoids for 3 days. Cells were then washed with PBS, and incubated with MTT (0.1 mg) at 37°C for 3 h. The reagent was then removed, and 150 μl DMSO was added to each well. The optical density of the solution was read at 540 nm in an enzyme-linked immunosorbent assay (enzyme-linked immunosorbent assay) plate reader. Since the generation of the blue product is proportional to mitochondrial dehydrogenase activity, decreases in absorbance at 540 nm provided a direct measure of cell death.

## Results

**Curcuminoids can inhibit the formations of Jun-Fos-DNA and endogenous AP-1-DNA** Eleven curcuminoids were synthesized using Pabon's method (Pabon, 1964); their structures are shown in Table 1. We examined whether these curcuminoids could inhibit the binding of a Jun-Fos dimer to DNA, using Jun and Fos truncated polypeptides, which both contained a leucine zipper domain and a DNA binding domain. These were expressed in *E. coli*(BL21) and purified by nickel affinity chromatography. Curcumin and three of the 11 curcuminoids (CHC007, CHC009 and CHC010) inhibited the formation of Jun-Fos-DNA (Fig. 1A). It has already been reported that the $IC_{50}$ of curcumin, CHC007, and CHC009 with respect to Jun-Fos dimmer DNA bindingare 0.48 mM, 0.38 mM, and 0.64 mM respectively (Hahm *et al*., 2002). In our experiment, excessive inhibitor quantities were used to produce complete inhibition. The ability of these curcuminoids to inhibit the formation of AP-1-DNA complexes was tested using AGS (a gastric cancer cell-line) nuclear extracts, which contained endogenous AP-1. In was observed that thesecurcuminoids inhibited the formation of endogenous AP-1-DNA completely, as did the truncated proteins (Fig. 1B).

**Table 1.** The functional groups of curcumin derivatives




| Inhibitors | A | B | C | D |
|---|---|---|---|---|
| CHC001 | H | $OCH_3$ | H | H |
| CHC002 | H | $OCH_3$ | $OCH_3$ | $OCH_3$ |
| CHC003 | H | $OCH_3$ | H | $OCH_3$ |
| CHC004 | H | $OCH_3$ | $OCH_3$ | H |
| CHC005 | H | H | $OCH_3$ | H |
| CHC006 | H | H | H | H |
| CHC007 | H | $NO_2$ | OH | H |
| CHC008 | H | OH | H | H |
| CHC009 | H | $NO_2$ | H | H |
| CHC010 | $NO_2$ | H | H | H |
| CHC011 | H | H | $NO_2$ | H |
| Curcumin | H | $OCH_3$ | OH | H |

**The binding of mutated Jun-Fos dimer to DNA was inhibited by curcumin and curcuminoids, but not by selenium** The inhibitory effects of curcumin and curcuminoids were tested using mutated Jun (JunM) and Fos (FosM) truncated polypeptides. $Cys^{272}$ was replaced by $Ser^{272}$ in JunM, and $Cys^{154}$ was replaced by $Ser^{154}$ in FosM. JunM and FosM were able to dimerize like native Jun and Fos. Moreover, the binding of cysteine-to-serine mutants to DNA is known not to be abolished by oxidizing agents, unlike that of the native

