Arch Pharm Res Vol 24, No 4, 349-354, 2001

Archives of Pharmacal Research
http://apr.psk.or.kr

Inhibition of Invasion and Induction of Apoptosis by Curcumin in H-ras-Transformed MCF10A Human Breast Epithelial Cells

Mi-Sung Kim, Hye-Jung Kang, and Aree Moon

College of Pharmacy, Duksung Women's University, Seoul 132-714, Korea

(Received June 5, 2001)

Curcumin, a dietary pigment in turmeric, posseses anti-carcinogenic and anti-metastatic properties. The present study was conducted to study *in vitro* chemopreventive effects of curcumin in transformed breast cells. Here, we show that curcumin inhibits H-ras-induced invasive phenotype in MCF10A human breast epithelial cells (H-ras MCF10A) and down-regulates matrix metalloproteinase (MMP)-2 dose-dependently. Curcumin exerted cytotoxic effect on H-ras MCF10A cells in a concentration-dependent manner. Curcumin-induced cell death was mainly due to apoptosis in which a prominent downregulation of Bcl-2 and upregulation of Bax were involved. We also suggest a possible involvement of caspase-3 in curcumin-induced apoptosis. Curcumin treatment resulted in the production of reactive oxygen species (ROS) in H-ras MCF10A cells. Apoptotic event by curcumin was significantly inhibited by pretreatment of an antioxidant *N*-acetyl-$_L$-cysteine (NAC), suggesting redox signaling as a mechanism responsible for curcumin-induced apoptosis in H-ras MCF10A cells. Taken together, our results demonstrate that curcumin inhibits invasion and induces apoptosis, proving the chemopreventive potential of curcumin.

Key words: Curcumin, Invasion, Apoptosis, Bcl-2, Bax, ROS

INTRODUCTION

A naturally occurring dietary pigment from the root of the plant turmeric (*Curcuma longa Linn*), curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)1,6-hepta-diene-3,5-dione] (depicked in Fig. 1), has been used as a coloring agent and/or spice in foods as well as in cosmetics and drugs (Wattenberg, 1990). Curcumin exhibits a wide range of biological and pharmacological activities including anti-cancer (Pereira *et al.*, 1996; Chun *et al.*, 1999), anti-inflammatory (Huang *et al.*, 1991) and anti-metastatic (Menon *et al.*, 1999) activities. Curcumin was demonstrated to inhibit SK-Hep-1 hepatocellular carcinoma cell invasion and suppress matrix metalloproteinase (MMP)-9 secretion (Lin *et al.*, 1998). Recent studies from our laboratory suggested a possible role of MMP-2, rather than MMP-9, in curcumin-induced invasive phenotype in human breast epithelial cells (MCF10A) transformed with H-ras (Kim and Moon, 1999; Moon *et al.*, 2000).

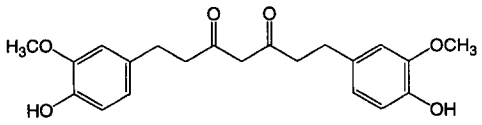

Fig. 1. Chemical structure of curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)1,6-hepta-diene-3,5-dione]

Efforts have been made to develop a chemoprevention strategy that triggers apoptosis in malignant/premalignant cancer cells (Kong *et al.*, 2000). Curcumin has been shown to exert anti-apoptotic activities in various cells including prostate (Dorai *et al.*, 2001), breast (Ramachandran and You, 1999) and rat histiocytic (Anjum and Khar, 2001) cells. The production of reactive oxygen species (ROS) was reported to be involved in curcumin-mediated apoptosis in AK-5 tumor cells (Bhaumik *et al.*, 1999). Caspase-3 was known to play an essential role in apoptosis induced by a variety of stimuli (Porter and Janick, 1999). Bcl-2, an anti-apoptotic oncoprotein, has been shown to act on mitochondria and prevent the release of cytochrome c and thus caspase activation (Yang *et al.*, 1997). Bax counteracts the anti-apoptotic effects of Bcl-2 by forming a heterodimer with Bcl-2 (Kobayashi *et al.*, 1998).

