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Suppression of Protein Kinase C and Nuclear Oncogene Expression as Possible Action Mechanisms of Cancer Chemoprevention by Curcumin

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Curcumin (diferuloylmethane) is a major naturally-occurring polyphenol of Curcuma species, which is commonly used as a yellow coloring and flavoring agent in foods. Curcumin has shown anti-carcinogenic activity in animal models. Curcumin possesses anti-inflammatory activity and is a potent inhibitor of reactive oxygen-generating enzymes such as lipoxygenase/ cyclooxygenase, xanthine dehydrogenase /oxidase and inducible nitric oxide synthase; and an effective inducer of heme oxygenase-1. Curcumin is also a potent inhibitor of protein kinase C (PKC), EGF(Epidermal growth factor)-receptor tyrosine kinase and IκB kinase. Subsequently, curcumin inhibits the activation of NF(nucleor factor)κB and the expressions of oncogenes including *c*-jun, *c*-fos, *c*-myc, NIK, MAPKs, ERK, ELK, PI3K, Akt, CDKs and iNOS. It is proposed that curcumin may suppress tumor promotion through blocking signal transduction pathways in the target cells. The oxidant tumor promoter TPA activates PKC by reacting with zinc thiolates present within the regulatory domain, while the oxidized form of cancer chemopreventive agent such as curcumin can inactivate PKC by oxidizing the vicinal thiols present within the catalytic domain. Recent studies indicated that proteasome-mediated degradation of cell proteins play a pivotal role in the regulation of several basic cellular processes including differentiation, proliferation, cell cycling, and apoptosis. It has been demonstrated that curcumin-induced apoptosis is mediated through the impairment of ubiquitin-proteasome pathway. Curcumin was first biotransformed to dihydrocurcumin and tetrahydrocurcumin and that these compounds subsequently were converted to monoglucuronide conjugates. These results suggest that curcumin-glucuronide, dihydrocurcumin-glucuronide, tetrahydrocurcumin-glucuronide and tetrahydrocurcumin are the major metabolites of curcumin in mice, rats and humans.

Key words: Curcumin, Dihydrocurcumin, Curcumin-glucuronide, Protein kinase C, *c*-Jun, *c*-Fos, *c*-Myc, Xanthine oxidase, NFκB, IKK

INTRODUCTION

It has been suggested that diet has an impact on cancer incidence and daily consumption of vegetables and fruits decreases the risk for human cancer (Armstrong and Doll, 1975; Phillips, 1975). Recently, efforts have been focused on identifying dietary phytochemicals which have the ability to inhibit the processes of carcinogenesis. Among these phytochemicals, curcumin has been demonstrated to be a promising cancer chemopreventive agents in animal systems (Huang *et al.*, 1988; 1991). Curcumin has been listed as the third generation of cancer chemopreventive agents by the Institute of Cancer Chemoprevention, NCI, NIH of the United States.

A recent study on phase I clinical trial of curcumin in patients with high risk or pre-malignant lesions has demonstrated that curcumin is not toxic to human up to 8000 mg/day when taken by normal for 3 months and a promising biologic effect of curcumin in the chemoprevention of several types of cancer (Cheng *et al.*, 2001).

Curcumin is the major yellow pigment isolated from the ground rhizome of Curcuma species, Zingiberaceae, which is commonly used as coloring and flavoring agent in several oriental foods. Seven major species of Curcuma including *Curcuma longa* Linn., *C. xanthorrhiza* Roxb., *C.*

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wenyujin (Y.H. Chen et C, Ling); *C. sichuanensis*; *C. kwangsiensis*; *C. aeruginosa* Roxb; and *C. elata* Roxb. were cultivated in China and their composition of curcuminoids were analyzed (Chen and Fang, 1997). Three major curcuminoids namely curcumin, demethoxycurcumin and bisdemethoxycurcumin occurred naturally in these Curcuma species. It seems that *C. longa* L. (turmeric) has the highest concentration of curcumin (2.03%) as compared with other species (0.01-1.79%).

Turmeric is widely used as a spice and coloring agent in several foods such as curry, mustard, bean cake cassava paste and potato chips as well as cosmetics and drugs. Another species namely *C. wenyujin* (Y.H. Chen et C. Ling) has been used for centuries in Chinese tradition medicine for the treatment of a variety of inflammatory conditions such as hepatitis and bile duct disorders.

Curcumin has been demonstrated to have potent antioxidant (Kunchandy and Rao, 1990; Subramanian *et al.*, 1994; Sreejayan,1994) and anti-inflammatory activities (Huang *et al.*, 1988, 1991, 1997; Shih and Lin, 1994), and it also inhibits the carcinogen-DNA adduct (Conney *et al.*, 1991) and tumorigenesis in several animal models (Huang *et al.*, 1992, 1994, 1995; Rao *et al.*, 1995).

