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Novel aspects for the application of Curcumin in chemoprevention of various cancers

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TABLE OF CONTENTS

1. ABSTRACT

Chemoprevention of malignant tumor growth is a novel and potentially powerful approach for tumor therapy. Recent *in vitro* and *in vivo* investigations provide increasing evidence that naturally occurring substances may exhibit significant chemopreventive activities. To this regard, the spice Curcumin, widely used in Indian cuisine, has been identified to show considerable anti-tumor effects. Most interestingly, numerous studies have not shown toxic side effects of this substance. Curcumin induces tumor cell apoptosis along with a reduction of tumor cell invasion and metastasis. Recent molecular studies provide evidence that Curcumin acts via a control of the NFkappaB pathway exerting most of the various modulating and moderating effects on malignant cells. Along with these *in vitro* studies, *ex vivo* and first clinical investigations confirm the anti-tumor effects of Curcumin, either as an isolated chemoprevention substance or in combination with chemotherapeutic agents as supportive measure reducing pharmaceutical resistance of tumor cells to certain chemotherapeutics. Despite our increasing knowledge on this interesting substance there still remain many unknown effects that deserve intense investigation.

2. INTRODUCTION

Malignant tumors represent currently the second most frequent cause of death in Western industrialized countries and the demographic rise in individual age strongly suggest even an increase in the incidence of cancer in the near future. In contrast to the increasing epidemiologic significance of malignancies, recent advances of early tumor detection, e.g. by systematic screening programs, and more and more efficient tumor therapies, e.g. by individualized chemo- and antibody therapies, limit the deleterious effects of malignancies. However, despite these improvements the prognosis of systemically spread tumors is still poor. Therefore, novel options of both tumor prevention and tumor therapy are required. One such strategy is represented by chemoprevention (1) such as hormone-depletion in postmenopausal women for the prevention of breast cancer (2). However, those strategies have shown until now only very small benefit along with considerable side-effects, such as enhanced bone demineralization (osteopenia) and others. Therefore, the search for further chemopreventive substances with little or no side-effects has focused on various natural plants, most coming from traditional Chinese and Indian medicine.

Curcumin (diferuloylmethane) is a naturally occurring polyphenol derived from the rhizome of the plant *Curcuma longa*. It has been used for many centuries in traditional Indian cuisine and its beneficial effects on inflammation are known for more than 2000 years. The substance is a potent antioxidant that possesses both anti-inflammatory and anti-tumor activities. As illustrated in more detail below, it has repeatedly been shown that Curcumin can suppress the initiation and progression of various tumors. Its anti-tumor properties in various cancer models and negligible toxicity in normal cells renders it to a promising chemotherapeutic candidate.

In vitro and *in vivo* studies on various tumor cell and animal models have partly unraveled the molecular regulation mechanisms by which Curcumin inhibits invasion (3-13), angiogenesis (8, 14-22) and metastasis (8, 10, 13, 15, 23). Since the hallmarks of tumor progression depend strongly on the regulation of matrix metalloproteinases (MMPs), a family of proteolytic enzymes that play a key role in the degradation of extracellular matrix proteins and the regulation of a series of pro-inflammatory cytokines and growth factors, the investigation of MMPs in Curcumin-treated models has been both an interesting target for the identification of the anti-cancer mechanism of Curcumin and an important aspect for future intervention. These studies provided circumstantial evidence that Curcumin shows indeed a systemic effect – despite the fact that Curcumin is a polyphenol and therefore poorly soluble in aqueous solution which may reduce the systemic availability of Curcumin in solid organ tumors. In the following, we will discuss the currently available information upon Curcumin on malignant tumors, their prevention and treatment, showing up a promising novel therapeutic approach with this agent in the future.

3. CURCUMIN –PLANT, CHEMISTRY AND BIOAVAILABILITY

Curcuma longa Linn, a member of the *Zingiberaceae*, is naturally occuring throughout tropical and subtropical regions of the world and is today widely cultivated in Middle and Far East Asian countries, especially in India and China. The plant is growing up to 1m height with a short stem and an oblong, ovate, pyriform and often short-branched rhizome. The powdered rhizome (called *tumeric*) is yellow colored and used for flavoring and coloring vegetarian and non-vegetarian food preparations, particularly in Indian cuisine. In the European Union Curcumin is admitted to the Food Supply List as "E100" and used as coloring substance in a variety of items including yellow mustard, cosmetics, pharmaceuticals and dyes of hair and fur.

Our own HPLC analysis revealed that commercially available Curcumin (e.g. Sigma-Aldrich, Steinheim, Germany) from the powdered dry rhizome, is composed of the three diarylhaptanoides called curcuminoids (Curcumin ≈82.38%, desmethoxycurcumin ≈15.03%, and bisdesmethoxycurcumin ≈2.59%) (24). All three polyphenols have a beta-diketon structure composed of two ferulic acid molecules linked via a methylene bridge at the C-atoms of the carboxyl groups. Due to the conjugated beta-diketone structure Curcuminoids undergo keto-enol tautomerization and exist entirely in the enol form in solution (25, 26) .

Curcumin is lipophilic and thus poorly water soluble; accordingly it has been shown to have low intestinal resorption rates in animals and/or humans. In plasma or tissues of most subjects, free Curcumin could not (or only in low levels) be detected, while levels of metabolites and conjugates were present (27, 28). Metabolites of curcuminoids, which can be found in the intestine and liver, originate from reductive processes (29), whereas oxidative metabolites could not be observed (30).

The major phase I metabolite for all three curcuminoids is the hexahydro-form, while lower amounts of dihydro-, tetrahydro- and octohydro curcuminoids could also be detected. Interestingly this group also found differences in gender concerning the formation of single metabolites and thereby showed that in male rats the hexa- and the octahydro-form whereas in females the tetra- and the hexahydro-form dominate (30) and they showed that there are diverse patterns of metabolites in different human cancer cell lines, but all cancer cell lines tested were sensitive to curcumin responding with an arrest in the G2/M phase and mitotic catastrophe (31).

In phase II curcumin as well as its phase I metabolites are conjugated to glucuronic acid and in lower amounts to sulfates with increased water solubility for renal secretion (30). There are two types of glucuronides existing, major phenolic ones and minor alcoholic ones (32). Several isoforms of UDP-glucuronosyl transferases (UGTs) from liver and intestine are responsible for the glucuronidation of curcuminoids, suggesting that the gastrointestinal tract substantially contributes to their metabolism (29). UGTs convert dihydrocurcumin and tetrahydrocurcumin to di-, tetra- and hexahydro-Curcumin-glucuronoside, which are colorless and have been detected in plasma (33). However, several studies have up to now detected only very low levels if any of free Curcumin in plasma (27, 28). Although it is most probable that Curcumin is biotransformed in the intestinal wall and the liver, the corresponding pathways have not yet been detected and some metabolism may also occur in the kidney and/or other organs (34).