Correspondence to: Dr. Aree Moon, College of Pharmacy, Duksung Womens University 419 Ssangmun-Dong, Tobong-Ku, Seoul 132-714, Korea
E-mail: armoon@center.duksung.ac.kr

In the present study, we attempted to study *in vitro* chemopreventive effects of curcumin in transformed breast cells. Here, we report that curcumin inhibits invasive phenotype in parallel with a specific downregulation of MMP-2 in a dose-dependent manner. We also show that curcumin efficiently inhibited H-ras MCF10A cell growth through induction of apoptosis in which downregulation of Bcl-2, upregulation of Bax, caspase-3 activation and redox signaling were possibly involved.

MATERIALS AND METHODS

Cell lines and culture condition

Establishment of H-ras MCF10A cells was previously described (Moon *et al.*, 2000). Cells were cultured in DMEM/F12 supplemented with 5% horse serum, 0.5 μg/ml hydrocortisone, 10 μg/ml insulin, 2 ng/ml EGF, 0.1 μg/ml cholera enterotoxin, 2 mM L-glutamine, 100 units/ml penicillin-streptomycin and 0.5 μg/ml fungizone. Cells were cultured in a humidified 5% CO_2 incubator at 37°C.

Materials

Curcumin was purchased from Sigma Co. (St. Louis, MO) and resuspended in DMSO to make 100 mM stock solution which was diluted with PBS before treatment. A caspase inhibitor, Ac-DEVD-CHO were purchased from Takara Co. (Shiga, Japan). *N*-acetyl-$_L$-cysteine (NAC) was from Sigma Co.

In vitro invasion assay

In vitro invasion assay was performed as described previously (Kim *et al.*, 2000) using a 24-well transwell unit with polycarbonate filters, 6.5 mm in diameter and a pore size of 8.0 μm (Corning Costar, Cambridge, MA). Fifty thousand cells pretreated with various concentrations of curcumin for 48 h were resuspended in 100 μl of DMEM/F12 and placed in the upper part of a transwell plate. The curcumin-pretreated cells were incubated for 17 h in the absence of curcumin and the invasive phenotypes were determined by counting the cells that migrated to the lower side of the filter with microscopy at ×400. Thirteen fields were counted for each assay. Each sample was assayed in triplicate.

Immunoblot analysis for Bcl-2 and Bax

Equal amounts (30 μg) of protein extracts in lysis buffer (0.5% Triton X-100, 0.15M NaCl, 50 mM Tris-HCl, pH7.4, 25 mM NaF, 20 mM EGTA, 1 mM DTT, 1 mM Na_3VO_4) containing protease inhibitor cocktail (Roche, Mannheim, Germany) were subjected to 12% SDS-PAGE analysis and transferred to nitrocellulose membrane. The levels of Bcl-2 and Bax were detected using anti-Bcl-2 (mouse monoclonal purchased from DAKO, Denmark) and anti-Bax (rabbit polyclonal from Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) antibodies.

MTT assay

Cells (1×10^4) cultured in a 96-well plate were treated with curcumin for 20 h. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) solution (5 mg/ml) was added to the media and the cells were further incubated for 4 h. After 100 μl of supernatant was replaced with 100 μl of DMSO, absorbance of each well was read at 540 nm with a micro-ELISA reader (Molecular Devices, Sunnyvale, CA). Percent of cell survival was defined as the relative absorbance of treated versus untreated cells.

DNA Fragmentation Assay

Cells in a 100-mm dish were treated with 50 μM curcumin for 48 h, trypsinized and collected with ice-cold PBS. After centrifugation (2,000 g) for 10 min at 4°C, cells were transferred to an eppendorf tube and recentrifuged at 15,000 rpm for 15 min at 4°C. Cell pellets were resuspended in 0.5-1 ml of isolation buffer (10 mM EDTA, 50 mM Tris-HCl, pH 8.0, 0.5% SDS, 0.5 mg/ml proteinase K) and incubated overnight at 50°C. The lysate was centrifuged at 15,000 rpm for 15 min at 4°C to separate the soluble fragmented DNA from the intact chromatin pellet. DNA was extracted with phenol/chloroform/isoamylalcohol (25:24:1) and precipitated with ethanol. Purified DNA was treated with 1μg/ml RNase A for 1 h at 37°C prior to electrophoresis on a 1.8% agarose gel containing ethidium bromide.