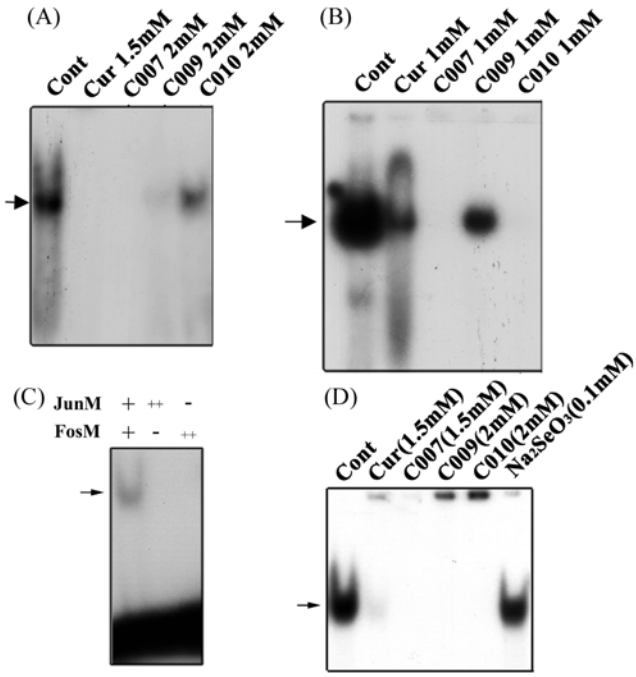



**Fig. 1.** The inhibitory effects of curcumin and curcuminoids on truncated Jun-Fos dimer, endogenous AP-1, and truncated JunM-FosM dimer. EMSA was performed by incubating truncated proteins (A,C,D) or AGS nuclear extracts (B) and $^{32}$P-labeled doubled-stranded AP-1 oligonucleotide with the inhibitors indicated above the gel picture for 30 min at room temperature. All inhibitors were dissolved in DMSO. Arrowheads indicate the positions of the retarded protein-DNA complexes representing the truncated Jun-Fos dimer(A) or Ed-confirm OK -please take care truncated JunM-FosM dimer(C,D) or AP-1 endogenous proteins (B). Curcumin and its derivatives inhibited the formation of JunM-FosM-DNA complex (D), and of Jun-Fos-DNA complex (A). They also suppressed the binding of endogenous AP-1 to DNA(B), indicating that the inhibitory mechanism involved is independent of cysteine residues. Cont; treated with DMSO alone.