In a recent study, Vareed and coworkers (35) have been able to identify Curcumin glucuronides and sulfates in a series of healthy human volunteers with a statistically half-life (t ½) of 6.77 +/- 0.83 h when a daily 10g- or a 12g-dose of Curcumin had been applied. Unexpectedly higher plasma levels of Curcumin conjugates in the 10g-dose than the 12g-dose have been suggested to result from saturation of a yet unknown transport mechanism. Recently, Baum and colleagues (36) measured the plasma Curcumin concentrations in humans in short term trial of 6 months. Here a mean level of 490 nmol/L of Curcumin could be reached in healthy volunteers, whereby Curcumin concentrations were higher after capsule than

Table 1. Short Overview of Presumed major direct and indirect antitumor effects of Curcumin (*in vitro* and *in vivo*)

Biological Process	Mediators	Effect
Control of transcriptional pathways	Several transcription factors (e.g., NFKappaB, AP-1, Notch, ERK1,2, □-catenin, Egr-1)	Down-regulation of pro-inflammatory and pro-metastatic enzymes – mainly MMPs – and cytokines; Inhibition of tumorprogression related gene expression and processes like proliferation, survival, invasion and metastasis
Cell apoptosis	Up-regulation of caspases and pro-apoptotic members of the bcl-2 gene family, etc.	Induction of apoptosis
Oxidative reactions	Up-regulation of antioxidants and detoxifying enzymes	Scavenge of oxygen radicals
Prostaglandin synthesis	Inhibition of NFκB	Inhibition of cyclooxygenase -2 (COX-2) and 5-lipoxygenase (LOX) transcription
Growth factor receptors	Inhibition of receptor ligation of EGFRs	Growth inhibition

adapted with persmission from (71, 154, 169)

powder administration. Curcumin consumption does not appear to have a significant effect on the serum lipid profile. However, the concentrations of plasma Curcumin and serum cholesterol were positively and significantly correlated. Thereby Curcumin consumption may modestly increase cholesterol.

Several formulation strategies are pursued to enhance the poor oral bioavailability of Curcumin like liposomes, nanoparticles, complexation with phospholipids and cyclodextrins or absorption enhancers like piperine. Complexation with phospholipids or cyclodextrins increases aqueous solubility of Curcumin as well as intermixture of alginates or gelatine at concentrations of 0,5% (w/v) at pH 5 (37). The combination of Curcumin with Piperine strongly enhances absorption rates and thus serum concentrations in rat and in human volunteers through the reduction of first pass effects by inhibiting Curcumin metabolising enzymes (38). On the other hand, solid lipid nanoparticles (SLNs) loaded with Curcumin did not show an improvement against pure Curcumin in absorption levels (39). Another approach was to encapsule Curcumin in a mixture of N-isopropylacrylamide (NIPPAM), N-vinyl-2-pyrrolidon (VP) and poly(ethylenglycol)monoacrylat (PEG-A). The so called "Nanocurcumin" demonstrates comparable therapeutic effects *in vitro* against various pancreatic cancer cell lines like pure Curcumin as there is an arrest of nuclear factor kappa B (NFkappaB) and various proinflammatory cytokines like tumor necrosis factor alpha (TNF alpha), IL-6 and IL-8 (40). *In vivo* studies are still missing. Very recently a group showed that there is a remarkably increase in absorption levels when Curcumin is orally administered as nanoparticles made of PLGA (polylactide-co-glycolide, Resomer). Bioavailability in rats is nine fold higher compared to Curcumin in combination with piperine as absorption enhancer and even higher than pure Curcumin (41). These data suggest that nanoparticles could be potent players for enhancement of the poor oral bioavailability of Curcumin.

Up to now, the biotoxicity and potential side-effects of the polyphenol have been tested in numerous studies. It has been shown, that Curcumin revealed only very little side-effects in healthy volunteers (e.g. (35)) and in cancer patients (see below, (27, 42)) with diarrhea and head ache being the most often indicated. However, there exists no report on a significant toxic reaction by Curcumin even up to daily dosage of 8 - 12 g confirming the innocuousness of this natural polyphenol.

In conclusion, although the polyphenol Curcumin has a very low resorption rate when administered orally, it has been proven that the Curcuminoids are taken up by the body mainly as glucuronides and sulfates. When the daily dosage is high enough (at present assumed to range at 8 – 10 g/day) plasma levels of Curcumin-glucuronides and –sulfates can be expected that may indeed exert pharmacological effects, particularly when those conjugates are deconjugated to free Curcumin as it has been shown for other polyphenols in inflammation (43, 44). This may also hold true for areas with malignant tumor growth. Extensive evidence has been gathered that even high-dosage Curcumin has no significant side-effects and does not induce toxic reactions (1).

4. PRESUMED CHEMOPREVENTIVE ANTI-CANCER ACTIVITIES OF CURCUMIN

Tumor progression is a complex process that comprises many steps and is driven by a sequence of molecular and cellular alterations affecting genes controlling cell proliferation, survival, and other attributes associated with the malignant phenotype. The crucial step of tumorigenesis is the transformation of premalignant into neoplastic cells, which is followed by the acquisition of increased invasivity and new blood vessel formation (angiogenesis) that are needed for metastasis (Figure 1). All these steps are potential targets for chemotherapy and chemoprevention.

At early stages of carcinogenesis cancer can be prevented by blocking the interaction of carcinogenic agents with cellular DNA causing genotoxic damage. This can be achieved by scavenging reactive oxygen species (ROS) or by inducing glutathione-S-transferase (GST) or other phase II conjugating enzymes to promote detoxification of the carcinogenic agent. During tumor promotion it is beneficial to slow down proliferation and to induce apoptosis in tumor cells. Later, during invasion and metastasis, prevention of proteolysis and degradation of extracellular matrix (ECM), as well as angiogenesis and the signaling pathways associated with these processes become targets of chemoprevention..