Assay of intracellular ROS

Dishes of confluent H-ras MCF10A cells treated with various concentrations of curcumin for 1 h were washed with serum-free DMEM/F12 media containing 5 mM 2',7'-Dichlorofluorescin diacetate (DCFH-DA) and incubated in a humidified atmosphere of 5% CO_2 at 37°C for 5 min (Bass *et al.*, 1983; Bae *et al.*, 1997). Culture dishes were transferred to a fluorescence microscope (Olympus 1 × 70, Olympus optical Co, Japan) and ROS generation was detected as a result of the oxidation of DCFH. All experiments were repeated at least three times.

RESULTS

Curcumin inhibits invasion and downregulates MMP-2 dose-dependently

To ascertain the effect of curcumin on invasion of transformed breast cells, *in vitro* invasion assay was performed on highly invasive H-ras MCF10A cells. As shown in Fig. 2, treatment of curcumin for 48 h significantly reduced the number of invaded cells through a reconstituted base-

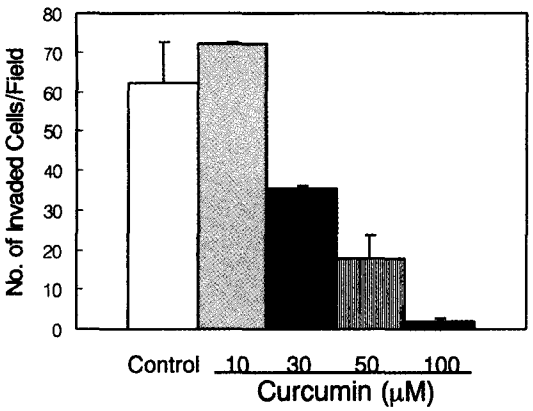

Fig. 2. Curcumin inhibits invasive phenotype of H-ras MCF 10A cells. Fifty thousand viable cells pretreated with curcumin for 48 hr were incubated in a transwell chamber for 17 h. Numbers of invaded cells per field were counted under 400x light microscopy. The mean values of triplicates were plotted and the error bars represent standard deviation of the mean of triplicate.

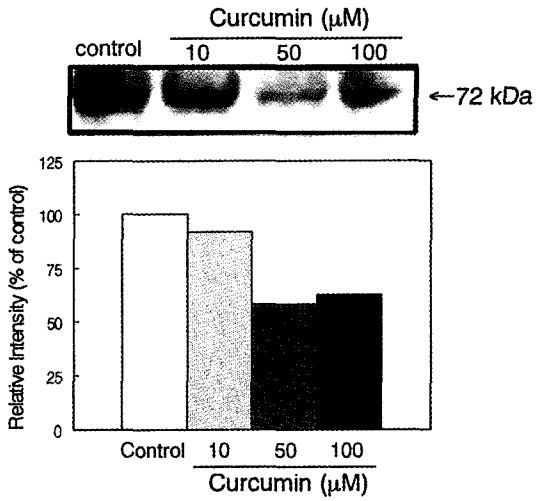

Fig. 3. Curcumin downregulates MMP-2 dose-dependently in H-ras MCF10A cells. Cells were treated with curcumin for 24 h and the conditioned media were subjected to the Immunoblot analysis using anti-MMP-2 antibody.

ment membrane in a dose-dependent manner. Because *in vitro* invasiveness depends on the number of viable cells, we examined the viability of curcumin-pretreated cells to exclude the possibility that the observed inhibition of H-ras-induced invasion was due to a growth effect. Survival of the curcumin-pretreated cells during 17 h (the same period of time for invasion assay) was 98% of control cells, indicating that the inhibition of invasion was not due to cytotoxic effect of curcumin. These findings demonstrate that curcumin inhibits H-ras-induced invasion in MCF10A cells.