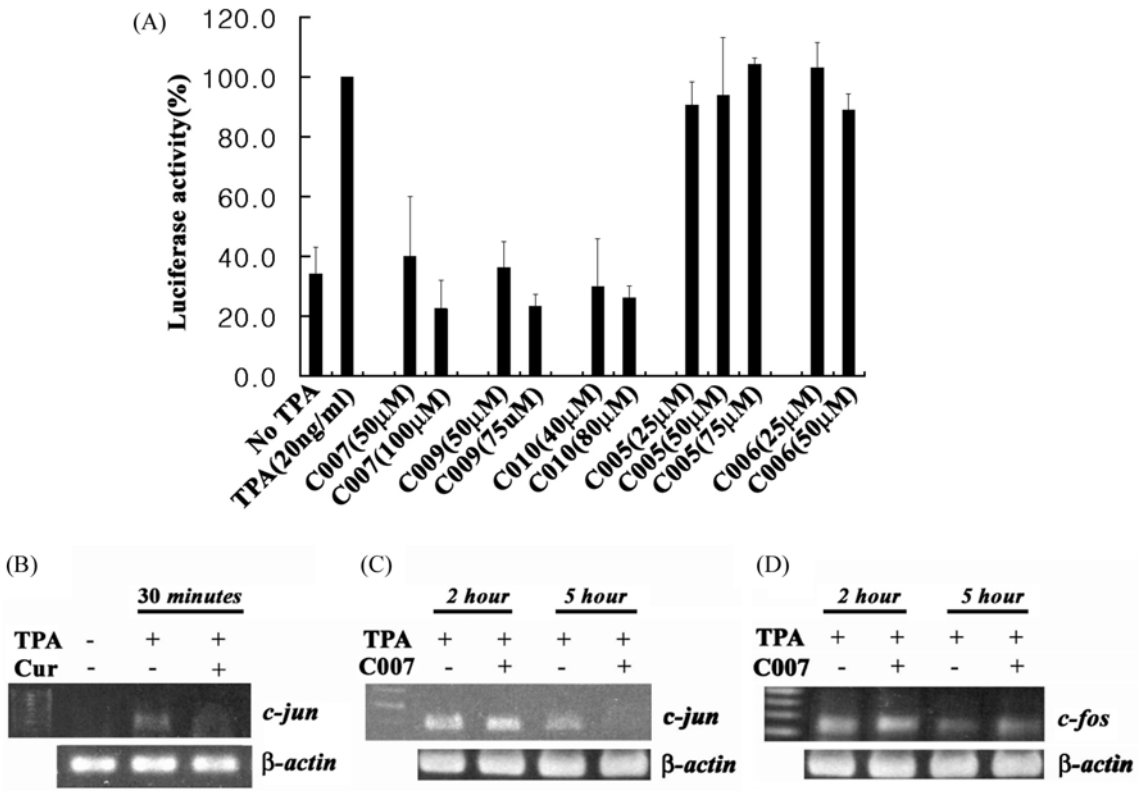



**Fig. 2.** Curcumin and Curcuminoids inhibit TPA-induced AP-1 transcriptional activity and downregulate *c-jun* mRNA, not *c-fos* mRNA, in JB6 cells. (A)JB6 cells, which were stably transfected with AP-1/luciferase, were treated with curcuminoids. Ed-pl. consider - only altered here in the paper. Bradford assay was used for normalization. JB6 cells were cultured and treated as described in *Materials and Methods*. Data are means±SD of triplicate experiments. Species that suppressed Jun-Fos-DNA complex formation *in vitro,* efficiently downregulated the transcriptional activity of AP-1. (B,C,D) A confluent monolayer of JB6 cells was trypsinized and $3 \times 10^5$ cells were seeded in 60mm cell-culture plates. The RT-PCR procedure is described in detail in *Materials and Methods*. Curcumin and C007 downregulated *c-jun*, but not *c-fos* transcripts levels. Lane 1, in Fig. 2 represent the DNA marker.

proteins (Abate *et al*., 1990b). Initially, we investigated whether that the band observed on EMSA gel was that of a JunM-FosM heterodimer or a JunM-JunM homodimer. As shown in Fig. 1C, no band appeared in the presence of only JunM (lane 2) or FosM (lane 3); however, in the presence of both JunM and FosM, the band appeared, thus indicating that the band in lane1 was of JunM-FosM heterodimer and not JunM-JunM homodimer. Excessive amounts of inhibitors (curcumin, C007, C009 and C010), i.e., exceeding their inhibitory $IC_{50}$'s, were added to JunM-FosM dimer. Although selenium, a well known Jun-Fos inhibitor, did not inhibit JunM-FosM dimer formation, the curcuminoids inhibited JunM-FosM-DNA complex formation, as did curcumin (Fig. 1D).

**Inhibition of the transcriptional activity of AP-1 by curcuminoids in JB6 cells** Luciferase assays were performed to determine whether curcuminoids can act as AP-1 inhibitors intracellulary JB6 cells, stably transfected with the AP-1luciferase gene, were treated with CHC007, CHC009 or CHC010, inhibitors of Jun-Fos dimer in an *in vitro* experiment, and with CHC005 and CHC006, which had much higher $IC_{50}$'s that the former inhibitors, 24 h prior to performing the luciferase assay. The $IC_{50}$'s of CHC005 and CHC006 against Jun-Fos dimer were >2.50 mM. The amount of luciferase expressed in the cells treated with CHC007, C009 and C010 decreased markedly in a concentration dependent manner. On the other hand, CHC005 and CHC006 did not reduce the amount of luciferase (Fig. 2A), indicating that the transcriptional activity of AP-1 in cells was reduced only by curcuminoids known to efficiently inhibit Jun-Fos activity *in vitro*.

**Curcumin or CHC007 reduced TPA-induced c-jun mRNA levels** To investigate the effect of CHC007 on *c-jun* and *c-fos* transcription, we used RT-PCR (Fig. 2). It is known that curcumin downregulates *c-jun* mRNA and that *c-fos* mRNA is not affected by curcumin in NIH3T3 cells (Huang *et al*., 1991). As shown in Fig. 2B, curcumin inhibited *c-jun* transcription in JB6 cells. To verify the effect of CHC007 on *c-jun* and *c-fos* mRNA, JB6 cells were treated with CHC007 for 5 h prior to TPA treatment. After 2 h or 5 h, RNA was isolated for RT-PCR. Figure 2C shows that the effect of CHC007 on *c-jun* mRNA was manifest after 5 h, that is, CHC007 suppressed *c-jun* transcription. However, CHC007 did not affect *c-fos* mRNA levels (Fig. 2D).