The chemopreventive activities of Curcumin are assumed to involve a whole variety of mechanisms that play a role in tumorigenesis and tumor progression (see Table 1). Out of those mechanisms a significant activation of programmed cell death (apoptosis), strong

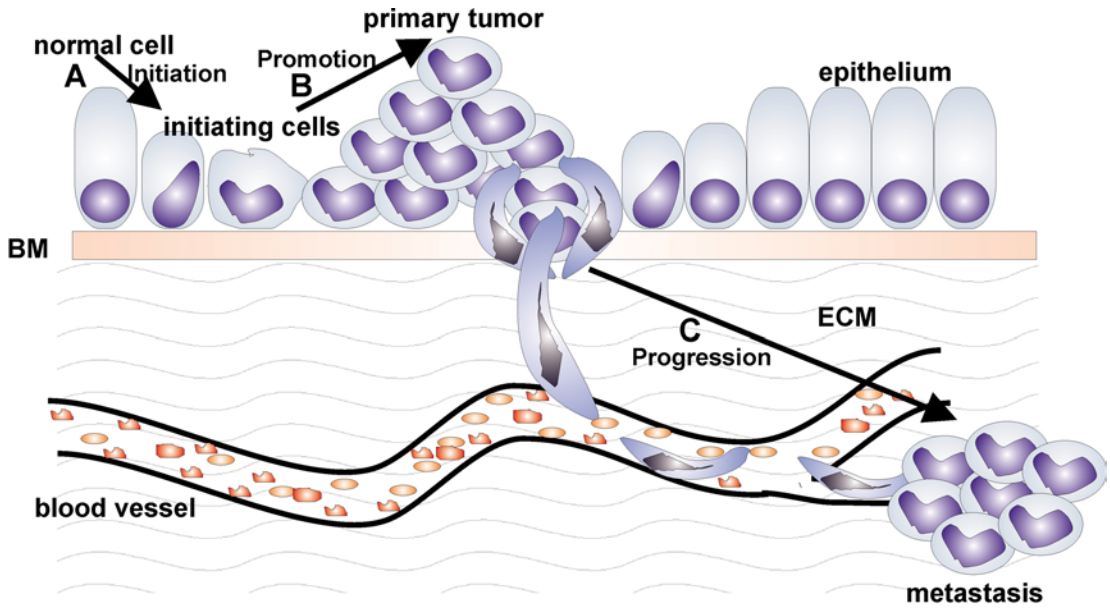

Figure 1. Multistage Carcinogenesis Process. Factors involved in malignant transformation and tumor progression: (A) activation of phase I and inactivation of phase II metabolizing enzymes, enhanced free radical generation; (B) oncogenes, overexpression of growth factors, cell cycle alterations; (C) MMPs, Cytokines; TNF alpha; COX2; adhesion molecules, angiogenic factors

antioxidant effect by direct scavenge of reactive oxygen species as well as up-regulation of carcinogen-detoxifying enzymes / antioxidants and the inhibition of various transcription factors with the inhibition of NFkappaB represent the most central issues. In particular, the inhibition of the NFkappaB pathway by Curcumin leads to the down-regulation of various pro-inflammatory cytokines and the inhibition of the mRNA expression of different pro-inflammatory and pro-metastatic enzymes, such as matrix metalloproteinases (MMPs). Similarly, other factors/enzymes, such as cyclooxygenase (COX)-2, are inhibited and down-regulated and several other important cellular effectors are influenced. In consequence, Curcumin has been shown to interfere with multiple signal pathways including apoptosis, proliferation (by growth factor receptor inhibition), cell cycle, cell survival, inflammation, invasion and metastasis (13, 24).

Subsequently, we will therefore look at the most important issues of the presumed Curcumin effectors in detail – keeping, however, in mind that the other interactions of Curcumin may in fact contribute to the pharmacological effects as well.

4.1. Apoptosis, proliferation and cell cycle arrest

The loss of a fully functional apoptotic program is one of the hallmarks of malignant tumor cells and apoptosis is therefore a major target of chemoprevention of cancer. Although the initiation of apoptosis by p53 is an important mechanism (intrinsic pathway) apoptosis can also be initiated through a variety of extra cellular signals (extrinsic pathway). Both the extrinsic and the intrinsic apoptotic pathways converge to activate the caspase cascade leading to the activation of caspases-3, -8 and -9. An indirect involvement in the regulation of apoptotic cell death comes from the signaling pathways of PI3K/AKT, MAPK, and NFkappaB, which control cell proliferation and survival.

Curcumin exerts significant anti-proliferative and pro-apoptotic effects against diverse tumors *in vitro* (45-48) and *in vivo*, as it has been found to suppress carcinogenesis of the breast (49) and other organs (50-52). This is determined by the repression of cell survival factors through the strong inhibition of the NFkappaB-pathway (see below), as well as by a Curcumin-induced release of cytochrome C and activation of caspases (53). In several colon cancer cell lines, Curcumin activated caspases-3, -7, -8 and -9, but reduced activation of caspases related to the mitochondrial pathway together with a partial blocking of apoptosis-inducing factor (AIF) (54). Accordingly, it has been shown that Curcumin mediates the opening of the permeability transition pores leading to increased permeability of the mitochondrial membrane and the collapse of its membrane potential. Squires and coworker additionally described that Curcumin inhibits the basal phosphorylation of Akt/protein kinase B (PKB) in breast cancer cells which constitutes a further important way to facilitate apoptosis (55).

The restriction point (R point) represents a crucial step in the cell cycle that is deregulated in most types of cancer leading to unconstrained cell proliferation through the deregulated expression of a variety of cyclins. Disturbance of cell cycle specific factors and proteins by Curcumin therefore constitutes a possibility to block continuous proliferation of tumor cells. In human colon and

bladder cells G2/M cell cycle arrest, down-regulation of cyclin A and up-regulation of the CDK inhibitor p21 and Cdc2 have been observed (56, 57). Moreover, Curcumin treatment reduced proliferation in lymphoma and keratinocyte cell cultures. It decreased cyclin and CDK1 levels and suppressed Bcl-X_L levels, resulting in a reduced mitochondrial membrane potential and increased cleavage of PARP (58, 59). It has also been reported, that Curcumin induces cell cycle arrest followed by the induction of apoptosis by cyclin-dependent kinase inhibitor $p21^{/WAF1/CIP1}$ in androgen-sensitive LnCaP as well as androgen-insensitive PC3 prostate carcinoma cells (60).

Further more it has been shown that Curcumin and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)/Apo2L interact to induce cytotoxicity in the prostate cancer cell line LnCaP (61). Extensive *in vivo* studies have shown that the polyphenol sensitizes even TRAIL-resistant prostate cancer cells to undergo apoptosis by TRAIL. The underlying molecular mechanisms, by which Curcumin sensitizes the resistant tumor cells involves up-regulation of the expression of of TRAIL-R1/DR4, TRAIL-R2/DR5, Bax, Bak, $p21^{/WAF1}$, and $p27^{/KIP1}$, and inhibition of NFkappaB and its gene products such as cyclin D1, VEGF, uPA, MMP-2, MMP-9, Bcl-2 and Bcl-X_L (15, 17), whereby Bax and Bak seem to be essential for maximum apoptotic response to Curcumin (62). Besides regulating Bcl-2 family members, Curcumin up-regulates the expression of p53 as well as its phosphorylation at serine 15, and acetylation in a concentration-dependent manner. Moreover, Curcumin inhibits expression of phosphatidylinositol-3 kinase (PI3K) p110 and p85 subunits, and phosphorylation of Ser 473 Akt/PKB. Through the action on Akt and the interaction with p53 and the mitochondrial death pathway, Curcumin induces apoptosis in prostate carcinoma cells (63).