We have previously shown that secretion of MMP-2, but not that of MMP-9, was significatly inhibited by curcumin treatment in H-ras MCF10A cells as evidenced by gelatin zymography (Kim and Moon, 1999). In this study, a dose response analysis of Western blotting was conducted to confirm the association of MMP-2 in the anti-invasive effect of curcumin in H-ras MCF10A cells. MMP-2 was downregulated by treatment of curcumin dose-dependently in H-ras MCF10A cells (Fig. 3). A prominent inhibition of MMP-2 activity was observed in H-ras MCF10A cells treated with 50 μM and 100 μM of curcumin. The data demonstrate that curcumin inhibits invasive phenotype of H-ras MCF10A human breast epithelial cells in which MMP-2 is possibly involved.

Curcumin downregulates Bcl-2 and upregulates Bax

In an attempt to unveil the molecular mechanism(s) underlying curcumin-induced apoptosis of H-ras MCF 10A cells, we measured the protein levels of two key apoptosis-linked gene products, Bcl-2 and Bax, which are known to regulate the cell death/survival in opposite manners. As shown in Fig. 4, expression of the anti-apoptotic oncoprotein Bcl-2 was greatly decreased whereas the death-promoting Bax expression was upregulated during the apoptosis process in H-ras MCF10A cells treated with curcumin for 24 h dose-dependently. A significant downregulation of Bcl-2 level was observed in cells treated with 30 μM of curcumin. Curcumin increased Bax level more efficiently: it significantly upregulated Bax at as low as 10 μM concentration. The results suggest that curcumin induce apoptotic cell death by prominently reducing the ratio of Bcl-2 to Bax.

Table I. Ac-DEVD-CHO attenuates the curcumin-induced growth inhibition in H-*ras* MCF10A cells.

Treatment	No. of viable cells (% of control)
Control	100
Curcumin	38.1 ± 0.60
Ac-DEVD-CHO+ Curcumin	54.4 ± 2.43*

H-*ras* MCF10A cells (1.5×10^5) in a 48-well plate were pre-incubated with 200 μM Ac-DEVD-CHO (a caspase-3 inhibitor) for 2 h followed by curcumin treatment (50 μM) for 20 h. Cell viability was determined by trypan blue exclusion assay. The results presented are means ± SE of triplicates. *Significantly different from curcumin-treated cells ($p<0.05$).

Since Bcl-2 was known to prevent caspase-3 activation (Yang *et al.*, 1997), we then examined if a caspase-3 inhibitor affects curcumin-induced apoptosis in H-ras MCF10A cells. Shown in Table I is the inhibitory effect of Ac-DEVD-CHO (Nicholson *et al.*, 1995) on curcumin-induced cell death in H-ras MCF10A cells. The data suggest the possible involvement of caspase-3 activation in the apoptotic event upon curcumin treatment.

Redox signaling may be responsible for curcumin-induced apoptosis

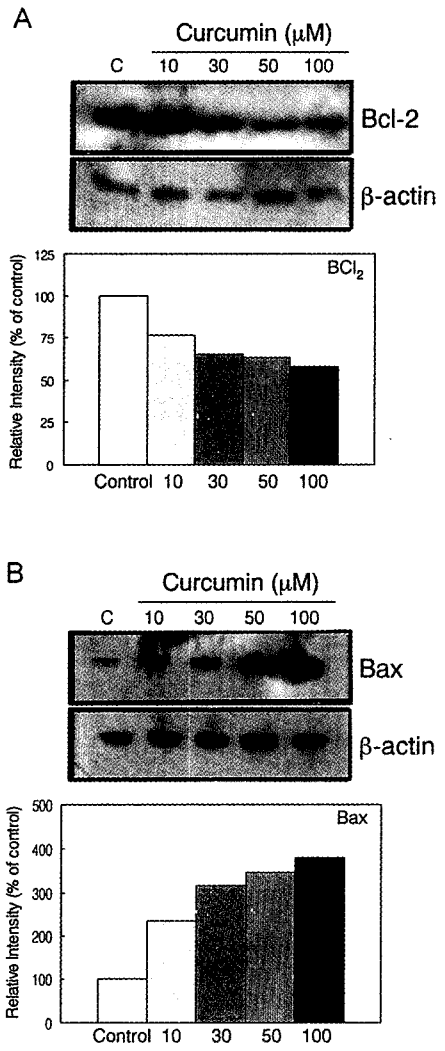

Fig. 4. Curcumin downregulates Bcl-2 and upregulates Bax. Immunoblot analysis for Bcl-2 and Bax was performed with lysates from cells treated with curcumin for 24 h. Relative band intensities were determined by quantitation of each band with an Image Analyzer (Vilber Lourmet, France).