**Cytotoxicity of curcuminoids in SW480, HCT116, and AGS cancer cell** To investigate the potential of using curcuminoids as candidate anti-cancer agents, we adopted an MTT assay approach (Fig. 3). Cancer cells (AGS, SW480, or HCT116) were incubated with curcumin or curcuminoids for 72 hr. Curcumin, CHC009, or CHC010 showed high cytotoxicities in SW480 (a colon cancer cell-line) cells at 20 μM; however, most SW480 cells remained viable in the presence of CHC007 even at 50 μM. Similar results were obtained for AGS gastric cancer cells and HCT116 colon cancer cells.

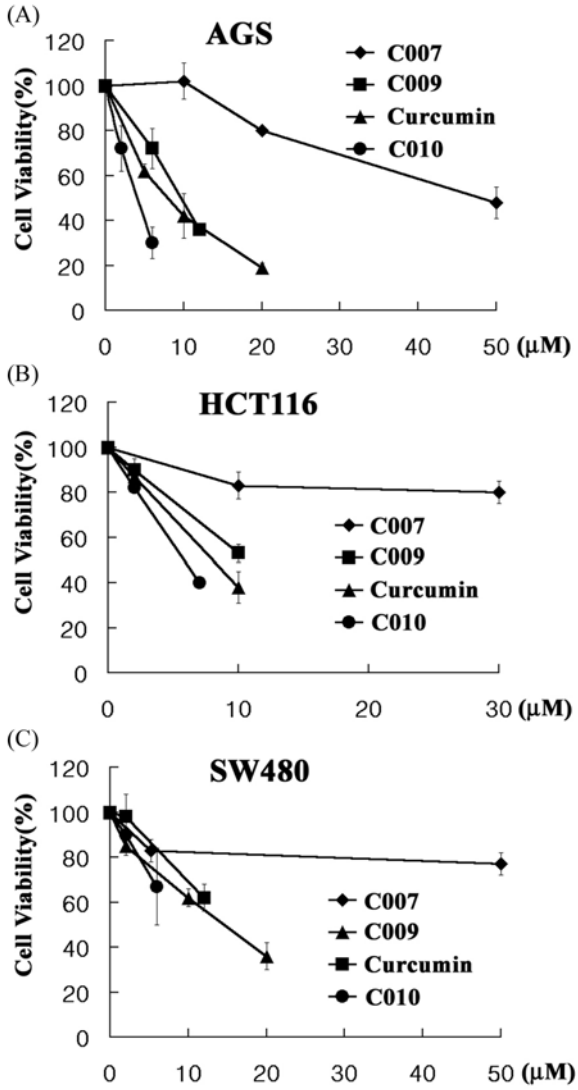



**Fig. 3.** Curcumin and curcuminoids had high cytotoxicity but CHC007 had little cytotoxicity in cancer cell-lines. To evaluate cell growth rates, cells were counted, and $7 \times 10^3$ were plated in 96-well culture plates in RPMI-1640 medium 24 h before being treated with curcumin or curcuminoids for 72 h. MTT assays was performed as described in *Materials and Methods*. Curcumin and the curcuminoids tested showed high cytotoxicity against cancer cells, except CHC007. Data are the means ± SD of triplicate experiments.

