MOLECULAR TARGETS OF CURCUMIN

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Abstract: 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 (iNOS); it is an effective inducer of heme oxygenase-1. Curcumin is also a potent inhibitor of protein kinase C (PKC), EGF-receptor tyrosine kinase, and IκB kinase. Subsequently, curcumin inhibits the activation of NF-κB and the expressions of oncogenes including c-jun, c-fos ,c-myc, NIK, MAPKs, ERK, ELK, PI3K, Akt, CDKs, and iNOS. It is considered that PKC, mTOR, and EGFR tyrosine kinase are the major upstream molecular targest for curcumin intervention, whereas the nuclear oncogenes such as c-jun, c-fos, c-myc, CDKs, FAS, and iNOS might act as downstream molecular targets for curcumin actions. It is proposed that curcumin might 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, whereas 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 the ubiquitin–proteasome pathway.

1. INTRODUCTION

Chemoprevention is the attempt to use dietary factors, synthetic pharmacological agents, and changes in lifestyle to intervene in the precancerous stages of carcinogenesis before the invasive disease begins.[1] It has been suggested that diet has an impact on cancer incidence and that daily consumption of vegetables and fruits decreases the risk for human cancer.[2,3] 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 agent in animal systems.[4,5] 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 study of

the clinical application of curcumin as a chemopreventive agent was intensively carried out at the NCI.[6] Our recent study on phase I clinical trial of curcumin in patients with high-risk or premalignant lesions has demonstrated that curcumin is not toxic to humans up to 8000 mg/day when taken for 3 months and has a promising biologic effect in the chemoprevention of several types of cancer.[7] 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*, has been used for centuries in Chinese traditional medicine for the treatment of a variety of inflammatory conditions such as hepatitis and bile duct disorders.[8] Curcumin has been demonstrated to have potent antioxidant[9–11] and anti-inflammatory activities,[4,5,12,13] and it inhibits the carcinogen–DNA adduct.[14] and tumorigenesis in several animal models.[15–18]

2. CANCER CHEMOPREVENTION BY CURCUMIN

Several studies have demonstrated that 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-dimethylbenz[*a*]anthracene (DMBA) in mouse epidermis.[14] The topical application of curcumin strongly inhibited tumor promotion in the skin of DMBA-initiated mice.[4,15,17] 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.[16] Further studies indicated that curcumin might inhibit BaP-induced forestomach cancer in mice by affecting both activation as well as inactivation pathways of BaP metabolism in the liver.[19] Feeding curcumin in the diet decreased the number of *N*-ethyl-*N*′-nitro-*N*-nitrosoguanidine (ENNG)-induced duodenal tumors per mouse.[16] Administration of curcumin in the diet decreased the number of azoxymethane (AOM)-induced colon tumors in mice[16] and rats.[18]

Curcumin is an effective agent for chemoprevention action at the radiation-induced initiation stage of mammary carcinogenesis.[20] A recent study in our laboratory also indicated that curcumin effectively inhibits diethylnitrosamine-induced hepatocarcinogenesis in mice.[21] It is suggested that the feasibility of using curcumin in the chemoprevention of human hepatocellular carcinoma should be further explored.[7] Recent studies have indicated the combined inhibitory effects of curcumin and phenethylisothiocyanate on the growth of PC-3 prostate xenografts in immunodeficient mice.[22]

3. METABOLISM OF CURCUMIN

The pharmacokinetic properties of curcumin have been investigated in mice.[23] 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 high-performane liquid chromatography (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 mass spectrometry–mass spectrometry (MS/MS) analysis.[23] The experimental results suggested that curcumin was first biotransformed to dihydrocurcumin and tetrahydrocurcumin and these compounds subsequently were converted to monoglucuronide conjugates. These results suggest that curcumin–glucuronide, dihydrocurcumin–glucuronide, tetrahydrocurcumin–glucuronide, and tetrahydrocurcumin are major metabolites of curcumin in mice.

The bioavailability of parent curcumin is low,[7] so its pharmacological activity can 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 generate species with reduced ability to inhibit cyclooxygenase (COX)-2 expression.[24] Because the gastrointestinal tract seems to be exposed more prominently to unmetabolized curcumin than any other tissue, the 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 humans and rats .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 humans 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.[25] Curcumin sulfate was identified in the incubation of curcumin with intact rat gut sacs. Curcumin was sulfated by human phenol sulfotransferase isoenzymes SULT1A1 and SULT1A3.