Treatment of H-ras MCF10A cells with curcumin (50 and 100 μM) resulted in the efficient production of ROS (Fig. 5). In order to study the role of ROS generation in the curcumin-induced apoptosis, effect of a known anti-

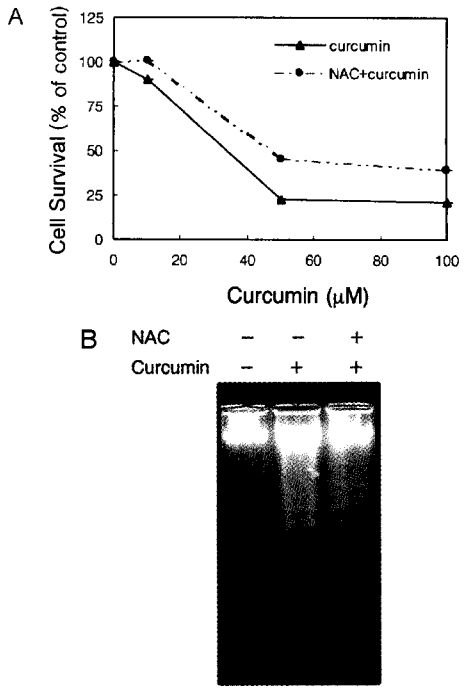

Fig. 6. NAC inhibits curcumin-induced cell death and DNA fragmentation. *A*. H-ras MCF10A cells (1×10^4) were pretreated with NAC 10 mM for 30 min followed by treatment of various concentrations of curcumin for 20 h. MTT assay was performed and the cytotoxicity was determined by relative absorbance normalized to the control cells. The results presented are means ± SE of triplicates. B. Cells were pretreated with 10 mM NAC for 30 min and then treated with 50 μM curcumin for 48 h. Cellular DNA was extracted and analyzed by 1.8% agarose gel electrophoresis.

oxidant, NAC, was determined on the cells treated with curcumin. Pretreatment of NAC inhibited the curcumin-induced cell death (Fig. 6A) and DNA fragmentation (Fig. 6B), indicating that the apoptotic event by curcumin was controlled by the antioxidant. These results suggest redox signaling as a mechanism responsible for curcumin-induced apoptosis in H-ras MCF10A cells.

DISCUSSION

In recent years there has been a growing body of evi-

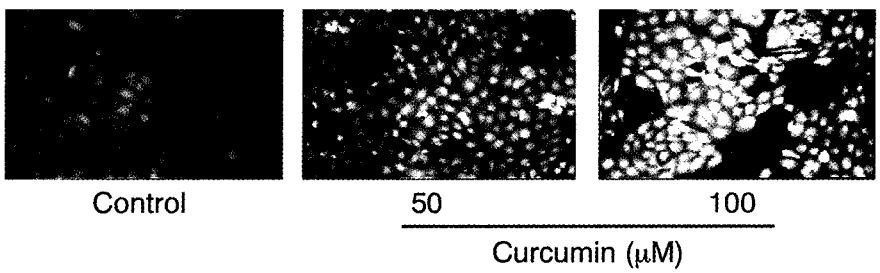

Fig. 5. Curcumin induces ROS in H-ras MCF10A cells. ROS generation was detected on the cells treated with curcumin for 1 h as a result of the oxidation of DCFH. The experiments were repeated at least three times.