## Discussion

Recently, there has been a marked increase in interest in curcumin derivatives. Ohtsu *et al.* reported that curcumin derivatives possessing a methoxy group are novel androgen receptor antagonists with anti-prostate cancer potential (Ohtsu *et al.*, 2002). In addition, it has been reported that some curcuminoids can inhibit the binding of Jun-Fos dimer to DNA more efficiently than curcumin. To understand their inhibitory mechanism *in vitro*, JunM(Cysteine → Serine) and FosM (Cysteine → Serine) were used. Cysteine residues in the DNA binding domains of Jun and Fos proteins are known to play important roles in DNA binding by Jun-Fos dimers (Abate *et al.*, 1990b), which bind to DNA even in the absence of a reducing agent. Curcumin and its derivatives were reported to have anti-oxidant effects *in vitro* and *in vivo* (Sugiyama *et al.*, 1996). However, other reports claim that curcumin has a pro-oxidizing nature and that it is capable of oxidative DNA cleavage, particularly in the presence of transition metal ions such as copper (Rahman *et al.*, 1989). In addition, Nikitovic *et al.* and Handel *et al.* reported that nitric oxide, gold, or selenium have an AP-1 inhibitory effect that is associated with cysteine residues, suggesting the thiol group oxidation. In these papers, the presence of this inhibitory mechanism was demonstrated by showing that NO, gold (I) thiomalate (AuTM), and $Na_2SeO_3$ all inhibited the formation of a Jun-Fos-DNA complex, but not the formation of a JunM-FosM-DNA complex (Handel *et al.*, 1995; Nikitovic *et al.*, 1998). Based on these results, we presumed that curcumin could oxidize the thiol group in the reduced cysteine residues in Jun and Fos proteins and thus inhibit the binding of Jun-Fos dimer to DNA. To investigate this presumption, curcumin and some curcuminoids, including CHC007, CHC009, and CHC010, which have been reported to inhibit the formation of Jun-Fos-DNA *in vitro*, were investigated to determine whether they have an inhibitory effect on JunM-FosM dimer and on Jun-Fos dimer. We found that curcumin and the curcuminoids, C007, C009 and C010, inhibited JunM-FosM-DNA formation (Fig. 1). This result indicates that curcumin and these curcuminoids, unlike nitric oxide, gold, or selenium, block the ability of Jun-Fos dimer to bind DNA regardless of the presence of conserved cysteine residues. This finding indicates that the mechanism whereby curcumin or curcuminoids inhibit Jun-Fos dimer differs from that of other well known AP-1 inhibitors. In order to clarify the details of this inhibitory mechanism, further studies are needed.

Next, we tested the inhibitory effect of curcuminoids on the oncogenic transcriptional activity of AP-1 *in vivo*. For this study, JB6 cells stably transfected with AP-1luciferase gene (Ding *et al.*, 2001) were used. Cells were treated with curcuminoids and then with TPA, and luciferase expressions were measured. The levels of luciferase in C007, C009, and C010 treated cells were significantly lower than that in DMSO treated cells. On the other hand, C005 and C006,