4. MAJOR TARGETS FOR THE BIOLOGICAL ACTIONS OF CURCUMIN

4.1. Antioxidative Effects Through Modulating Related Enzyme Systems

Curcumin possesses anti-inflammatory activity and is a potent inhibitor of reactive-oxygen-generating enzymes, such as lipoxygenase (LOX)/cyclooxygenase (COX), xanthine dehydrogenase/oxidase, and inducible nitrogen oxide synthase (iNOS).[26] Simultaneous administration of 2 and 10 μM curcumin with 100 ng/mL

trifluoroacetic acid (TPA) inhibits TPA-induced increases in xanthine oxidase activity measured 30 min later by 22.7% and 36.5%, respectively.[27] 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.[27]

It is interesting to note that curcumin induces heme oxygenase-1 (HO-1) and protects endothelial cells against oxidative stress.[28] 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 that is not necessary linked to changes in glutathione but might depend on redox signals sustained by specific and targeted sulfhydryl groups.[29]

Curcumin is a potent scavenger of a variety of reactive oxygen species (ROS), including superoxide anion,[9] hydroxyl radical, singlet oxygen,[10] 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.[9] Curcumin is a potent inhibitor of ROS-generating enzymes cyclooxygenase and lipoxygenase in mouse epidermis.[5]

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%).[30] 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, 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.[31]

4.2. Metabolic Enzyme Induction

Metabolic studies showed that curcumin significantly inhibited CYP1A1-mediated benzo(a)pyrene diol bioactivation in both oral squamous cell carcinoma cells and intact oral mucosa.[32] Because CYP1A1 is one of the primary carcinogen-activating enzymes in oral mucosa, the use of curcumin as an oral cavity chemopreventive agent have significant clinical impact via its ability to inhibit

carcinogen bioactivation. Curcumin exhibits anticancer activity in rodents and in humans. Its efficacy appears to be related to induction of GST enzymes, inhibition of prostaglandin E_2 (PGE_2) production, or suppression of oxidative DNA adduct formation.[33] Curcumin and a number of naturally occurring and synthetic analogues are phase II 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 II enzyme induction plays a significant role in the chemopreventive and antioxidant activities of these curcuminoids.[34]

It has been demonstrated that coordinate induction of phase II 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 antioxidant response element (ARE) of phase II genes and accelerate their transcription.[35] This finding suggests 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 sensors of inducers of the phase II enzyme system.[35]

4.3. Induction of Apoptosis

We have demonstrated that curcumin (30 μM) induces apoptosis in several tumor cell lines.[36] 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.[36] Treatment of NIH 3T3 cells with the protein kinase C (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 human promyelocytic HL-60 cells. The apoptosis-inducing activity of curcumin appeared in a dose- and time-dependent manner.[37] 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.[38] The antioxidants

N-acetyl-L-cysteine (NAC), L-ascorbic acid, α-tocopherol, catalase, and superoxide dismutase 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) remarkably induced 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.[39]

4.4. Inhibition of Mammalian Target of Rapamycin

Curcumin inhibits proliferation/growth, induces apoptosis, and suppresses motility of cells by inhibition of mammalian targets of rapamycin (mTOR)-mediated signaling pathways in rhabdomyosarcoma cells. Recently, two mTOR complexes (m-TORmLST8-raptor and mTOR-mLST8-rictor) have been identified. The former is rapamycin-sentsitive, whereas the latter is rapamycin-insensitive. Treatment with curcumin promoted dissociation of raptor from mTOR in a concentration-dependent manner, but did not affect association of mLST8 with mTOR. Furthermore, unlike rapamycin, curcumin also induced the dissociation of rictor from mTOR. Therefore, curcumin might represent a novel class of mTOR inhibitor.[40] It is found that curcumin induced sustained hyperphosphorylation of c-jun and activation of JNK, which might be responsible for curcumin-induced apoptosis of the rhabdomyosarcoma cells.

The conserved checkpoint protein kinase mTOR is a key regulator of cell growth and proliferation and increasing evidence supports that the mTOR pathway plays a central role in the genesis of cancer. Curcumin has been shown to inhibit carcinogenesis and tumor cell proliferation. Curcumin inhibits PKB/Akt, one of the upstream regulators of mTOR; furthermore, curcumin also activates AMPK1, which senses cellular ATP levels and inhibits mTOR indirectly. Inhibition of mTOR signaling by curcumin is mediated by the inhibition of Akt and activation of AMPK, and the inhibition of mTOR signaling is important for the inhibition of PC-3 cell proliferation by curcumin.[41]

Human tumors that overexpressed ErbB2, which have been previously shown to have higher VEGF expression, showed significantly higher p70S6K phosphorylation as well. Increased vascular epithelial growth factor (VEGF) expression also significantly correlated with higher levels of Akt and mTOR phosphorylation. Additionally, patients with tumors having increased p70S6K phosphorylation showed a trend for worse disease-free survival and increased metastasis. These findings show that ErbB2 increases VEGF protein production by activating p70S6K in cell lines, xenografts, and human cancers and suggest that these signaling molecules might serve as targets for antiangiogenic and antimetastatic therapies.[42]