BIOTRANSFORMATIONS OF CURCUMIN

The pharmacokinetic properties of curcumin have been investigated in mice (Pan *et al.*, 1999). After intraperitoneal adminstration of curcumin (0.1 g/kg) to mice, about 2.25 μg/mL of curcumin appeared in the plasma in the first 15 min. One hour after administration the levels of curcumin in the intestine, spleen, liver and kidneys were 177, 26, 27, and 7.5 μg/g, respectively. Only traces (0.41 μg/g) were observed in the brain at 1 h. To clarify the nature of the metabolites of curcumin, the plasma was analyzed by reversed-phase HPLC, and two putative conjugates were observed. Further treatment of the plasma with β-glucuronidase resulted in a decrease in the levels of these two putative conjugates and the concomitant appearance of the tetrahydrocurcumin and curcumin, respectively. To investigate the nature of these glucuronide conjugates *in vivo*, the plasma was analyzed by electrospray. The chemical structures of these metabolites were determined by MS/MS analysis (Pan *et al.*,1999). The experimental results suggested that curcumin was first biotransformed to dihydrocurcumin and tetrahydrocurcumin and these compounds subsequently were converted to monoglucuronide conjugates as illustrated in Fig. 1. These results suggest that curcumin-glucuronide, dihydrocurcumin-glucuronide, tetrahydrocurcumin-glucuronide and tetrahydrocurcumin are major metabolites of curcumin *in vivo*.

The systemic bioavailability of curcumin is low (Cheng *et al.*, 2001), so that its pharmacological activity may be mediated, in part, by curcumin metabolites. The major products of curcumin biotransformation by hepatocytes occur only at low abundance in rat plasma after curcumin administration and metabolism of curcumin by reduction or conjugation generates species with reduced ability to inhibit COX-2 expression (Ireson *et al.*, 2001). Because the gastrointestinal tract seems to be exposed more prominently to unmetabolized curcumin than any other tissue, these metabolic results support the clinical evaluation of curcumin as a colorectal cancer chemopreventive agent.

Curcumin glucuronide was identified in intestinal and hepatic microsomes, and curcumin sulfate, tetrahydrocurcumin and hexahydrocurcumin were found as curcumin metabolites in intestinal and hepatic cytosol from human and rats (Fig. 1). The extent of curcumin conjugation was much greater in intestinal fractions from humans than in those from rats, whereas curcumin conjugation was less extensive in hepatic fractions from human than in those from rats. The curcumin-reducing ability of cytosol from human intestinal and liver tissue exceeded that observed with the corresponding rat tissue by factors of 18 and 5, respectively (Ireson *et al.*, 2002). Curcumin sulfate was identified in incubation of curcumin with intact rat gut sacs. Curcumin was sulfated by human phenol sulfotransferase isoenzymes SULT1A1 and SULT1A3. Equine alcohol dehydrogenase catalyzed the reduction of curcumin to hexahydrocurcumin.

Cancer chemoprevention by curcumin

Curcumin inhibited chemical carcinogenesis in different tissue sites in several experimental animal models. Curcumin inhibited the tumor initiation by benzo[a]pyrene (BaP) and 7,12-dimethyl-benz[a]anthracene (DMBA) in mouse epidermis (Conney *et al.*, 1991). Topical application of curcumin strongly inhibited tumor promotion in the skin of DMBA-initiated mice (Huang *et al.*, 1988; 1992; 1995). Feeding 0.5% -2.0% curcumin in the diet decreased BaP-induced forestomach tumors per mouse by 51% -53% when administered during the initiation period and 47%-67% when administered during the postinitiation period (Huang *et al.*, 1994). Further studies indicate that curcumin may inhibit BaP-induced forestomach cancer in mice by affecting both activation as well as inactivation pathways of BaP metabolism in the liver (Singh *et al.*, 1998). Feeding curcumin in the diet decreased the number of *N*-ethyl-*N*-nitro-*N*-nitrosoguanidine (ENNG)-induced duodenal tumors per mouse (Huang *et al.*, 1994). Administration of curcumin in the diet decreased the number of azoxymethane (AOM)-induced colon tumors in mouse (Huang *et al.*, 1994) and in rats (Rao *et al.*, 1995).

Curcumin is an effective agent for chemoprevention action at the radiation-induced initiation stage of mammary carcinogenesis (Inano *et al.*, 2000). Recent study in our

laboratory also indicates that curcumin effectively inhibits diethylnitrosamine-induced hepatocarcinogenesis in mice (Chuang *et al.*, 2000). It is suggested that the feasibility of curcumin in the chemoprevention of human hepatocellular carcinoma should be further explored (Cheng *et al.*, 2001).