dence that phytochemicals in our diet can reduce the risk of cancers. Chemoprevention is arguably one of the major weapons in the anticancer arsenal (Ferguson, 1994; Stavric, 1994). In our laboratory, we have reported that several potential chemopreventive agents including capsaicin and allylthiopyridazine derivatives induce apoptosis of malignant cells in which Bcl-2 downregulation and caspase-3 activation were involved (Jung *et al.*, 2001a; 2001b). In the present study, we demonstrate that curcumin inhibits invasive phenotype and induces apoptosis in H-ras-transformed MCF10A human breast epithelial cells. Taken in conjunction with the fact that uncontrolled ras activation is probably the most common genetic defect in human cancer cells, our findings may be critical for chemopreventive potential of curcumin. Our results correlate well with the previous finding by Mehta *et al.* (1997) that curcumin was a potent antiproliferative agent for breast tumor cells although they failed to observe apoptosis or any change in the expression of apoptosis-related genes in the curcumin-induced cell death.

We confirm our previous finding (Kim and Moon, 1999) that MMP-2 is more likely associated with curcumin-inhibited invasive phenotype. MMP-2 was downregulated in the cells treated with curcumin in a dose-dependent manner. Given that H-ras mediated transformation and invasiveness were shown to be associated with enhanced expression of MMP-9 mRNA and protein in rat and human embryonic fibroblasts (Bernhard *et al.*, 1994) and in human fibrosarcoma cells (Moon *et al.*, 1996; Kim *et al.*, 1998), the role of MMP-2 and/or MMP-9 on H-ras-signaling may be cell type-specific.

In an effort to elucidate mechanism(s) involved in curcumin-induced apoptosis of H-ras MCF10A cells, we examined the expressions of apoptosis-related genes in curcumin-treated cells. The ratio of Bcl-2 to Bax, rather than the levels of the individual proteins, is considered to be critical in determining the survival/death of cells (Oltvai *et al.*, 1993; Fukamachi *et al.*, 1998). We show that the ratio of Bcl-2 to Bax was prominently decreased by curcumin which may induce apoptotic response in H-ras MCF10A cells. A marked increase in Bax expression was observed in curcumin-treated cells. The results suggest that the increased level of pro-apoptotic Bax protein as well as the downregulation of Bcl-2 may accelerate caspase-3 activation during curcumin-induced apoptosis in H-ras MCF10A cells.

Opposing results have been reported regarding ROS generation by curcumin in different cell systems. Curcumin (10 μM) were shown to scavenge oxygen free radicals and lower the extent of ROS generation in rat peritoneal macrophages (Joe and Lokesh, 1994). In contrast, at a relatively high concentration (50 μM), curcumin treatment resulted in the hyperproduction of ROS in AK-5 tumor cells (Bhaumik *et al.*, 1999). It is plausible that curcumin lowers the generation of ROS at a low concentration while the higher concentration of curcumin might have the opposing effect on ROS production. A possibility that the cell-type difference may be responsible for the apparent controversy should not be excluded. In this study, we show that curcumin resulted in the generation of ROS at concentrations of 50 and 100 μM in H-ras MCF10A cells. The apoptotic events in curcumin-treated H-ras MCF10A cells were controlled by the antioxidant NAC. Taken together, our study suggests redox signaling and caspase-3 activation as mechanisms responsible for the induction of apoptosis by curcumin in H-ras-transformed MCF10A human breast epithelial cells.

ACKNOWLEDGEMENTS

This study was supported by a 2000 research grant from Research Institute for Pharmaceutical Science, Duksung Women's University, and by the Korean Science and Engineering Federation (KOSEF) through the Intellectual Biointerface Engineering Center at the Seoul National University. We thank Dr. Young-Joon Surh (College of Pharmacy, Seoul National University, Seoul, Korea) for helpful discussion.

REFERENCES

Alnemri, E. S., Mammalian cell death protease: a family of highly conserved aspartate specific cytosine proteases. *J. Cell Biochem.*, 64, 33-42 (1997).

Anjum, R. and Khar, A., Differential regulation of apoptosis in AK-5 tumor cells by the proto-oncogene Bcl-2: presence of Bcl-2 dependent and independent pathways. *FEBS Lett.*, 499, 166-70 (2001).

Bass, D. A., Parce, J. W., Dechatelet, L. R., Szejda, P., Seeds, M. C., and Thomas, M., Flow cytometric studies of oxidative product formation by neutrophils: a graded response to membrane stimulation. *J. Immunol.*, 130 (4), 1910-7 (1983).