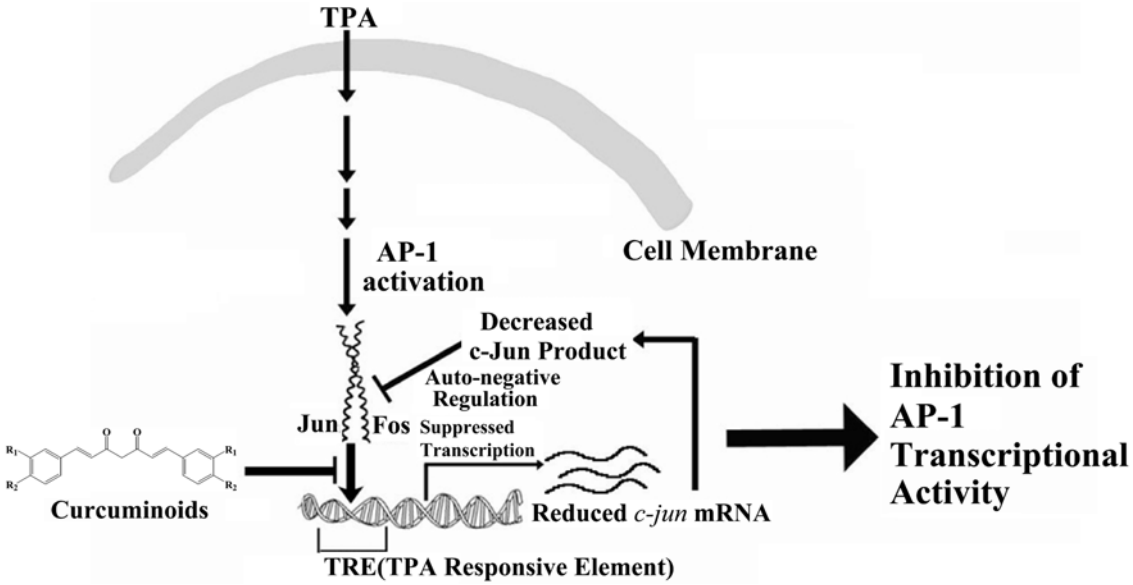



**Fig. 4.** Mechanism of AP-1 inhibition by curcuminoids.

which had little inhibitory effect on Jun-Fos dimer by EMSA, did not inhibit AP-1 transcriptional activity (Fig. 2A). This result has two important implications; first, the inhibition of AP-1 transcriptional activity by curcumin or curcuminoids, *in vivo*, is due to interference with the formation of the Jun-Fos-DNA complex. Huang *et al*. showed that curcumin suppresses AP-1 activity in NIH3T3 cells (Huang *et al*., 1991), and Hahm *et al*. reported that curcumin inhibits Jun-Fos-DNA complex formation *in vitro* (Hahm *et al*., 2002). The present study shows for the first time an important relationship between these two earlier studies, that is to say, it demonstrates that the inhibition of Jun-Fos to DNA binding by curcumin or curcuminoids is a direct cause of AP-1 inhibition *in vivo*. Secondly, the study also demonstrates that EMSA is an excellent tool for determining the identities of species that inhibit transcriptional activators, because EMSA can test protein to DNA binding, which is the primary function of a transcription activator.

To determine the cytotoxicities of curcuminoids that inhibit AP-1 transcriptional activity, we used the MTT assay (Fig. 3). Gastric and colon cancer cell lines treated with curcumin died at a curcumin concentration of 10ìM, and other curcumin derivatives also showed excellent cytotoxicity at *ca.* 20ìM. These data indicate that curcumin and curcumin derivatives have anti-cancer potential.

In addition, RT-PCR showed that one of the curcuminoids, CHC007, like curcumin, suppressed *c-jun* transcription (Fig. 2C). Since *c-jun* is the target gene of AP-1 (Angel *et al*., 1988), *c-jun* mRNA attenuation was attributed to suppressed AP-1 transcriptional activity by curcumin or curcuminoids. Because it is known that *c-jun* is positively autoregulated by its product c-Jun/AP-1(Angel *et al*., 1988), we were able to summarize the mechanism underlying AP-1 inhibitory by curcuminoids, as shown in Fig. 4. Summarizing, curcumin or curcuminoids on entering a cell (observed by fluorescence microscopy - data not shown) interfere with AP-1/DNA binding inducedby TPA activation, regardless of cysteine residues in Jun and Fos proteins. This reduced DNA/AP-1 binding leads to inhibited AP-1 transcriptional activity and to the suppression of AP-1 target genes, including *c-jun*, which reduces the level of c-Jun product, which reinforces reduced AP-1 transcriptional activity (Fig. 4).

**Acknowledgments** We thank Dr. T. Curran (St. Jude's Children's Research Hospital, USA) for generously donating the wild and mutant wbjunand wbfos genes, and Dr. V. Vallyathan (National Institute for Occupational Safety and Health, USA) for the JB6 cells stably transfected with AP-1 luciferase. This research was supported by the Korean Ministry of Science and Technology (Grant no., KRF 2001-015-DP0344). We also gratefully acknowledge the financial support provided by the Brain Korea 21 program.

## References

Abate, C., Luk, D., Gentz, R., Rauscher, F. J. III. and Curran, T. (1990a) Expression and purification of the leucine zipper and DNA-binding domains of Fos and Jun: Both Fos and Jun contact DNA directly. *Proc. Natl. Acad. Sci. USA* **87**, 1032-1036.

Abate, C., Patel, L., Rauscher, F. J. III. and Curran, T. (1990b) Redox regulation of fos and jun DNA-binding activity *in vitro*. *Science* **249**, 1157-1161.

Ammon, H. P. and Wahl, M. A. (1991) Pharmacology of Curcuma longa. *Plata. Med.* **57**, 1-7.

Angel, P., Hattori, K., Smeal, T. and Karin, M. (1988) The jun proto-oncogene is positively autoregulated by its product, Jun/AP-1. *Cell* **55**, 875-885.