BIOCHEMICAL AND MOLECULAR EFFECTS OF CURCUMIN

Antioxidative effects through modulating related enzyme systems

Curcumin possesses an anti-inflammatory activity and is a potent inhibitor of reactive oxygen generating enzymes, such as lipoxygenase/cyclooxygenase, xanthine dehydrogenase/oxidase and inducible nitrogen oxide synthase (Lin and Lin-Shiau, 2001). Simultaneous administration of 2 and 10 μM curcumin with 100 ng/mL TPA inhibits TPA-induced increases in xanthine oxidase activity measured 30 min later by 22.7% and 36.5% respectively (Lin and Shih, 1994). Based on these findings, induction of xanthine oxidase activity is deemed to be one of the major causative elements in TPA-mediated tumor promotion, and the major inhibitiory mechanism of curcumin on TPA-induced increases in xanthine dehydrogenase/oxidase enzyme activities is through direct inactivation in the protein level (Lin and Shih, 1994).

It is interesting to note that curcumin induces heme oxygenase-1 (HO-1) and protects endothelial cells against oxidative stress (Motterlin *et al.*, 2000). Exposure of bovine aortic endothelial cells to curcumin (5-15 μM) resulted in both a concentration- and time-dependent increase in HO-1 mRNA, protein expression and heme oxygenase activity. Interestingly, prolonged incubation (18 h) with curcumin in normoxic or hypoxic conditions resulted in enhanced cellular resistance to oxidative damage, this cytoprotective effect was considerably attenuated by tin protoporphyrin IX, an inhibitor of heme oxygenase activity. Regulation of HO-1 expression by curcumin and other polyphenols is evoked by a distinctive mechanism which is not necessary linked to changes in glutathione but might depend on redox signals sustained by specific and targeted sulfhydryl groups (Scapagini *et al.*, 2002).

Curcumin is a potent scavenger of a variety of reactive oxygen species (ROS) including superoxide anion (Kunchandy and Rao, 1990), hydroxyl radical, singlet oxygen (Subramanian *et al.*, 1994), nitric oxide and peroxynitrite. Curcumin has the ability to protect lipids, hemoglobin, and DNA against oxidative degradation. Pure curcumin has more potent superoxide anion scavenging activity than demethoxycurcumin or bisdemethoxycurcumin (Kunchandy and Rao, 1990). Curcumin is also a potent inhibitor of ROS-generating enzyme cyclooxygenase and lipoxygenase in mouse epidermis (Huang *et al.*, 1991).

Supplementation with *curcuma longa* extract reduces oxidative stress and attenuates the development of fatty streaks in male New Zealand white rabbits fed a high cholesterol diet (1.3%) (Quiles *et al.*, 2002). Many studies have shown the capacity of curcumin to prevent lipid peroxidation, a key process in the onset and progression of many diseases including atherosclerosis. It has been observed that curcumin reduces plasma lipid peroxides; it reduces the susceptibility of LDL to oxidation, inhibits the proliferation of vascular smooth muscle cells, has an antithrombotic effect and inhibits platelet aggregation *in vivo* and *ex vivo*.

Curcumin prevents colon cancer in rodent models. It inhibits lipid peroxidation and cyclooxygenase-2 (Cox-2) expression and induces glutathione S-transferase (GST). The total GST activity and adducts of malondialdehyde with DNA in colon mucosa, liver and blood leukocytes were significantly inhibited by curcumin (Sharma *et al.*, 2001).

Phase 2 enzyme induction

Curcumin and a number of naturally occurring and synthetic analogs are phase 2 enzyme inducers, as demonstrated by their ability to elevate the enzyme activity of quinone reductase in murine hepatoma cells. It is reasonable to assume that phase 2 enzyme induction plays a significant role in the chemopreventive and anti-oxidant activities of these curcuminoids (Dinkova-Kostova and Talalay, 1999).

It has been demonstrated that coordinate induction of phase 2 proteins and elevation of glutathione protect cells against the toxic and carcinogenic effects of electrophiles and oxidants. All inducers react covalently with thiols at rate that are closely related to their potencies. Inducers disrupt the cytoplasmic complex between the actin-bound protein Keap1 and the transcription factor Nrf2, thereby releasing Nrf2 to migrate to the nucleus where it activates the anti-oxidant response element (ARE) of phase 2 genes and accelerate their transcription (Dinkova-Kostova *et al.*, 2002). This finding suggestes that reaction of cysteine thiols is followed by rapid formation of protein disulfide linkages. The most reactive residues of Keap1 (C^{257}, C^{273}, C^{288} and C^{297}) were identified by mapping the hexamethasone-modified cysteines by mass spectrometry of tryptic peptides. The residues are located in the intervening region between BTB and Kelch repeat domains of Keap1 and probably are the direct sensor of inducers of the phase 2 enzyme system (Dinkova-Kostova *et al.*, 2002).