4.5. Suppression of Akt Signaling

Althugh 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, might facilitate apoptosis in the tumor cell line.[43] Recent studies have demonstrated that curcumin downregulates nuclear factor (NF-κB) through inhibiting IκBα, Bcl-2, Bcl-xL, cyclin D1, and interleukin (IL)-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.[44] Curcumin causes dose-dependent apoptosis and DNA fragmentation of Caki cells, which is preceded by the sequential dephosphorylation of Akt, downregulation of the antiapoptotic 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.[45]

4.6. Inhibition of MDM2 Oncogene Action

The anti-cancer effect of curcumin might be through direct inhibition of the MDM2 oncogene action. It is well established that the MDM2 oncogene plays a major role in human cancer development and progression. Its tumorigenic properties are associated with both p53-dependent and p53-independent pathways. It has been demonstrated that in a dose-dependent manner, curcumin inhibited DMD2 expression in various human cancer cell lines with different p53 backgrounds. Curcumin inhibited MDM2 expression at the transcription level, affecting DMD2 promoter activity. The levels of p21 and Bax were increased and E2F1 and Bcl2 decreased. Curcumin induced apoptosis and inhibited proliferation in PC3 cells. The chemosensitization and radiosensitization effects of curcumin *in vitro* and *in vivo* are tightly associated with its MDM2 inhibitory effects.[46]

4.7. 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.[47] 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 ultraviolet B (UVB) light-induced expression of c-jun and c-fos in JB6 cells and in mouse epidermis.[48] Recent studies indicated that curcumin treatment attenuated TPA-stimulated NF-κB 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.[49] TPA treatment resulted in rapid activation via phosphorylation of ERK1/2 and p38 MAP kinases, which are upstream of NF-κB. The MEK1/2 inhibitor UO126 strongly inhibited NF-κB activation, whereas the p38 inhibitor SB203580 failed to block TPA-induced NF-κB 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 NF-κB activation might provide the molecular basis for the antitumor-promoting effects of curcumin in mouse skin carcinogenesis.[49,50] Curcumin has potent antineoplastic activity in several tumor types and is thought to exert anti-inflammatory effects in part through inhibition of NF-κB, which has been linked to inhibition of the cytokine IL-8, production of PGE_2, and VEGF. IL-8, PGE_2, and VEGF are expressed in ovarian cancers and are associated with increased angiogenesis and poor prognosis. Curcumin is cytotoxic in ovarian cancer cells and exerts its effects by inhibiting NF-κB activation and decreasing levels of IL-8 and VEGF. The inhibition of these angiogenic factors along with the induction of apoptosis might have broad clinical benefits to ovarian cancer patients.[51]

4.8. 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.[52]

Dietary antioxidants are important in cancer prevention. The conventional view held for a long time is that antioxidants act by scavenging free radicals. Although these actions of antioxidants are certainly important in preventing promutagenic DNA damage caused by oxidants, other actions of antioxidants, particularly those influencing cell signaling mechanisms, have also recently come to light. Antioxidants are believed to induce their own effects on cell signaling, such the PKC pathway in the precancer cells to decrease tumor promotion, a critical stage in carcinogenesis.[53] By having different oxidation susceptible regions, PKC can respond to both oxidant tumor promoters and cancer preventive antioxidants to elicit apposite cellular responses. The oxidant tumor promoter (such as TPA) activates PKC by reacting 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 the tumor promoter at the first step itself.[54]

4.9. 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 phosphorylation of EGF-receptors in A431 cells.[55] 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.[56] Curcumin has been shown to suppress the expression of iNOS *in vivo*.[57] The 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 NF-κB is inactivated by curcumin and sodium salicylate.[58] Under the NF-κB 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 NF-κB (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.[58]

4.10. Proteasome System in Cell Proliferation and Apoptosis

It has been proposed that curcumin mediates growth arrest in breast cancer cells by targeting the proteasome[59] Evidence points to curcumin as a potent antioxidant and anti-inflammatory agent, both desirable properties that have been reported to play key roles in inhibiting carcinogenesis. Using a panel of three breast cancer cell culture models (MDA-MB-231, MDA-MB-436, and Hs578T), it has been shown that curcumin mediates its cell cycle inhibitory activities by blocking the chymotrypsin-like activity of the proteasome *in vitro*. It is suggested that curcumin might mediate G1 arrest and possible cytostasis and apoptosis by blocking the proteasome activity and upregulating the p21 protein in breast cancer cells.[59] 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.[60] It is thus clearly important to recognize that a potent mitogen like EGF also sends out apoptotic signals and identifies conditions in which these signals are regulated. NF-κB inhibition makes A431 cells more susceptible to EGF-induced apoptosis, whereas Rel A protects 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 NF-κB activation, indicating that EGF-induced NF-κB activation requires proteasome-dependent IκB degradation. Furthermore, the EGF-induced DNA-binding complex of NF-κB in A431 was found to be composed of p50/Rel A heterodimers, but not c-Rel.[61]