4.2. Antioxidant activities

Reactive oxygen species (ROS) are important mediators of inflammation; oxidative stress plays an important role in the pathogenesis of various diseases including cancer. Curcuminoids have long been established as strong antioxidants in foods and biological systems (64) and the antioxidant activity of Curcumin is ranked as comparable to that of vitamins C and E (65). From a structural point of view, the curcuminoids have been shown (mainly by their beta-diketone moiety) to be potent scavengers of reactive oxygen species including superoxide anion radicals, hydroxyl radicals and nitrogen dioxide radicals (66-68). Experimental *in vivo* studies in animals identified Curcumin to inhibit lipid peroxidation (69). Vascular endothelial cells treated with Curcumin prevented oxidant mediated injuries by increased heme oxygenase production (70). Furthermore, numerous studies have used *in vitro* and animal models and all have identified a beneficial effect of Curcumin in hypoxia-, oxygen-radical- or hemorrhage-induced models of oxidative lesion (71).

4.3. Inhibition of tumor-progression on the level of transcription

Uncontrolled cell proliferation, escape from the apoptotic pathways, cell invasion, metastasis, angiogenesis and resistance to chemotherapy and radiotherapy, which are the major hallmarks of tumor progression, are under the control of the transcriptional pathways of AP-1 (activator protein 1), NFkappaB, beta-catenin, EGR-1 (early growth response gene 1), and Notch-1. Several of these pathways are up-regulated in many cancers and are targeted by Curcumin. Therefore the interference with transcription pathways is obviously one of the central issues of Curcumin's anti-tumoral effect. Out of numerous potential activation ways and factors, the inhibition of the NFkappaB-pathway seems to be the most efficient and thereby the most important one. In the majority of normal cells as yet investigated, NFkappaB exists in an inactive form in the cytoplasm bound to the inhibitory proteins referred to as IkappaB. Several IkappaBs, including IkappaB alpha (MAD3), IkappaB alpha, p105/IkappaB gamma, p100/IkappaB delta and IkappaB epsilon have been identified (72). Upon phosphorylation by the corresponding kinase IKK, IkappaB alpha is rapidly degraded via the ubiquitin-proteasome pathway, permitting activation and translocation of NFkappaB into the nucleus where it binds to its cognate sequences in the promoter regions of multiple genes. NFkappaB activity is associated with anti-proliferative effects, as well as indicated above with the induction of apoptosis (73, 74). Several signal transduction pathways converge on NFkappaB and its regulators to mediate the transcriptional control of apoptosis and cell-cycle control (75, 76). NFkappaB is required for prevention of cell death induced by TNF alpha and its ability to induce anti-apoptotic genes such as *bcl2* and *birc5/survivin* protects cancer cells from apoptosis (77, 78). As a consequence, activation of NFkappaB constitutes a crucial step in tumor promotion and progression, angiogenesis, inflammation, invasion, and metastasis (79). Constitutive NFkappaB expression correlates with the metastatic potential of breast tumors and has been proposed as both a prognostic marker and a drug target (80). Curcumin acts through the inhibition of phosphorylation of IκB which prevents its proteolytic degradation, as well as phosphorylation of the p65 subunit of NFkappaB. As a consequence translocation of NFkappaB from the cytoplasm into the nucleus is prevented and transcriptional activation of tumor associated genes is inhibited (13, 24, 81). Curcumin thus inhibits NFkappaB regulated expression and activation of matrix metalloproteinases (MMPs), which without Curcumin's action would enable invasive growth through the degradation of the peritumoral matrix (82). Likewise, our group has shown this effect in the breast cancer cell line MDA-MB-231 where the application of Curcumin reduced the expression of the major MMPs expressed by the cells probably due to reduced NFkappaB activity (13, 24).

Additionally, we have shown that the transcription factor AP-1, which is also important for MMP biosynthesis and therefore for matrix degradation, is down-regulated by Curcumin. There exists a crosstalk between the c-jun subunit of AP-1 and the p65 subunit of NFkappaB collaborating within the molecular action of Curcumin. Acting through both pathways together in a synergistic way, rather than acting only on one of them alone multiplies and guarantees the efficacy of the polyphenol (13, 24).

In pancreatic cancer cells it could be shown, that Curcumin down-regulates Notch-1, a factor playing a critical role in maintaining the balance between cell proliferation, differentiation and apoptosis. Again, in this study it has been shown that Curcumin acts in a synergistic way on Notch-1 and NFkappaB (83).

One of the novel molecular targets of Curcumin' s chemopreventive action is beta-catenin, whose signaling pathway is disrupted in many cancer cells, such as those of colorectal cancer, hepatocellular carcinoma, and gastric carcinoma (84-86). beta-catenin either enters the nucleus to transactivate the TCF/LEF transcription factor, leading to upregulation of many genes responsible for cell proliferation, or binds to the E-cadherin adhesion complex. Reduction or loss of E-cadherin and/or increased localization of beta-catenin in the nucleus is associated with invasive metastatic cancer progression and poor prognosis (87, 88). Curcumin has been found to decrease nuclear beta-catenin and TCF-4 and hence inhibit beta-catenin/TCF signaling in various cancer cell lines (89). Curcumin induces G(2)/M phase arrest and apoptosis in colon cancer cells by impairing Wnt signaling and decreasing transactivation of beta-catenin/TCF/LEF (90), subsequently attenuating tumor progression.

The early growth response gene product (Egr-1) is a nuclear transcription factor and is implicated in the regulation of a number of genes that are involved in inflammation, growth, development, and differentiation (91-93). Significantly reduced Egr-1 expression has been observed during tumor formation in various mammalian cells and tissues (94).

It has been shown, that the induction of the cell cycle inhibitor p21 by Curcumin is mediated by transactivation of Egr-1 (95). Furthermore pretreatment of endothelial cells and fibroblasts with Curcumin suppresses phorbol 12-myristate 13-acetate and serum-induced Egr-1 binding activity and Curcumin inhibits phorbol 12-myristate 13-acetate-induced de novo synthesis of Egr-1 protein in endothelial cells (96). Another group reported that Curcumin reduced expression of EGFR in human colon cancer derived cells, including Moser, Caco-2 and HT-29, by inhibition of the transcription factor Egr-1 as trans-activator (97).