Curcumin inhibited experimental allergic encephalomyelitis in association with a decrease in IL-12 production from macrophage/microglial cells and differentiation of

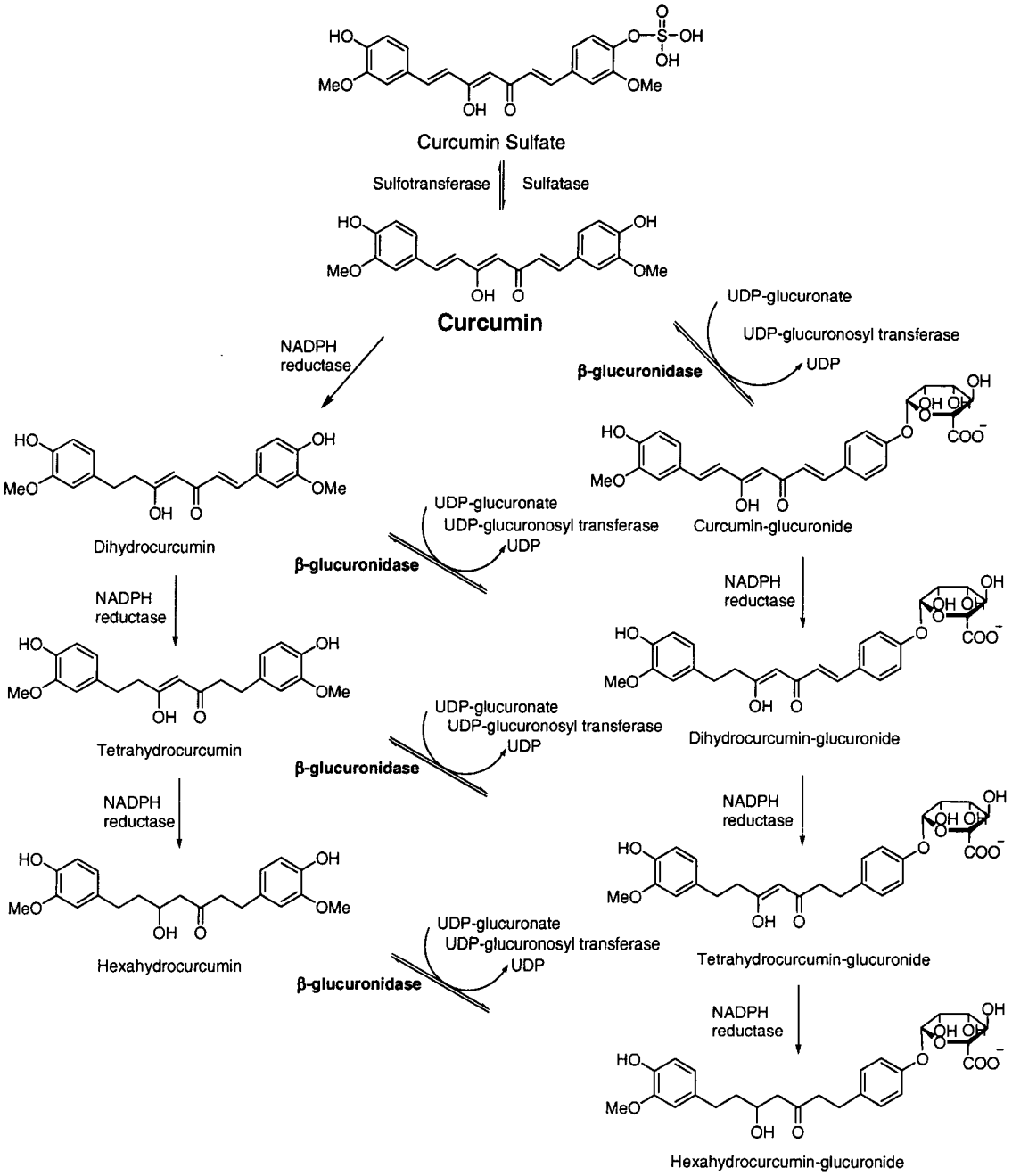

Fig. 1. Biotransformations of curcumin in mouse. Two main biotransformation pathways namely reduction and glucuronidation of curcumin are depicted. Our preliminary results indicate that NADPH is required for reduction reaction, while nature of the reductase is unknown. Furthermore, most conjugated derivatives are hydrolyzed by β-glucosidase and identified as glucuronides in the mouse plasma (Data from Pan *et al.*, 1999; Ireson *et al.*, 2002).

neural Ag-specific Th1 cells. *In vivo* treatment of activated T cells with curcumin inhibited IL-12-induced tyrosine phosphorylation of Janus kinase 2, tyrosine kinase 2, and STAT 3 and STAT 4 transcription factors. The inhibition of Janus kinase-STAT pathway by curcumin resulted in a decrease in IL-12 induced T cell proliferation and Th 1 differentiation. These findings suggest the use of curcumin in the treatment of multiple sclerosis and other Th 1 cell-mediated inflammatory diseases (Natarajan *et al.*, 2002).