Angel, P. and Karin, M. (1991) The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. *Biochim. Biophys. Acta.* **1072**, 129-157.

Bhaumik, S., Anjum, R., Rangaraj, N., Pardhasaradhi, B. V. and Khar, A. (1999) Curcumin mediated apoptosis in AK-5 tumor cells involves the production of reactive oxygen intermediates. *FEBS Lett.* **456**, 311-314.

Bohmann, D. and Tjian, R. (1989) Biochemical Analysis of Transcriptional Activation by Jun: differential activity of C- and v-Jun. *Cell* **59**, 709-717.

Conney, A. H., Lysz, T., Ferraro, T., Abidi, T. F., Manchand, P. S., Laskin, J. D. and Huang, M. T. (1991) Inhibitory effect of curcumin and some related dietary components on tumor promotion and arachidonic acid metabolism in mouse skin. *Adv. Enzyme Regul.* **31**, 385-396.

Devary, Y., Gottlieb, R. A., Lau, L. F. and Karin, M. (1991) Rapid and preferential activation of the *c-jun* gene during the mammalian UV response. *Mol. Cell. Biol.* **11**, 2804-2811.

Devary, Y., Gottlieb, R. A., Smeal, T. and Karin, M. (1992) The Mammalian ultraviolet response is triggered by activation of src tyrosine kinases. *Cell* **71**, 1081-1091.

Ding, M., Shi, X., Lu, Y., Huang, C., Leonard, S., Roberts, J., Antonini, J., Castranova, V. and Vallyathan, V. (2001) Induction of activator protein-1 through reactive oxygen species by crystalline silica in JB6 cells. *J. Biol. Chem.* **276**, 9108-9114.

Distel, R. J., Ro, H. S., Rosen, B. S., Groves, D. L. and Spiegelman, B. M. (1987) Nucleoprotein complexes that regulate gene expression in adipocyte differentiation: direct participation of c-fos. *Cell* **49**, 835-844.

Hahm, E. R., Cheon, G., Lee, J. H., Kim, B. J., Park, C. H. and Yang, C. H. (2002) New and known symmetrical curcumin derivatives inhibit the formation of Fos-Jun-DNA complex. *Cancer Lett.* **184**, 89-96.

Han, S. S., Keum, Y. S., Soe, H. J. and Surh, Y. J. (2002) Curcumin suppresses activation of NF-κB and AP-1 induced by phorbol ester in cultured human promyelocytic leukemia cells. *J. Biochem. Mol. Biol.* **35**, 337-342.

Handel, M. L., Watts, C. K., DeFazio, A., Day, R. O. and Sutherland, R. L. (1995) Inhibition of AP-1 binding and transcription by gold and selenium involving conserved cysteine residues in Jun and Fos. *Proc. Natl. Acad. Sci. USA* **92**, 4497-4501.

Huang, M. T., Deschner, E. E., Newmark, H. L., Wang, Z. Y., Ferraro, T. A. and Conney, A. H. (1992) Effect of dietary curcumin and ascorbyl palmitate on azoxymethanol-induced colonic epithelial cell proliferation and focal areas of dysplasia. *Cancer Lett.* **64**, 117-121.

Huang, M. T., Lou, Y. R., Ma, W., Newmark, H. L., Reuhl, K. R. and Conney, A. R. (1994) Inhibitory effects of dietary curcumin on forestomach, duodenal, and colon carcinogenesis in mice. *Cancer Res.* **54**, 5841-5847.

Huang, T. S., Lee, S. C. and Lin, J. K. (1991) Suppression of *c-jun*/AP-1 activation by an inhibitor of tumor promotion in mouse fibroblast cells. *Proc. Natl. Acad. Sci. USA* **88**, 5292-5296.

Kouzarides, T. and Ziff, E. (1988) The role of the leucine zipper in the fos-jun interaction. *Nature* **336**, 646-651.

Lee, W., Mitchell, P. and Tjian, R. (1987) Purified transcription factor AP-1 interacts with TPA-inducible enhancer elements. *Cell* **49**, 741-752.