In order to gain a complete picture of all transcriptional processes affected by Curcumin, our group has screened the effects of Curcumin in MDA-MB-231 breast cancer cells using microarray gene expression analyses of several thousands of genes. The subsequent differential display between Curcumin-treated and non-treated cells revealed 45 genes to be statistically significantly regulated by the polyphenol among which we recognized several targets of the transcription factor NFkappaB (Table 2).

The microarray data were validated by quantitative real time RT-PCR. Some of these genes are in the group of metastases associated genes like e.g. EGR1 with reduced expression in Curcumin treated cells (97). Other genes that were found to be up-regulated in the breast cancer cells by Curcumin were linked to anti-oxidant responses like e.g. HMOX1 (hemeoxygenase-1) or GCLM and have been shown to be induced also by other chemopreventive anti-oxidants (98).

EGR1 has been shown to mediate the induction of the cyclin-dependent kinase inhibitor $p21^{Waf1/Cip1}$ by Curcumin in human glioma cells (95). Proliferation is thus targeted by NFkappaB mediated down-regulation of the expression of S-phase cyclins (99) and by EGR1 dependent regulation of p21. Activation of p21 transcription is p53 independent and the effect of Curcumin on proliferation is therefore expected to be active also in p53 mutated cells, an important feature for chemoprevention since about half of human tumors carry mutated forms of p53. In breast cancer cells, EGR1 is down-regulated by Curcumin (see Table 2). When we validated the microarray expression value for this gene by real time PCR we observed discordant results dependent on the region of the transcript that was analyzed: Primers located in the 5' region of the gene revealed EGR1 up-regulation as observed for glioma cells whereas primers in the 3' region, where our microarray probes were located, confirmed the array data (our unpublished observation). Most probably, Curcumin induces transcription of a different transcript from the EGR1 locus instead of the transcript present in untreated cells.

Additionally, Curcumin significantly down-regulated the transcriptional level of the inflammatory cytokines CXCL1 and –2 (GROalpha and –beta) ending up with a reduction of the related proteins. The two cytokines are regulated by NFkappaB since they were down-regulated by silencing of the subunit p65 of NFkappaB and up-regulated by silencing of IkappaB alpha.

4.4. The anti-cancer effects of Curcumin by interfering with microRNAs

Beyond the aforementioned mechanisms, recent approaches have identified novel target pathways the role of which is as yet only incompletely identified. One such new system covers the microRNAs (miRNA), highly conserved, non-coding RNAs composed of 20 to 22 nucleotides, which are encoded in the genome of plants, animals and humans. These small molecules have recently been identified as a major regulatory gene family in eukaryotic cells. It has been speculated that they serve as rivals to the usually known system of transcription factors. It is now clear that miRNAs are involved in numerous processes of development, differentiation, apoptosis and proliferation and specific miRNAs have been implicated in human cancer pathogenesis with some miRNAs being up- and others down-regulated (100-102). To the best of our knowledge, the effect of Curcumin on miRNA expression has been addressed in a single study, so far. In a microchip test for the most important – and potentially relevant – miRNAs, Sun and colleagues (103) tested human pancreatic cancer cells (cell line PxBC-3) for the effects of Curcumin on miRNA expression. In their system, they detected an up-regulation of miRNA-22 and down-regulation of miRNA-199a suggesting that Curcumin also interferes with the miRNA-system in human cancer cells providing an additional anti-cancer effect yet to be explored in depth.

Table 2. Gene expression after Curcumin treatment in human breast cancer cells - Curcumin-responsive genes

Gene Name	Description	Fold Change
Up- regulated >2		
HMOX1	*heme oxygenase (decycling) 1*	10,92
GCLM	*glutamate-cysteine ligase, modifier subunit*	4,24
GCLM	*glutamate-cysteine ligase, modifier subunit*	3,81
GCLM	*glutamate-cysteine ligase, modifier subunit*	3,39
MRCL3	MYOSIN REGULATORY LIGHT CHAIN MRCL3	3,12
TRIM16	*tripartite motif-containing 16*	2,61
HIST1H2BD	histone 1, H2bd	2,32
NA	NA	2,26
MGC14376	HYPOTHETICAL PROTEIN MGC14376	2,26
LOC399959	NA	2,26
LOC399959	NA	2,23
LOC51315	NA	2,21
RNPC2	RNA-binding region (RNP1, RRM) containing 2	2,2
SLC7A11	solute carrier family 7, (cationic amino acid transporter, y+ system) member 11	2,17
DNAJB9	DnaJ (Hsp40) homolog, subfamily B, member 9	2,17
LOC399959	NA	2,17
SFRS1	splicing factor, arginine/serine-rich 1 (splicing factor 2, alternate splicing factor)	2,11
FNTB	farnesyltransferase, CAAX box, beta	2,1
MGC16275	HYPOTHETICAL PROTEIN MGC16275	2,07
LOC51315	NA	2,07
DNAJB9	DnaJ (Hsp40) homolog, subfamily B, member 9	2,06
HSZFP36	ZFP-36 FOR A ZINC FINGER PROTEIN	2,06
TBC1D8	TBC1 domain family, member 8 (with GRAM domain)	2,05
RPL31	ribosomal protein L31	2,03
SLC30A1	solute carrier family 30 (zinc transporter), member 1	2,03
Down-regulated <0.5		
NA	NA	0.50
NHLRC2	NHL repeat containing 2	0.50
LOC400027	HYPOTHETICAL GENE SUPPORTED BY BC047417	0.49
NA	NA	0.48
RFXAP	regulatory factor X-associated protein	0.48
SFRS5	splicing factor, arginine/serine-rich 5	0.47
KIAA1223	ANKYRIN REPEAT DOMAIN 50	0.47
C1orf79	chromosome 1 open reading frame 79	0.47
KIAA1838	KIAA1838	0.45
ERN1	endoplasmic reticulum to nucleus signalling 1	0.45
HNRPD	heterogeneous nuclear ribonucleoprotein D (AU-rich element RNA binding protein 1, 37kDa)	0.45
RGS7	regulator of G-protein signalling 7	0.44
DDIT4L	DNA-damage-inducible transcript 4-like	0.43
SUI1	EUKARYOTIC TRANSLATION INITIATION FACTOR 1	0.42
MGC16121	NA	0.42
NA	NA	0.42
NR4A2	nuclear receptor subfamily 4, group A, member 2	0.41
NANOS1	nanos homolog 1 (Drosophila)	0.39
MGC16121	NA	0.38
EDG2	endothelial differentiation, lysophosphatidic acid G-protein-coupled receptor, 2	0.37
KRTAP2-1	keratin associated protein 2-1	0.37
HNRPA1	heterogeneous nuclear ribonucleoprotein A1	0.36
PTGS2	*prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)*	*0.36*
NA	REGULATING SYNAPTIC MEMBRANE EXOCYTOSIS 2	0.34
CXCL1	*chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)*	*0.34*
NA	FUSION (INVOLVED IN T(12;16) IN MALIGNANT LIPOSARCOMA)	0.34
MGC16121	NA	0.33
FOSL2	FOS-like antigen 2	0.32
NR4A2	nuclear receptor subfamily 4, group A, member 2	0.31
IL6	*interleukin 6 (interferon, beta 2)*	*0.28*
PTGS2	*prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)*	*0.27*
EGR1	*early growth response 1*	*0.24*
KLF10	Kruppel-like factor 10	0.23
DAF	decay accelerating factor for complement (CD55, Cromer blood group system)	0.20
CXCL2	*chemokine (C-X-C motif) ligand 2*	*0.19*
FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog	0.18
EGR1	*early growth response 1*	*0.18*