Induction of apoptosis

We have demonstrated that curcumin (30 μM) induces apoptosis in several tumor cell lines (Jiang *et al.*, 1996). The curcumin-induced apoptosis is highly dependent on the origin and malignancy of cell lines. It appears that the typical apoptosis can only be induced in immortalized mouse embryo fibroblast NIH 3T3, erbB2 oncogene-transformed NIH 3T3, mouse Sarcoma 180, human colon cancer cell HT29, human kidney cancer cell 293, and

human hepatocellular carcinoma HepG2 cells; but not in primary cultures of mouse embryonic fibroblast C3H 10T1 /2, rat embryonic fibroblast and human foreskin fibroblast cells (Jiang *et al.*, 1996). Treatment of NIH 3T3 cells with the PKC inhibitor staurosporine, the tyrosine kinase inhibitor herbimycin A or arachidonic acid metabolism inhibitor quinacrine induces typical apoptosis. These results suggest that blocking the cellular signal transduction in immortalized or transformed cells might trigger the induction of apoptosis.

We have also demonstrated that curcumin (3.5 μg/mL) induces apoptosis in human promyelocytic HL-60 cells. This apoptosis-inducing activity of curcumin appeared in a dose- and time-dependent manner (Kuo *et al.*, 1996). Flow cytometric analysis showed that the hypodiploid DNA peak of propidium iodide-stained nuclei appeared at 4 h after 7 μg/mL curcumin treatment. The action mechanism has been demonstrated to be through cytochrome c release and activation of caspases (Pan *et al.*, 2001). The antioxidants, *N*-acetyl-L-cysteine (NAC), L-ascorbic acid, alpha-tocopherol, catalase and superoxide dismutase, all effectively prevented curcumin-induced apoptosis.

The combined treatment of LNCaP prostate cancer cells with curcumin (10 μM) and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL, 20 ng/mL) induced remarkably apoptosis through inducing cleavage of procaspase-3, procaspase-8 and procaspase-9, truncation of Bid and release of cytochrome c from the mitochondria, indicating that both the extrinsic (receptor-mediated) and intrinsic (chemical-induced) pathways of apoptosis are triggered in prostate cancer cells treated with a combination of curcumin and TRAIL. These findings define a potential use of curcumin to sensitize prostate cancer cells for TRAIL-mediated immunotherapy (Deeb *et al.*, 2003).

While curcumin has several different molecular targets within the MAPK and PI3K/PKB signaling pathways that could contribute to inhibition of proliferation and induction of apoptosis, inhibition of basal activity of Akt/PKB, but not ERK, may facilitate apoptosis in the tumor cell line (Squires *et al.*, 2003). Recent studies have demonstrated that curcumin down-regulates NFκB through inhibiting IκBα, Bcl-2, Bcl-xL, cyclin D1 and interleukin-6 in human multiple myeloma cells leading to the suppression of proliferation and induction of apoptosis, thus providing the molecular basis for the treatment of multiple myeloma patients with this pharmacologically safe agent (Bharti *et al.*, 2003).

Curcumin causes dose-dependent apoptosis and DNA fragmentation of Caki cells, which is preceeded by the sequential dephosphorylation of Akt, down regulation of the anti-apoptotic Bcl-2, Bcl-xL and IAP proteins, release of cytochrome c and activation of caspase 3, cyclosporin A, as well as caspase inhibitor, specifically inhibit curcumin-induced apoptosis in Caki cells. Pretreatment with *N*-acetylcysteine markedly prevented dephosphorylation of Akt and cytochrome c release, and cell death, suggesting a role for ROS in this process (Woo *et al.*, 2003).

Suppression of *c*-jun and *c*-fos expression

In 1991, we have made an interesting finding that the phorbol ester TPA induced transcriptional factor *c*-jun/AP-1 in mouse fibroblast cells is suppressed by curcumin (Huang, 1991). Elevated expression of gene transcriptionally induced by TPA is among the events required for tumor promotion. Functional activation of transcriptional factor *c*-Jun/AP-1 is believed to play an important role in signal transduction of TPA-induced tumor promotion. Suppression of the *c*-jun/AP-1 activation by curcumin (10 μM) is observed in mouse fibroblast cells. These findings show for the first time that the effect of curcumin on TPA-induced inflammation/tumor promotion could be studied at the molecular level.

Curcumin also inhibits the TPA- and UVB light-induced expression of *c*-jun and *c*-fos in JB6 cells and in mouse epidermis (Lu *et al.*, 1994). Recent studies indicated that curcumin treatment attenuated TPA-stimulated NFκB activation in mouse skin, which was associated with its blockade of degradation of the inhibitory protein IκBα and also of subsequent translocation of the p65 subunit to nucleus (Chun *et al.*, 2003). TPA treatment resulted in rapid activation via phosphorylation of ERK1/2 and p38 MAP kinases, which are upstream of NFκB. The MEK1/2 inhibitor UO126 strongly inhibited NFκB activation, while p38 inhibitor SB203580 failed to block TPA-induced NFκB activation in mouse skin. It is suggested that curcumin inhibits the catalytic activity of ERK1/2 in mouse skin and its suppression of COX-2 expression by inhibiting ERK activity and NFκB activation may provide the molecular basis for antitumor promoting effects of curcumin in mouse skin carcinogenesis (Chun, *et al.*, 2003; Singh and Aggarwal, 1995).