Mohan, R., Sivak, J., Ashton, P., Russo, L. A., Pham, B. Q., Kasahara, N., Raizman, M. B. and Fini, M. E. (2000) Curcuminoids inhibit the angiogenic response stimulated by fibroblast growth factor-2, including expression of matrix metalloproteinase gelatinase B. *J. Biol. Chem.* **275**, 10405-10412.

Nikitovic, D., Holmgren, A. and Spyrou, G. (1998) Inhibition of AP-1 DNA binding by nitric oxide involving conserved cysteine residues in Jun and Fos. *Biochem. Biophys. Res. Commun.* **242**, 109-112.

Ohtsu, H., Xiao, Z., Ishida, J., Nagai, M., Wang, H. K., Itokawa, H., Su, C. Y., Shih, C., Chiang, T., Chang, E., Lee, Y., Tsai, M. Y., Chang, C. and Lee, K. H. (2002) Antitumor Agents. 217. Curcumin analogues as novel androgen receptor antagonists with potential as anti-prostate cancer agents. *J. Med. Chem.* **45**, 5037-5042.

Pabon, H. J. J. (1964) A synthesis of curcumin and related compounds. *Rec. Trav. Chim. Pays. Bas.* **83**, 379-386.

Park, J. E., Lee, K. Y., Do, S. I. and Lee, S. S. (2002) Expression and characterization of β-1,4-galactosyltransferase from *Neisseria meningitidis* and *Neisseria gonorrhoeae*. *J. Biochem. Mol. Biol.* **35**, 330-336.

Rahman, A., Shahabuddin, H. S. M., Parish, J. H. and Ainley, K. (1989) Strand scission in DNA induced by quercetin and Cu(II): role of Cu(I) and oxygen free radicals. *Carcinogenesis* **10**, 1833-1839.

Rao, C. V., Rivenson, A., Simi, B. and Reddy, B. S. (1995) Chemoprevention of colon carcinogenesis by dietary curcumin, a naturally occurring plant phenolic compound. *Cancer Res.* **55**, 259-266.

Satoskar, R. R., Shah, S. J. and Shenoy, S. G. (1986) Evaluation of anti-inflammatory property of curcumin (dieferuloyl methane) in patients with postoperative inflammation. *Int. J. Clin. Pharmacol. Ther. Toxicol.* **24,** 651-654.

Sharma, O. P. (1976) Antioxidant activity of curcumin and related compounds. *Biochem. Pharmacol.* **25**, 1811-1812.

Shim, M. J., Kim, H. J., Yang S. J., Lee, I. S., Choi, H. I. and Kim, T. U. (2002) Arsenic trioxide induces apoptosis in chronic myelogenous leukemia K562 cells: possible involvement of p38 MAP kinase. *J. Biochem. Mol. Biol.* **35**, 377-383.

Singh, A. K., Sidhu, G. S., Deepa, T. and Maheshwari, R. K. (1996) Curcumin inhibits the proliferation and cell cycle progression of human umbilical vein endothelial cell. *Cancer Lett.* **107**, 109-115.

Srimal, R. C. and Dhawan, B. N. (1973) Pharmacology of diferuloyl methane (curcumin), a non-steroidal anti-inflammatory agent. *J. Pharm. Pharmacol.* **25**, 447-452.

Sugiyama, Y., Kawakishi, S. and Osawa, T. (1996) Involvement of the beta-diketone moiety in the antioxidative mechanism of tetrahydrocurcumin. *Biochem. Pharmacol.* **52**, 519-525.

Toda, S., Miyase, T., Arichi, H., Tanizawa, H. and Takiyano, Y. (1985) Natural antioxidants, III: antioxidative components isolated from rhizome of curcuma longa L. *Chem. Pharm. Bull.* **33**, 1725-1728.

Zhang, F., Altorki, N. K., Mestre, J. R., Subbaramaiah, K. and Dannenberg, A. J. (1999) Curcumin inhibits cyclooxygenase-2 transcription in bile acid - and phorbol ester-treated human gastrointestinal epithelial cells. *Carcinogenesis* **20**, 445-451.