It has been demonstrated that curcumin-induced apoptosis is mediated through the impairment of the ubiquitin–proteasome system. Exposure of curcumin to the mouse neuro2a cells causes a dose-dependent decrease in proteasome activity and an 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.[62] 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 Jurkat T-cells through inhibiting proteasome activity and elevating $p27^{kip1}$, $P21^{cip1/WAF1}$, and Bax proteins.[63]

Proteasome-mediated degradation of cell proteins plays a pivotal role in the regulation of several basic cellular processes, 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 and neurodegenerative and myodegenerative disases, suggesting that the proteasome might be a novel target for anticancer therapy.[64]

4.11. 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.[65] 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 might 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.[65]

4.12. Suppression of Hepatocellualr Carcinoma Invasion by Inhibiting MMP-9

An *in vitro* assay, without or with the 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 has a significant anti-invasion activity in SK-Hep-1 cells and that this effect is associated with its inhibitory action on MMP-9 secretion.[66]

Osteopontin (OPN) is a member of the extracellular matrix protein; it is a non-collagenous, sialic acid-rich, and glycosylated phosphoprotein. OPN stimulates tumor growth and activation of promatrix metalloproteinase-2 (ProMMP-2) through NF-κB-mediated induction of membrane type-1 matrix metalloproteinase (MT1-MMP) in murine melanoma cells.[67] 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, NF-κB DNA-binding, and NF-κB transcriptional activity. Curcumin also inhibited OPN-induced cell proliferation, cell migration, extracellular matrix invasion, and synergistically induced apoptotic morphology with OPN in these cells.[68]

5. MECHANISM OF ACTION OF CURCUMIN IN CHEMOPREVENTION

Multiple evidences have 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, wereas 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 II drug-metabolizing activities and scavenging of ROS in the target tissue. Meanwhile, these compound might act as suppressing agents to suppress carcinogenesis involving modulation of signal transduction that leads to altered gene expression, cell cycle arrest, or apoptosis.[69] 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.[70]

Recent intensive studies on the action mechanisms of curcumin in various biological systems have indicated that this compound has engaged in multiple antitumor-promoting pathways.[48,71] It has been demonstrated that the TPA-induced tumor promotion is significantly inhibited by curcumin.[4,12,18] Angiogenesis is a key component of cancer metastasis. ErbB2- overexpressing breast tumors tend to be more angiogenic than other breast tumors. One of the most potent inducers of angiogenesis is VEGF, which induces endothelial cell proliferation and migration. In human breast tumors, overexpression of ErbB2 is correlated with increased VEGF expression and the transcription factor hypoxia-inducible factor-1α has been postulated to mediate the ErbB2 upregulation of VEGF.[22] It is conceivable that the molecular mechanism of action of curcumin is quite complicated and dispersed. The primary target of curcumin could be on the plasma membrane where the activity of PKC is first inhibited.[52] In addition, the activity of EGF receptor tyrosine kinase is also inhibited.[55] Some PKC-mediated nuclear protein factors, such as IκB kinase and NF-κB, are then inhibited through various signal transduction pathways. The TRE-binding activity of c-Jun/AP-1 is then repressed[47] 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, COX

and LOX. It appears that activation of calcium-dependent protein kinases (such as PKC) or inhibition of protein phosphatases results in tumor promotion.[72] In the case of tumor promoters, it appears that a common final effect is to increase the phosphorylation of the 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 might acquire the ability to drive the cell into unchecked proliferation and lead to tumor promotion. Curcumin might attenuate or suppress the hyperactivity of these components of signal transduction and maintain the normal cell function.[73]

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REFERENCES

1. G. J. Kelloff, C. W. Boone, V. E. Steele, et al., Progress in cancer chemoprevention: Prospective on agent selection and short term clinical intervention trials.*Cancer Res* **54(Suppl)**, 2015s–2024s (1994).
2. B. Armstrongand R. Doll, Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices. *Int J Cancer*,**15**, 617–631 (1975).
3. R. L. Phillips, Role of life-style and dietary habits in risk of cancer among Seventh-Day Adventists. *Cancer Res* **35**, 3513–3522 (1975).
4. M. T. Huang, R. C. Smart, C. Q. Wong, and A. H. Conney, Inhibitory effect of curcumin, chlorogenic acid, caffeic acid and ferulic acid on tumor promotion in mouse skin by 12-O-tetradecanoylphorbol-13-acetate. *Cancer Res* **48**, 5941–5946 (1988).
5. M. T. Huang, T. Lysz, T. Ferraro, T. F. Abidi, J. D. Laskin, and A. Conney,. Inhibitory effects of curcumin in vivo lipoxygenase and cyclooxygenase activities in mouse epidermis. *Cancer Res* **51**, 813–819 (1991).
6. G. J. Kelloff, J. A. Crowell, E. T. Hawk, et al., Stragegy and planning for chemopreventive drug–development: Clinical development. *J Cell Biochem*. **26(Suppl),** 54–71 (1996).
7. A. L. Cheng, C. H. Hsu, J. K. Lin, M. M. Hsu, Y. F. Ho, T. S. Shen, TJ. Y. Ko, J. T. Lin, B. R. Lin, W. Ming-Shiang, H. S. Yu, S. H. Jee, G. S. Chen, T. M. Chen. C. A. Chen, M. K. Lai, . Y. S. Pu, M. H. Pan, Y. J. Wang, C. C. Tsai, and C. Y. Hsieh, Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high risk or pre-malignant lesion. *Anti-cancer Res* **21**(4B), 2895–2900 (2001). .
8. C. M. Chen and H. C. Fang, chemical analysis of the active principles of curcuma species In: C. Y. Sung, ed. *Modern Treatise on Chinese Herbal Medicines*. Beijing:

The Institute of Pharmaceutical Sciences, Medical Academia, 1997,Vol. III, pp 95–105.
9. E. Kunchandyand M. N. A. Rao, Oxygen scavenging activity of curcumin. *Int J Pharm* **38**, 239–240 (1990) .
10. M. Subramanian, M. N. A. Sreejayan Rao, T. P. A. Devasagyam, and B. B. Singh, Diminution of singlet oxygen induced DNA-damage by curcumin and related antioxidants. *Mutat Res* **311**, 249–255 (1994).
11. M. N. A. Sreejayan Rao, Curcuminoids as potent inhibitors of lipid peroxidation. *J Pharm Pharmacol* **46**, 1013–1016 (1994).
12. M. T. Huang, W. Ma, P. Yen, J. G. Xie, J. Han, K. D. Fenkel, K. D. Grunberger, and Conney, Inhibitory effects of topical application of low doses of curcumin on TPA-induced tumor promotion and oxidized DNA bases in mouse epidermis. *Carcinogenesis* **18**, 83–88 (1997).
13. C. A. Shih and J. K.Lin, Inhibition of 8-hydroxydeoxyguanosine formation by curcumin in mouse fibroblast cells. *Carcinogenesis* **14**, 709–712 (1994).
14. A. H. Conney, T. Lysz, T. Ferraro, T. F. Abidi, P. S. Manchand, J. D. Laskin, and M. T. Huang, Inhibitory effect of curcumin and some related dietary compounds on tumor promotion and arachidonic acid metabolism in mouse skin. *Adv Enzyme Regul* **31**, 385–389 (1991).
15. M. T. Huang, Z. Y. Wang, C. A. Georgiadis, J. D. Laskin, and A. H. Conney, Inhibitory effect of curcumin on tumor initiation by benzo[a]pyrene and 7,12- dimethylbenz[a]anthracene. *Carcinogenesis* **13**, 947–954 (1992).
16. M. T. Huang, Y. R. Lou, YW. Ma, H. L. Newmark, K. R. Reuhl, and A, H. Conney, Inhibitory effect of dietary curcumin on forestomach, duodenal and colon carcinogenesis in mice. *Cancer Res* **54**, 5841–5847 (1994).
17. M. T. Huang, W. Ma, Y. P. Lu, YR. L. Chang, C. Fischer, P. S. Manchand, H. L. Newmark, and H. H. Conney, Effects of curcumin, demethoxy- curcumin,bisdemethoxycurcumin and tetrahydrocurcumin on TPA-induced tumor promotion. *Carcinogenesis* **16**, 2493–2497 (1995).
18. C. V. Rao, A. B. Riven, A. B.Simi, and B. S. Reddy, Chemoprevention of colon carcinogenesis by dietary curcumin, a naturally occurring plant phenolic compound.*Cancer Res* **55**, 259–266 (1995).
19. S. V. Singh, X. Hu, S. K. Srivastava, M. Singh, H. Xia, J. L. Orchard, and H. A. Zaren, Mechanism of inhibition of benzo[a]pyrene-induced forestomach cancer in mice by dietary curcumin. *Carcinogenesis* **19**(8), 1357–1360 (1998).
20. H. Inano, M. Onoda, N. Inafuku, et al., Potent protective action of curcumin on radiation-induced initiation of mammary tumorigenesis in rats. *Carcinogenesis* **21**(10), 1835–1841 (2000).
21. S. E. Chuang, M. L. Kuo, C. H. Hsu, C. R. Chen, J. K. Lin, G. M. Lai, C. Y. Hsieh, and A. L. Cheng, Curcumin-containing diet inhibits diethylnitrosamine- induced murine hepatocarcinogenesis.*Carcinogenesis* **21**(2), 331–335 (2000).
22. K. S. Klos, S. L. Wyszomierski, M. Sun, et al., ErbB2 increases vascular endothelial growth factor protein synthesis via activation of mammalian target of rapamycin/p70S6K leading to increased angiogenesis andspontaneous metastasis of human breast cancer cells. *Cancer Res* **66**, 2028–2037 (2006).
23. M. H. Pan, T. M. Huang, Tand J. K. Lin, Biotransformation of curcumin through reduction and glucuronizationm in mice. *Drug Metab Dispos* **27**, 486–494 (1999).
24. C. Ireson, S. Orr, D. J. Jones, R. Verschoyle, C. K. Lim, J. L. Luo, L. Howells, S. Plummer, R. Jukes, M. Williams, W. P. Steward, and A.Gescher, Characterization of