Inhibition of protein kinase C

Treatment with 15 or 20 μM curcumin for 15 min inhibited TPA-induced PKC activity in particulate fractions by 26 or 60% and did not affect the level of PKC protein. However, the inhibitory effect of curcumin was reduced after preincubation with the thiol compounds (Liu *et al.*, 1993).

Dietary antioxidant are important in cancer prevention. The conventional view held for a long time is that anti-oxidants act by scavenging free radicals. Although these actions of anti-oxidants are certainly important in preventing promutagenic DNA damage caused by oxidants, other actions of anti-oxidants particularly those influencing cell signaling mechanisms, have also recently come into light. Antioxidants are believed to induce their own effects on cell signaling such as protein kinase C (PKC) pathway in

the precancer cells to decrease tumor promotion, a critical stage in carcinogenesis (Gopalakrishna and Jaken, 2000). By having different oxidation susceptible regions, PKC can respond to both oxidant tumor promoters and cancer-preventive antioxidants to elicit apposite cellular responses. Oxidant tumor promoter (such as TPA) activates PKC by reating with zinc-thiolates present within the regulatory domain. In contrast, the oxidized forms of some cancer-preventive agents, such as polyphenolics (curcumin, ellagic acid and 4-hydroxytamoxifen) and seleno compounds, can inactivate PKC by oxidizing the vicinal thiols present within the catalytic domain. This brings an efficient counteractive mechanism to block the signal transduction induced by tumor promoter at the first step itself (Gopalakrishna and Gundimeda, 2002).

Suppression of EGF receptor tyrosine kinase activity

Curcumin (10 μM) inhibits EGF receptor kinase activity up to 90% in a dose- and time-dependent manner and also inhibits EGF-induced tyrosine auto phosphorylation of EGF-receptors in A431 cells (Korutla and Kumar, 1994). Treatment of NIH 3T3 cells with a saturating concentration of EGF for 5-15 min induced increased EGF-R tyrosine phosphorylation by 4 to 11-fold and this was inhibited by curcumin, which also inhibited the growth of EGF-stimulated cells (Korutla *et al.*, 1995). Curcumin has been shown to suppress the expression of inducible nitric oxide synthase (iNOS) *in vivo* (Chan *et al.*, 1998).

EGF is a well-known mitogen, but it paradoxically induces apoptosis in cells that overexpress its receptor. It has been demonstrated that the EGF-induced apoptosis is accelerated if NFκB is inactivated by curcumin and sodium salicylate (Anto *et al.*, 2003). Under the NFκB inactivated condition, A431 cells were more sensitive to EGF with decreased cell viability and increased externalization of phosphatidylserine on the cell surface, DNA fragmentation and activation of caspases (3 and 8 but not 9), typical features of apoptosis. These results were further supported by the potentiation of the growth inhibitory effects of EGF by chemical inhibitors of NFκB (such as curcumin and sodium salicylate) and the protective role of Rel A evidenced by the resistance of A431-Rel A cells (stably transfected with Rel A) to EGF-induced apoptosis (Anto *et al.*, 2003).

Proteasome system in cell proliferation and apoptosis

In addition to the mechanisms by which the growth factors exhibit both stimulatory and inhibitory activity in a single cell depending on the context of the other signal molecules present, the final outcome is presumably influenced by a host of regulatory molecules other than the growth factors and their receptors (Sporn and Roberts, 1988). It is thus clearly important to recognize that a potent mitogen like EGF also sends out apoptotic signals and identify conditions in which these signals are regulated. NFκB inhibition make A431 cells more susceptible to EGF-induced apoptosis whereas Rel A protect them against it. EGF-stimulation in A431 cells enhances the degradation of IκBα, but not IκBβ and proteasome inhibitors such as ALLN or MG132, block EGF-mediated NFκB activation, indicating that EGF-induced NFκB activation requires proteasome-dependent IκB degradation. Furthermore, EGF-induced DNA-binding complex of NFκB in A431 cells was found to be composed of p50/Rel A heterodimers but not *c*-Rel (Sun and Carpenter, 1998).

It has been demonstrated that curcumin-induced apoptosis is mediated through the impairment of ubiquitin-proteasome system. Exposure of curcumin to the mouse neuro2a cells causes dose-dependent decrease in proteasome activity and increase in ubiquitinated proteins. Curcumin exposure also decreases the turnover of the destabilized enhanced green fluorescence protein, a model substrate for proteasome and cellular p53 protein (Jana *et al.*, 2004). In our laboratory, a similar effect was observed in another polyphenolic, pentagalloylglucose (5GG). It is interesting to note that 5GG induces G1 arrest and apoptosis in human Jarkart T cells through inhibiting proteasome activity and elevating $p27^{kip1}$, $P21^{cip1/WAF1}$, and Bax proteins (Chen and Lin, 2004).