Bae, Y. S., Kang, S. W., Seo, M. S., Baines, I. C., Tekle, E., Chock, P. B., and Rhee, S. G., Epidermal growth factor (EGF)-induced generation of hydrogen peroxide. Role in EGF receptor-mediated tyrosine phosphorylation. *J. Biol. Chem.*, 272(1), 217-21 (1997).

Bernhard, E. J., Gruber, S. B., and Muschel, R. J., Direct evidence linking expression of matrix metalloproteinase 9 (92-kDa gelatinase/ collagenase) to the metastatic phenotype in transformed rat embryo cells. *Proc. Natl. Acad. Sci.*, 91, 4293-7 (1994).

Bhaumik, S., Anjum, R., Rangaraj, N., Pardhasaradhi, B. V., and Khar, A., Curcumin mediated apoptosis in AK-5 tumor cells involves the production of reactive oxygen intermediates. *FEBS Lett.*, 456, 311-4 (1999).

Chun, K. -S., Shon, Y., Kim, H. -S., Kim, O. H., Park, K.-K., Lee, J. -M., Lee, J., Lee, J. -Y., Moon, A., Lee, S. S., and Surh, Y. -J., Anti-tumor promoting potential of

naturally occurring diarylheptanoids structurally related to curcumin. *Mutat. Res.*, 428, 49-57 (1999).

Cooper, J. D., Michaelidis, T. M., Tzimagiorgis, G. Z., Sendtner, M., Meyer, M., and Thoenen, H., Cholinergic neuron-specific overexpression of Bax in ChAT Bax transgenic mice. *Soc. Neurosci. Abstr.*, 22, 570 (1996).

Dorai, T., Cao, Y. C., Dorai, B., Buttyan, R., and Katz, A. E., Therapeutic potential of curcumin in human prostate cancer. III. Curcumin inhibits proliferation, induces apoptosis, and inhibits angiogenesis of LNCaP prostate cancer cells in vivo. *Prostate*, 47, 293-303 (2001).

Ferguson, L. R., Antimutagens as cancer chemopreventive agents in the diet. *Mut. Res.*, 307, 395-410 (1994).

Fukamachi, Y., Karasaki, Y., Sugiura, T., Itoh, H., Abe, T., Yamamura, K. and Higashi, K., Zinc suppresses apoptosis of U937 cells induced by hydrogen peroxide through an increase of the Bcl-2/Bax ratio. *Biochem. Biophys. Res. Commun.*, 246, 364-9 (1998).

Huang, M. -T., Lysz, T., Ferraro, T., Abidi, T. F., Laskin, J. D., and Conney, A. H., Inhibitory effects of curcumin on in vitro lipoxygenase and cyclooxygenase activities in mouse epidermis. *Cancer Res.*, 51, 813-9 (1991).

Joe, B. and Lokesh, B. R., Role of capsaicin, curcumin and dietary n-3 fatty acids in lowering the generation of reactive oxygen species in rat peritoneal macrophages. *Biochim. Biophys. Acta*, 1224, 255-63 (1994).

Jung, M. -Y., Kang, H. -J., and Moon, A., Capsaicin-induced apoptosis in SK-Hep-1 hepatocarcinoma cells involves Bcl-2 downregulation and caspase-3 activation. *Cancer Lett.*, 165, 139-145 (2001a).

Jung, M. -Y., Kwon, S. -K., and Moon, A., Chemopreventive allylthiopyridazine derivatives induce apoptosis in SK-Hep-1 hepatocarcinoma cells through a caspase-3-dependent mechanism. *Eur. J. Cancer*, In press (2001b).

Kim, M. -S., Son, M. -W., Kim, W. -B., Park, Y. I., and Moon, A., Apicidin, an inhibitor of histone deacetylase, prevents H-*ras*-induced invasive phenotype. *Cancer Lett.*, 157, 23-30 (2000).

Kim, M. -S. and Moon, A., Curcumin inhibits invasive phenotype of H-ras transformed human breast epithelial cells and suppresses matrix metallo-proteinase-2 activity. *J. Kor. Assoc. Cancer Prevention*, 4, 119-26 (1999).