metabolites of the chemopreventive agent curcumin in human and rat hepatocytes and in the rat in vivo, and evaluation of their ability to inhibit phorbol ester-induced prostaglandin E2 production. *Cancer Res* **61**(3), 1058–1064 (2001).

25. C. Ireson, D. J. Jones, S. Orr, M. W. Coughtrie, D. J. Hoocock, M. L. Williams, P. B. Farmer, W. P. Steward, and A. Gescher, Metabolism of the cancer chemopreventive agent curcumin in human and rat intestine. *Cancer Epidermiol Biomarkers Prev* **11**(1), 105–111 (2002).
26. J. K.Lin and S. Y. Lin-Shiau, Cancer chemoprevention by curcumin. *Proc Natl Sci Counc Repub China B* **25**(2), 59–66 (2001) .
27. J. K. Lin, T. S. HuangC. A. Shih, and J. L. Liu, Molecular mechanism of action of curcumin. In: C. T. Ho, T. Osawa, M. T. Huang, and R. T. Rosen, eds. *Food Phyto-Chemicals for Cancer Prevention II.* ACS Symposium Series 547. Washington, DC: Amercian Chemical Society, Washington, 1994**,** pp.196–203.
28. R. Motterlin, R. Foresti, R.,\ Bassi, and C. J. Green, Curcumin, an antioxidant and anti-inflammatory agent, induces heme oxygenase-1 and protects endothelial cells against oxidative stress. *Free Radical Biol Med* **28**(8), 1303–1312 (2000).
29. G. Scapagnini, R Foresti, V. Calabrese, A. M. Giuffrida Stella, C. J. Green, and R. Motterlin, Caffeic acid phenethyl ester and curcumin: A novel class of heme oxygenase-1 inducers. *Mol Pharmacol* **3**, 554–561 (2002).
30. J. L.Quiles, M. Dolores Mesa, C. L. Ramirez-Tortosa, C. M. Anguilera, M. Battino, A. Gil, and M. Carmen Ramirez-Tortosa, Curcuma longa extract supplementation induces oxidative stress and attenuates aortic fatty streak development in rabbits. *Arterioscler Thromb Vasc Biol* **22**, 1225–1231 (2002).
31. R. A. Sharma, C. R. Ireson, R. D. Verschoyle, K. A. Hill, M. L. Williams, C. Leuratti, M. M. Manson, L. J. Marett, W. P. Steward, and A. Gescher, Effect of dietary curcumin on glutathione S-transferase and malondialdehyde-DNA adducts in rat liver and colon mucosa: Relationship with drug levels. *Clin Cancer Res* **7**, 1452–1458 (2001).
32. A. L. Rinaldi, M. A. Monge, H. W. Fields, et al., Curcumin activates the arylhydrocarbon receptor yet significantly inhibits (-)-benzo(a)pyrene-7R-trans-7,8-dihydrodiol bioactivation in oral squamous cell carcinoma cells and oral mucosa. *Cancer Res* **62**, 5451–5456 (2002).
33. R. A. Sharma, S. A. Euden, S. L. Platton, et al., Phase I clinical trial of oral curcumin: Biomarkers of systematic activity and compliance. *Clin Cancer Res* **10**, 6847–6854 (2004).
34. A. Dinkova-Kostova and P. Talalay, Relation of structure of curcumin alalogs to their potencies as inducers of phase 2 detoxification enzymes. *Carcinogenesis* **20**(5), 911–914 (1999).
35. A. Dinkova-Kostova, W. D. Holtzclaw, R. N. Cole, K. Itoh, N. Wakabayashi, Y. Katoh, M. Yamamoto, and P. Talalay, Direct evidence that sulfhydryl groups of Keap 1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and antioxidants. *Proc Natl Acad Sci USA* **99**(18), 11,908–11,913 (2002).
36. M. C. Jiang, H. F. Yang-Yen, J. J. Yen, and J. K. Lin, Curcumin induces apoptosis in immortalized NIH 3T3 and malignant cancer cell lines. *Nutr Cancer* **26**, 111–120 (1996).
37. M. L. Kuo, T. S. Huang, and J. K. Lin, Curcumin, an antioxidant and anti-tumor promoter, induced apoptosis in human leukemia cells.*Biochim Biophys Acta* **1317**, 95–100 (1996).