Proteasome-mediated degradation of cell proteins play a pivotal role in the regulation of several basic cellular processs including differentiation, proliferation, cell cycling, apoptosis, gene expression and signal transduction. Imbalances in proteasome-mediated protein degradation contribute to various human diseases such as cancer, neurodegenerative and myodegenerative disases, suggesting that the proteasome may be a novel target for anticancer therapy (Naujokat and Hoffman, 2002).

Modulation of Ca^{+2} and cellular p53 protein

When COLO205 colorectal carcinoma cells were treated with curcumin (60 μM), the appearance of apoptotic DNA ladders was delayed about 5 h and G1 arrest was detected (Chen *et al.*, 1996). The reduction of p53 gene expression was accompanied by the induction of HSP70 gene expression in the curcumin-treated cells. These findings suggest that curcumin may induce the expression of the HSP70 gene through the initial depletion of intracellular Ca^{+2} followed by the suppression of p53 gene function in the target cells (Chen *et al.*, 1996).

Suppression of hepatocellualr carcinoma invasion by inhibiting MMP-9

An *in vitro* assay, without or with Matrigel matrix, was

used to quantitate cellular migration and invasion. Gelatin-based zymography was adapted to assay the secretion of matrix metalloproteinase-9 (MMP-9). We found that curcumin at 10 μM inhibited 17.4 and 70.6% of cellular migration and invasion of SK-Hep-1 cells, respectively.

Compared with a less invasive human hepatocellular carcinoma cell line Huh 7, SK-Hep-1 showed a much higher MMP-9 secretion. Furthermore, parallel with its anti-invasion activity, curcumin inhibited MMP-9 secretion in SK-Hep-1 in a dose-dependent fashion. We conclude that curcumin possesses a significant anti-invasion activity in SK-Hep-1 cells and that this effect is associated with its inhibitory action on MMP-9 secretion (Lin *et al.*, 1998).

Osteopontin (OPN) is a member of the extracellular matrix protein, and it is a non-collagenous, sialic acid rich and glycosylated phosphoprotein. OPN stimulates tumor growth and activation of promatrix metalloproteinase-2 (ProMMP-2) through NFκB-mediated induction of membrane type-1 matrix metalloproteinase (MT1-MMP) in murine melanoma cells (Phillip *et al.*, 2001). Recently, it has been shown that curcumin inhibited the OPN-induced IκBα phosphorylation and degradation by inhibiting the IKK activity. Moreover, curcumin inhibited the OPN-induced translocation of p65, NFκB DNA-binding and NFκB transcriptional activity. Curcumin also inhibited OPN-induced cell proliferation, cell migration, extracellular matrix invasion, and synergistically induced apoptotic morphology with OPN in these cells (Phillip and Kundu, 2003).

Action mechanisms of curcumin in chemoprevention

Muttiple evidence has been indicated that many dietary constituents are chemopreventive in animal models, and experiments with cultured cells are revealing various potential action mechanisms. Several compounds classified as blocking agents can prevent, or greatly reduce, initiation of carcinogenesis, while suppressing agents affect later stages of the promoting process by reducing cell proliferation. Many naturally occurring compounds such as curcumin, catechins, theaflavins, and others have both types of activity. These compounds exhibit their blocking mechanisms through alteration of phase 2 drug metabo-