Kim, M. -S., Won, J. -H., and Moon, A., Roles of matrix metalloproteinase-2 and -9 on the H-ras-induced invasive phenotype in human breast epithelial cells and human fibrosarcoma cells. *J. Toxicol. Pub. Health*, 14, 569-75 (1998).

Kobayashi, T., Ruan, S., Clodi, K., Kliche, K. O., Shiku, H., Andreeff, M., and Zhang, W., Overexpression of Bax gene sensitizes K562 erythroleukemia cells to apoptosis induced by selective chemotherapeutic agents. *Oncogene*, 16, 1587-91 (1998).

Kong, A. N., Yu, R., Chen, C., Mandlekar, S., and Primiano, T., Signal transduction events elicited by natural products: role of MAPK and caspase pathways in homeostatic response and induction of apoptosis. *Arch. Pharm. Res.*, 23, 1-16 (2000).

Lin, L. -I., Ke, Y. -F., Ko, Y. -C., and Lin, J. -K., Curcumin inhibits SK-Hep-1 hepatocellular carcinoma cell invasion in vitro and suppresses matrix metallo-proteinse-9 secretion. *Oncology*, 55, 349-53 (1998).

Menon, L. G., Kuttan, R. and Kuttan, G., Anti-metastatic activity of curcumin and catechin. *Cancer Lett.*, 141, 159-165 (1999).

Mehta, K., Pantazis, P., McQueen, T., and Aggarwal, B. B., Antiproliferative effect of curcumin (diferuloylmethane) against human breast tumor cell lines. Anticancer Drugs, 8, 470-81 (1997).

Moon, A., Kim, M. -S., Kim, T. G., Kim, S. H., Kim, H. E., Chen, Y. Q., and Kim, H. -R. C., H-*ras*, but not N-*ras*, induces an invasive phenotype in human breast epithelial cells: A role for MMP-2 in the H-*ras* induced invasive phenotype. *Int. J. Cancer*, 85, 176-81 (2000).

Moon, A., Park, S. H., and Lee, S. H., Expression of type IV collagenase genes and *ras* oncogene in various human tumor cell lines. *J. Biochem. Mol. Biol.*, 29, 484-7 (1996).

Nicholson, D. W., Ali, A., Thornberry, N. A., Vaillancourt, J. P., Ding, C. K., Gallant, M., Gareau, Y., Griffin, P. R., Labelle, M., Lazebnik, Y. A., Munday, N. A., Raju, S. M., Smulson, M. E., Yamin, T.-T., Yu, V. L., and Miller, D. K., Identificaion and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis. *Nature (Lond.)*, 376, 37-43 (1995).

Oltvai, Z. N., Milliman, C. L., and Korsmeyer, S. J., Bcl-2 heterodimerizes *in vivo* with a conserved homolog, Bax, that accelerates programmed cell death. *Cell*, 74, 609-19 (1993).

Pereira, M. A., Grubbs, C. J., Barnes, L. H., Li, H., Olson, G. R., Eto, I., Juliana, M., Whitaker, L. M., Kelloff, G. J., Steele, V. E., and Lubet, R. A., Effects of the phytochemicals, curcumin and quercetin, upon azoxymethane-induced colon cancer and 7,12-dimethylbenz[α] anthracene-induced mammary cancer in rats. *Carcinogenesis*, 17, 1305-11 (1996).

Porter, A. G. and Janike, R. U., Emerging roles of caspase-3 in apoptosis. *Cell Death Differ.*, 6, 99-104 (1999).

Ramachandran, C. and You, W., Differential sensitivity of human mammary epithelial and breast carcinoma cell lines to curcumin. *Breast Cancer Res. Treat*, 54, 269-78 (1999).

Wattenberg, L. W., Inhibition of carcinogenesis by minor anutrient constituents of the diet. *Proc. Natl. Acad. Sci.*, 49, 173-83 (1990).

Yang, J., Liu, X., and Bhalla, K., Prevention of apoptosis by bcl-2: release of cytochrome c from mitochondria blocked. *Science*, 275, 1129-32 (1997).