38. M. H. PanW. L. Chang, S. Y. Lin-Shiau, C. T. Ho, and J. K. Lin, Induction of apoptosis by garcinol and curcumin through cytochrome c release and activation of caspases in human leukemia HL-60 cells. *J Agric Food Chem* **49**(3), 1464–1474 (2001).
39. D. Deeb, Y. X. Xu, H. Jiang, X. Gao, N. Janakiraman, R. A. Chapman, and S. C. Gautam, Curcumin enhances tumor necrosis factor-related apoptosis-inducing-ligand-induced apoptosis in LNCaP prostate cancer cells. *Mol Cancer Ther* **2**(1), 95–103 (2003).
40. C. S. Beevers and S. Huang, Curcumin disrupts the complexes of mammalian target of rapamycin. *Proc Am Assoc Cancer Res* **7**, 356 (2006)
41. S. Yu, G. Shen, and T. A. Kong, Curcumin inhibits mTOR signaling by inhibiting protein kinase B/Akt and activating AMP-activated protein kinase (AMPK) in prostate cancer cell line PC-3.*Proc Am Assoc Cancer Res* **47**, 538 (2006).
42. T. O. Khor, Y. S. Keum, W. S. Lin, et al., Combined inhibitory effects of curcumin and phenethylisothiocyanate on the growth of human PC-3 prostate xenografts in immunodeficient mice. *Cancer Res* **66**(2), 613–621 (2006).
43. M. S. Squires, E. A. Hidson, L. Howells, S. Sale, C. E. Houghton, J. L. Jones, L. H. Fox, M. Dickens, S. A. Prigent, and M. M. Manson, Relevance of mitogen activated protein kinase (MAPK) and phosphatidylinositol 3-kinase/protein kinase B (PI3K/PKB) pathways to induction of apoptosis by curcumin in breast cells. *Biochem Pharmacol* **65**(3), 361–376 (2003).
44. A. C. Bharti, N. Donato, S. Singh, and B. B.Aggarwal, Curcumin down-regulates the constitutive activation of nuclear factor κB and IκBα kinase in human multiple myeloma cells leading to suppression of proliferation and induction of apoptosis. *Blood* **101**, 1053–1062 (2003).
45. J. H. Woo, Y. H. Kim, Y. J. Choi, D. G. Kim, K. S. Lee, J. H. Hae, D. S. Min, J. S. Chang, Y. J. Jeong, Y. S. Lee, J. W. Park, and J. K. Kwon, Molecular mechanisms of curcumin-induced cytotoxicity: Induction of apoptosis through generation of reactive oxygen species, down-regulation of Bcl-XL and IAP , the release of cytochrome c and inhibition of Akt. *Carcinogenesis* **24**(7), 1199–1208 (2003).
46. M. Li, H. Wang, Z. Zhang, et al., Curcumin, a multifunctional chemopreventive agent, inhibits MDM2 oncogene which is associated with its anti-cancer, chemosensitization and radiosensitization effects. *Proc Am Assoc Cancer Res* **47**, 538 (2006).
47. T. S. Huang, S. C. Lee, and J. K. Lin, Suppression of c-Jun/AP-1 activation by an inhibitor of tumor promotion *Proc Natl Acad Sci USA* **88**, 5292–5296 (1991).
48. Y. P. Lu, R. L. Cahng, Y. R. Lou, M. T. Huang, H. L. Newmark, K. R. Reuhl, and A. H. Conney, Effect of curcumin on TPA- and ultraviolet B light induced expression of c-jun and c-fos in JB6 cells and in mouse epidermis. *Carcinogenesis* **15**, 2363–2370 (1994).
49. K. S. Chun, Y. S. Keum, S. S. Han, Y. S. Song, S. H. Kim, and Y. J. Surh, Curcumin inhibits phorbal ester-induced expression of cyclooxygenase-2 in mouse skin through expression of extracellular signal-regulated kinase activity and NFκB activation. *Carcinogenesis* **24**(9), 1515–1524 (2003) .
50. S. Singhand B. B. Aggarwal, Activation of transcription factor NFκB is suppressing by curcumin.*J Biol Chem* **270**(42), 24,995–25,000 (1995).
51. S. K. Fogoros, M. Choi, and J. R. Liu, Curcumin mediates angiogenic factors through inhibition of NFκκn ovarian cancer cells. *Proc Am Assoc Cancer Res* **47**, 312 (2006).
52. J. Y. Liu, S. J. Lin, and J. K. Lin, Inhibitory effects of curcumin on protein kinase C activity induced by TPA in NIH 3T3 cells. *Carcinogenesis* **14**, 857–861 (1993).