NA = not annotated. Reproduced with permission from (24)

4.5. MMP expression

We and others have shown that the expression of major MMPs correlates with the growth behavior of tumor cells in vitro and in vivo. Tumor cell models of malignant keratinocytes (104-106) and breast carcinoma cells (107-109) showed increasing MMP expression levels along with increasing aggressiveness of tumor cell growth and metastatic potential.

The vast number of biological processes in which MMPs are involved comprises many other aspects of tumor

progression, such as activation of growth factors (reviewed in (110)), tumor growth, invasion, metastasis, angiogenesis and tumor associated inflammation (111, 112). The control of MMP synthesis and activation can therefore be regarded as an important target for the prevention of tumor progression.

Due to its central issue, over the past years, the effect of Curcumin on expression and activity of MMPs has been studied *in vitro* and *in vivo* in various inflammatory diseases (113, 114) and various cancer cell lines (12, 13, 115-118). Well in accordance with the above mentioned down-regulation of NFkappaB- and AP-1, Curcumin suppressed the synthesis of various MMPs in different tumor cell lines. Numerous studies have investigated the effect of Curcumin on transcription, protein expression and activity of MMPs providing in most instances consistent data.

We have shown that Curcumin treatment of breast cancer cells resulted in strongly diminished levels of MMP-1 and MMP-2 mRNA already after 2h leading to 3.45 fold (MMP-1) and 2.5 fold (MMP-2) reduction after 20h. MMP-3 and -9 expression levels, on the other hand, did not show any considerable decrease (13). This was confirmed by Western blot data showing diminished MMP-1, -2 and -3 protein concentrations in cell supernatants. The enzymatic activity of MMP-2 and -9 (evidenced as gelatinolytic activity by zympgraphy) was similarly down-regulated upon Curcumin treatment. Recently, Yodkeeree and coworkers (119) comparatively examined the influence of three curcuminoids on the expressions of uPA, MMP-2, MMP-9, membrane Type 1 MMP (MT1-MMP), tissue inhibitor of metalloproteinases (TIMP-2), and *in vitro* invasiveness of human fibrosarcoma cells showing that the three forms significantly inhibited MMP-2 and MMP-9, but not uPA activity. In another study Curcumin down-regulated MMP-2 expression and activity and expression of integrin receptors, FAK, and MT1-MMP to almost background levels in laryngeal squamous carcinoma cells. MMP-2 (but not MMP-9) mRNA expression was abolished upon Curcumin treatment, indicating specific inhibition of MMP-2. The invasive potential of the tumor cells was also significantly reduced. After drug withdrawal, expression of MMP-2, integrin receptors, MT1-MMP, and FAK returned to control levels (115). A study on human colon cancer cells revealed that Curcumin inhibited MMP-2 levels and promoted MMP-9 levels, but did not affect MMP-7 levels, based on Western blotting assays (116). These effects were also confirmed by cDNA microarray on the mRNA levels. In prostate cancer cells (DU-145) treated with Curcumin MMP-2 and MMP-9 activities were significantly reduced along with impaired *in vitro* cellular invasion and, moreover, tumorigenicity was diminished in a xenograft model (117). RNase protection assays of malignant glioma cells showed that Curcumin inhibited the PMA-induced mRNA expression of MMP-1, -3, -9, and -14 (120) and a study on head and neck squamous carcinoma cells revealed that various cell survival and cell proliferative genes including Bcl-2, cyclin D1, IL-6, COX-2 and MMP-9 were suppressed (121). Treatment of highly metastatic murine melanoma cells B16F10 with Curcumin (118) for 15 days significantly inhibited MMP-2 activity. Expression of MT1-MMP and focal adhesion kinase (FAK), an important component of the intracellular signaling pathway, were also reduced to almost background levels. MMP-2, MT1-MMP and FAK did not return to control levels even after 28 days of drug withdrawal. Moreover it has been demonstrated that Curcumin inhibits the expression of estrogen receptor (ER) downstream genes including pS2 and TGF in ER-positive MCF-7 cells, and that this inhibition is also dependent on the presence of estrogen (122). Curcumin also decreased the regulation of MMP-2 along with an upregulation of TIMP-1 (122). There exist several more studies providing widely similar data on various cell lines.

The inhibitory effect of Curcumin on MMP expression occurs most likely already at the level of gene transcription. The promoter regions of the genes encoding MMP-1, -2, -3, -7, -9, -12 and -13 have been analyzed. All of them contain a proximal activating-protein-1 (AP-1) binding site approximately 70 bp 5' to the transcription start (123, 124). Moreover, it is known that also other transcription factors influence MMP expression as their promoter regions contain NFkappaB-like elements (125, 126). As described above, Curcumin is capable of regulating the expression and the activity of many transcription factors as e.g. AP-1 and NFkappaB, resulting in altered MMP synthesis and proteolytic activity.

Taking all these observations into account, there is clear evidence that Curcumin indeed reduces the levels of major MMPs with some differences in the efficiency and some variety with respect to the MMPs affected. This may be caused by differences in responsiveness and partial resistance to Curcumin in some cell types. In conclusion, however, by reducing the expression and activity of MMPs, the most important matrix degrading systems is considerably affected by Curcumin proving a rationale for reduced tumor invasion and growth.