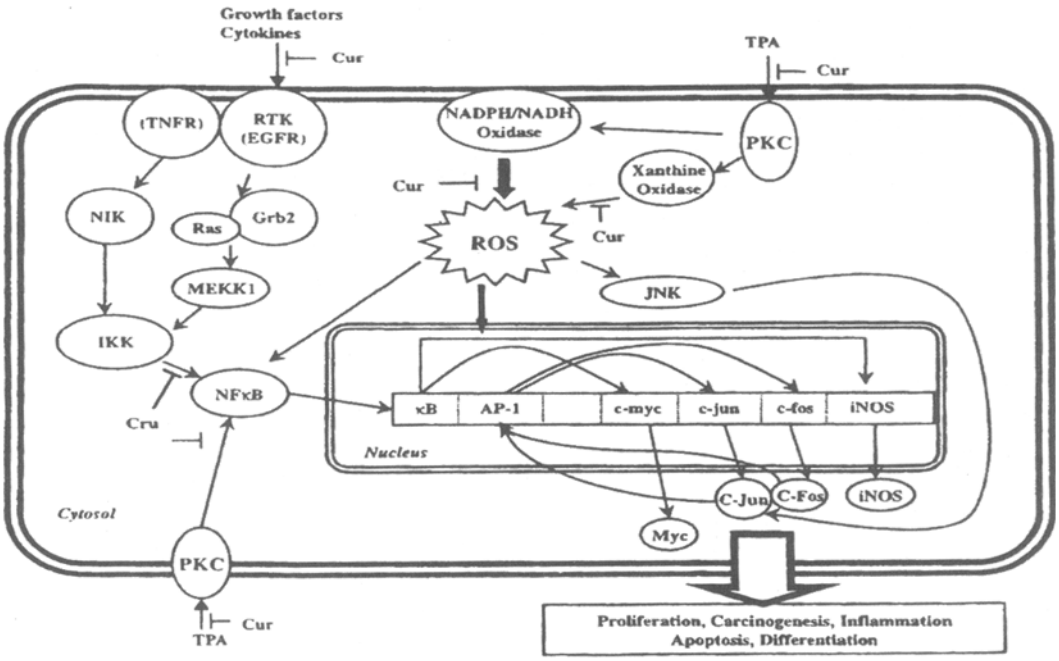

Fig. 2. Proposed action mechanisms of cancer chemoprevention by curcumin. Several exogenous stimuli namely extracellular growth factors, cytokines or tumor promoter 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) binds to membrane receptors such as epidermal growth factor receptor (EGFR), tumor necrosis factor receptor (TNFR), or protein kinase C(PKC), resulting in the activation of a number of serine, threonine or tyrosine kinases, which include ras, NFκB inducing kinase (NIK), mitogen activated protein kinase (MAPK), extracellular response kinase (ERK), MAPK/ERK kinase kinase (MEK), IκB kinase (IKK) and *c*-jun *N*-terminal kinase (JNK). JNK is activated by MAPK kinase (MKK_4), causing activation of the *c*-jun protein which forms a heterodimer with the *c*-fos protein thus enhancing the activity of the transcription factor AP-1. Recent studies demonstrate that both IKK and PKC are important for activation of NFκB which leads to enhancement of the expression of *c*-myc, iNOS and other cellular proliferation genes (Lin and Lin-Shiau, 2001). Reactive oxygen species (ROS) are considered to be endogenous mitogenic factors (or apoptotic factors under certain conditions) that can activate NFκB and other transcription factors in the nucleus. Ultimately, activation of the MAPK family members caused activation of specific transcription factors, such as NFκB, AP-1, serum response factor (SRF) and others which help determine the fate of cell such as proliferation, carcinogenesis, inflammation or apoptosis. Curcumin (Cur) has been demonstrated to block several sites of these multiple signal transduction pathways as indicated by the blockade symbol (⊥).

lizing activities and scavenging of ROS in the target tissue. Meanwhile, these compound may act as suppressing agents to suppress carcinogenesis involving modulation of signal transduction that leads to altered gene expression, cell cycle arrest or apoptosis (Manson *et al.*, 2000).

Although curcumin alone had little or no effect on cellular differentiation, when it was combined with all-trans retinoic acid or 1α-25-dihydroxyvitamin D_3, a synergistic effect was observed. It is possible that many dietary chemicals in fruits, vegetables and other edible plants can prevent cancer by synergizing with endogenously produced stimulators of differentiation such as all-trans retinoic acid, 1α-25-dihydroxyvitamin D_3 and butyrate (Conney *et al.*, 1997).

Recent intensive studies on the action mechanisms of curcumin *in various* biological systems have indicated that this compound has engaged in multiple anti-tumor promoting pathways (Lin *et al.*, 1994; Lin and Lin-Shiau, 2001). It has been demonstrated that the TPA-induced tumor promotion is significantly inhibited by curcumin (Huang *et al.*,1988; 1997; Rao *et al.*, 1995).

It is conceivable that the molecular mechanism of curcumin is quite complicated and dispersed as illustrated in Fig. 2. The primary target of curcumin could be on the plasma membrane where the activity of PKC is first inhibited (Liu *et al.*, 1993). In addition, the activity of EGF receptor tyrosine kinase is also inhibited (Korutla and Kumar, 1994). Some PKC-mediated nuclear protein factors, such as IκB kinase and NFκB are then inhibited through various signal transduction pathways. The TRE binding activity of *c*-Jun/AP-1 is then repressed (Huang, 1991) and finally the transcription of genes essential for cell proliferation are suppressed as indicated by the inhibition of related enzymes such as ornithine decarboxylase, PKC, cyclooxygenase and lipoxygenase.

It appears that activation of calcium-dependent protein kinases (such as PKC) or inhibition of protein phosphatases results in tumor promotion (Haystead *et al.*, 1989). In case of tumor promoters, it appears that a common final effect is to increase phosphorylation of protein substrate on serine or threonine residues. It appears that when any essential component of a signal transduction pathway is rendered hyperactive or autonomous, it may acquire the ability to drive the cell into unchecked proliferation and lead to tumor promotion. Curcumin may attenuated or suppressed the hyperactivity of these components of signal transduction and maintain coordinatively the normal cell function.

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