53. R. Gopalakrishna and S. Jaken, Protein kinase C signaling and oxidative stress. *Free Radical Biol Med* **28**, 1349–1361 (2000).
54. R. Gopalakrishna and U. Gundimeda, Antioxidant regulating protein kinase C in cancer prevention.*J Nutr* **132**, 3819s–3823s (2002).
55. L. Korutla and R. Kumar, Inhibitory effects of curcumin on epidermal growth factor receptor kinase activity in A431 cells.*Biochim Biophys Acta* **1224**, 597–600 (1994) .
56. L. Korutla, J. Y. Cheung, J. Mendelsohn, and R. Kumar, Inhibition of ligand–induced activation of epidermal growth factor receptor tyrosine phosphorylation by curcumin.*Carcinogenesis* **16**, 1741–1745 (1995).
57. M. M. Y. .Chan, H. I. Huang, M. R. Fenton, and D. Fong, In vivo inhibition of nitric oxide synthase gene expression by curcumin, a cancer preventive natural product with anti-inflammatory properties.*Biochem Pharmacol* **55**, 1955–1962 (1998).
58. R. J. Anto, M. Venkatraman, and D. Karunagaran, Inhibition of NFκB sensitizes A431 cells to epidermal growth factor-induced apoptosis, whereas its activation by ectopic expression of Rel A confers resistance. *J Biol Chem* **278**(28), 25,490–25,498 (2003).
59. E. T. Efuet and K. Keyomarsi, Curcumin and simvastatin mediate growth arrest in breast cancer cells by targeting proteasome. *Proc Am Assoc Cancer Res* **47**, 1092 (2006).
60. M. B. Sporn and A. B. Roberts, Peptide growth factors are multifunctional. *Nature* **332**, 217–219 (1998).
61. L. Sun and G. Carpenter, Epidermal growth factor activation of NF-κB is mediated through IκBα degradation and intracellular free calcium. *Oncogene* **16**, 2095–2102 (1998).
62. N. R. Jana, P. Dikshit, A. Goswami, and W. Nukina, Inhibition of proteasomal function by curcumin induces apoptosis through mitochondrial pathway. *J Biol Chem* 279(12), 11,680–11,685 (2004) .
63. W. J. Chen and J. K. Lin, Induction of G1 arrest and apoptosis in human Jurkat T cells by pentagalloylglucose through inhibiting proteasome activity and elevating p27, p21 and bax protein. *J Biol Chem* **279**(14), 13,496–13,525 (2004).
64. C. Naujokat and S. Hoffmann, Role and function of 26S proteasome in proliferation and apoptosis. *Lab Invest* **82**, 965–980 (2002).
65. Y. C. Chen, T. C. Kuo, S. Y. Lin-Shiau, and J. K. Lin, Induction of HSP70 gene expression by modulation of calcium ion and cellular p53 protein by curcumin in colorectal carcinoma cells.*Mol Carcinog* **17**, 224–234 (1996).
66. L. I. Lin, Y. F. Ke, Y. C. Ko, and J. K. LinCurcumin inhibits SK-Hep-1 hepatocellular carcinoma cell invasion in vitro and suppresses matrix metalloproteinase 9 secretion. *Oncology* **55**, 349–353 (1998).
67. S. Phillip, A. Bulbule, and G. C. Kundu, Osteopontin stimulates tumor growth and activation of promatrix metalloproteinase-2 through nuclear factor-κB mediated induction of membrane type 1 matrix metalloproteinase in murine melanoma cells. *J Biol Chem* **276**, 44,926–44,935 (2001).
68. S. Phillip and G. C. Kandu, Osteopontin induces nuclear factor κB-mediated promatrix metalloproteinase-2 activation through IκBα/IKK signaling pathways and curcumin down regulate these pathways. *J Biol Chem* **278**(16), 14,487–14,497 (2003).
69. M. M. Manson, A. Gescher, E. A. Hudson, S. M. Plummer, M. S. Squires, and S. A. Prigent, Blocking and suppressing mechanisms of chemoprevention by dietary constituents. *Toxicol Lett* **112–113**, 499–505 (2000) .

70. A. H. Conney, Y. R. Lou, J. G. Xie, T. Osawa, H. L. Newmark, Y. Liu, R. L. Chang, and M. Huang, Some perspectives on dietary inhibition of carcinogenesis: Studies with curcumin and tea. *Proc Soc Exp Biol Med* **216**(2), 234–245 (1997) .
71. J. K. Lin and C.A. Shih, Inhibitory effect of curcumin on xanthine dehydrogenase/oxidase induced by TPA in NIH 3T3 cells. *Carcinogenesis* **15**, 1717–1721 (1994).
72. R. A. Haystead, A. T. Sim, and D. Carling, Effects of the tumor promoter okadaic acid on intracellular protein phosphorylation and metabolism. *Nature* **337**, 78–81 (1989).
73. A. H. Conney, Enzyme induction and dietary chemicals as approaches to cancer chemoprevention: The Seventh Dewitt Goodman Lecture. *Cancer Res* **63**, 7005–7031 (2003).