4.6. Tumor cell invasion

In addition to the inhibitory effect on matrix degradation by blocking of various MMPs, but also other enzymes, the over-all effect on invasive properties of Curcumin has to be looked at. This can be tested *in vitro* by tests, such as the matrigel invasion assay, and *in vivo* in experimental animal models, such as tumor xenograft experiments. There exists a long list of experiments under various settings – using a broad range of different cell lines and cellular models. Out of the large bulk of data, three main results can be extracted.

1. Consistent data show that Curcumin inhibits invasion of numerous tumor cell lines whereas data on migration properties is less consistent.
2. Curcumin reduces tumor growth in a series of tumor cell xenograft models.
3. Synergistic effects between Curcumin and other chemotherapeutic agents, such as paclitaxel, or gamma-radiation seem to be very likely; the addition of Curcumin to the therapeutic protocol appears to provide benefits in terms of less resistance to other drugs and/or prolonged tumor control.

First reports on an anti-invasive effect of Curcumin came from an *in vitro* study investigating a highly invasive human hepatocellular carcinoma cell line in assays without or with Matrigel to quantify cellular migration and invasion (12). Thereby, Curcumin inhibited significantly both parameters along with secretion of MMP-9. Similar effects could be observed on invasion of B16F-10 melanoma cells across the collagen matrix in the Boyden chamber, which was impaired by Curcumin although the treatment did not inhibit the motility of the tumor cells (127). By means of an *in vitro* model of malignantly transformed keratinocytes Santibanez and colleagues (128) demonstrated that Curcumin inhibited the TGFbeta-1-induced synthesis of fibronectin, an early responsive gene to the growth factor along with a reduction of the TGFbeta-1-stimulated cell migration and invasiveness. Another study revealed that Curcumin inhibits the H-ras-induced invasive phenotype in MCF10A human breast epithelial cells and down-regulates MMP-2 dose-dependently (11). Synthetic Curcumin analogs exert a strong inhibitory effect on human fibrosarcoma invasion along with a down regulation of important invasion factors, such as MMP-9 and VEGF (129). Further studies demonstrated that Curcumin reduces invasion of colorectal cancer cell (116, 130).

In an experiment of colon cancer cell xenografts in nude mice, randomized into four groups, which were treated either with a non-reactive vehicle, Curcumin, gamma-radiation with the vehicle, and Curcumin in combination with gamma-radiation, Curcumin significantly enhanced the efficacy of fractionated radiation therapy by prolonging the time to tumor re-growth and by reducing the Ki-67 proliferation index. Moreover, Curcumin suppressed NFkappaB activity and the expression of NFkappaB regulated gene products (cyclin D1, c-myc, Bcl-2, Bcl-xL, cellular inhibitor of apoptosis protein-1, COX-2, MMP-9, and vascular endothelial growth factor), many of which were induced by radiation therapy and mediate radioresistance. The combination of Curcumin and radiation therapy also suppressed angiogenesis. These results suggest that Curcumin potentiates the antitumor effects of radiation therapy in colorectal cancer (8).

In an *in vitro* model of murine melanoma cells (B16F10) Curcumin reduced invasion along with impaired migration and enhanced apoptosis (131, 132). In a xenograft model of prostatic cancer Curcumin treatment resulted not only in a significant reduction of MMP-2 and MMP-9 expression, but also inhibited the invasive ability of the tumor cells *in vitro*. In addition Curcumin reduced markedly the tumor volume along with MMP-2, and MMP-9 activity in the tumor-bearing site and the metastatic nodules *in vivo* were significantly fewer in the treatment group in contrast to the untreated control group (117).

In our own previous studies (13, 24) we had shown that the application of Curcumin resulted in a statistically significant reduction of breast cancer cell invasion through a reconstituted basement membrane which correlated well with the inhibition of several MMPs resulting in a reduced formation of breast cancer metastasis.

Aggarwal and coworkers hypothesized that Curcumin would potentiate the effect of chemotherapy in advanced breast cancer. They tested this hypothesis using paclitaxel (Taxol)-resistant breast cancer cells and a human breast cancer xenograft model. Curcumin suppressed the paclitaxel-induced expression of antiapoptotic (XIAP, IAP-1, IAP-2, Bcl-2, and Bcl-xL), proliferative (COX-2, c-myc, and cyclin D1), and metastatic proteins (vascular endothelial growth factor, MMP-9, and intercellular adhesion molecule-1) (133).

However, a previous study described even adverse effects of Curcumin application *in vitro* when the polyphenol was added to chemotherapeutica (camptothecin, a cytotoxic topoisomerase 1-inhibitor, mechlorethamine or doxorubincin). They showed that Curcumin blocked the generation of reactive oxygen species otherwise induced by the chemotherapeutica, thereby preventing breast cancer cells *in vitro* from apotosis. However, it remains unclear why in this model the application of Curcumin itself did not induce apoptosis (134). Finally, the review by Garg and colleagues (81) clearly summarizes several studies that indicate a positive effect of Curcumin as chemosensitizer (in multiple myeloma (135); in prostate cancer cells (136)), radiosensitizer (in prostate carcinoma cells (137)) and cell protector from chemo-toxic effects (e.g. in lung cancer cells (138); whole-body irradiation (139, 140); doxorubicin toxicity (141, 142)).

Taking all experimental observations into account, one may conclude that the application of Curcumin significantly reduced the tumor cell´s capacity to invade the surrounding stroma – mainly by a control of matrix degrading enzymes (see also 4.5. “MMP expression”).

4.7. Metastasis

Finally, data emerge from various models that also indicate an anti-metastatic effect of Curcumin on tumor cells. As early as in 1995, first experiments used Curcumin as an anti-metastatic agent. Menon and coworkers (143) had tested mice for the inhibition of B16F10 melanoma cell lung metastases by Curcumin. Oral administration of polyphenols such as Curcumin and catechin at “physiological” concentrations were found to inhibit the formation of lung metastases by 80%. Curcumin proved to be the strongest drug out of a dozen substances. Consequent to the inhibition of the lung tumor nodules, the life span of animals treated with Curcumin was also found to be increased by approximately 150%. In another study, orthotopically implanted metastatic cells of Lewis lung carcinoma (LLC-MLN), which were isolated by an *in vivo* selection method, resulted in greater metastatic growth in mediastinal lymph nodes as compared with that of the original LLC cells (144). Oral administration of Curcumin significantly inhibited the mediastinal lymph node metastasis of those implanted LLC cells in a dose-dependent manner, although it did not affect the tumor growth at the implantation site. Combined treatment with Curcumin and the anti-cancer drug cis-diamine-

dichloroplatinum (CDDP) inhibited markedly tumor growth at the implanted site and of lymphatic metastasis, and a significantly prolonged the survival.

In a rat model of liver tumor resulting from copper-induced oxidative stress, the effect of Curcumin on lipid peroxidation and DNA damage was tested (145). Although Curcumin did not influence the tumor incidence of the primary liver tumors, it significantly reduced the tumor incidence at other organ sites and suppressed formation of metastases. Similarly, other tumor models such as prostatic carcinoma cells (DU-145) injected into immunodeficient mice revealed significantly fewer metastases when treated with Curcumin (117) and in the already previously mentioned study on Curcumin effects in paclitaxel (Taxol) resistant breast cancer cells (24) a significant reduction in the incidence of breast cancer metastasis was reported.

Finally, two studies from our group (13, 24) provided clear evidence for the statistically significant reduction of breast cancer metastasis *in vivo*. Five weeks after injection of metastasizing MDA-MB-231 breast cancer cells, Curcumin treated mice contained significantly less lung metastases. Surprisingly, four Curcumin treated and none of the control animals remained metastases free. While the number of hematogenous metastases obtained through the injection of MDA-MB-231 cells directly into the heart is significantly lower in Curcumin treated animals, size and morphology of the metastases formed are indistinguishable from those seen in control animals.

Subsequently we unraveled the underlying molecular mechanisms by analyzing all genes differentially expressed between Curcumin treated and un-treated MDA-MB-231 breast cancer cells (see also Table 2). Amongst the strongest down-regulated genes in the Curcumin treated cells we detected the two pro-inflammatory cytokines CXCL1 and –2. Both are known to be associated with migration of breast cancer cells *in vitro* (146) and tumor growth, metastasis, as well as angiogenesis in mouse squamous cell carcinoma (147), Well in line Massaguè and co-workers have shown that highly metastatic subclones derived from MDA-MB-231 cells by dilution cloning overexpress CXCL1 and –2 leaving the question unanswered inasmuch expression of these chemokines is causally linked to invasion and metastasis (148).

5. CURCUMIN'S ANTI-INFLAMMATORY ACTION AND ITS IMPACT ON TUMORPROGRESSION

The contribution of inflammation to tumor progression, initially proposed by Rudolf Virchow, has recently been confirmed by new evidence (149). Tumor inflammation can be induced by both, intrinsic (activated oncogenes) and extrinsic (inflammation, infections) factors leading to the activation of three major transcription factors: NFkappaB, STAT3 (signal transducers and activators of transcription 3) and HIF1 alpha (hypoxia induced factor 1 alpha) (150). Evidence for the effects of Curcumin on NFkappaB is sound (74, 81), perhaps most of the anti-inflammatory activities of the polyphenol can be attributed to the inhibition of the IkappaB kinase, IKK. IKK phosphorylates IkappaB and induces its degradation in the proteasome. In the absence of IkappaB, NFkappaB translocates to the nucleus where it can activate transcription of a large number of inflammation and survival related genes. Effects on STAT3 (135, 151) as well as on HIF1 alpha itself (22) and on the aryl hydrocarbon receptor nuclear translocator needed for HIF1 alpha (152) have been described. IL-6, a downstream target of STAT3 is included in the list of Curcumin regulated genes (Table 2).

Curcumin reduces the activities of these transcription factors and thus determines a reduced expression of inflammatory cytokines which in turn reduces the accrual of inflammatory cells into the tumor. Tumor associated macrophages, eosinophils and neutrophils secrete their own set of inflammatory cytokines and create a vicious cycle of cross-talk between the tumor cell and the tumor infiltrate that leads to increased cell proliferation, angiogenesis and lymphangiogenesis, tumor cell migration and invasion thereby altering the tumor's response to the adaptive immunity and to chemotherapy (150).

TNF alpha, NFkappaB and its downstream target COX2 are involved as key players in inflammation, since they also promote proliferation, anti-apoptotic activity, angiogenesis and metastasis (153). We and others have extensively studied the inhibitory effect of Curcumin on NFkappaB expression and activation, a key event in inflammation and cancer progression, and the decreased levels of COX2 after treatment with the polyphenol (13, 24, 56, 81, 133). The TNF alpha pathway is also directly targeted by Curcumin (154). A variety of inflammatory cytokines, which have been shown to mediate tumorigenesis, are modulated by Curcumin through the suppression of NFkappaB. Recent studies reported that Curcumin inhibits the expression of a variety of interleukins (IL-1, -2, -5, -8, -12, -18) (155-159), which play an important role in the induction of adhesion molecules, MMPs, and signaling pathways related to invasion and angiogenesis like NFkappaB and STATs.

We have also reported that Curcumin reduces the expression of CXCL1 and -2 in breast cancer by inhibiting NFkappaB (24). Silencing of the p65 subunit of NFkappaB reduced the expression of these two chemokines in breast cancer cells. Furthermore, we showed that this mechanism requires intact IkappaB alpha expression upstream of NFkappaB.

Inactivation of NFkappaB may explain the relatively strong chemopreventive effect of a drug with otherwise modest effects, since it occurs in both, the tumor cell and in the inflammatory cell and most likely synergistically interrupts the cross-stimulatory effect of cytokine production.

Interestingly, down-regulation of CXCL1 leads to reduced expression of CXCR4, the receptor for CXCL12/SDF1 (24), a metastasis promoting axis that has been recognized as a target for drug development (160).

Table 3. Cancer Rates in India and the United States (Rates are per 100,000 population)

	India		United States	
	Male	Female	Male	Female
Cancer Rates all sites, except skin	99,0	104,4	361,4	286,2
Oral	12,8	7,5	6,3	3,7
Oesophagus	7,6	5,1	4,9	1,4
Stomach	5,7	2,8	7,3	3,6
Lung	9,0	2,0	58,6	34,0
Colon	4,7	3,2	40,6	30,7
Breast		19,1		91,4
Ovary		4,9		10,6
Cervix		30,7		7,8
Endometrial		1,7		15,5
Prostate	4,6		104,3	
Liver	2,3	2,0	4,2	1,7
Bladder	3,2	0,7	23,4	5,4
Kidney	1,2	0,5	11,2	6,0
Melanoma of the skin	0,3	0,2	4,2	1,7

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CXCL1 is expressed by many cancers and has been included in the lung metastasis signature (148) although it is expressed by only very few breast cancers (161). Curcumin might therefore be more suited for other types of cancer that frequently overexpress CXCL1 and the expression of the cytokine probably is a suitable response marker for secondary prevention.

6. EPIDEMIOLOGY OF CURCUMIN CONSUMPTION AND CANCER – AN INDIRECT APPROACH TO ANTI-TUMOR EFFECTS

Besides the yet identified molecular pathways that seem to be involved in the activity of Curcumin and its metabolites (see below), there is indirect evidence on the potential anti-tumor activity of curcuminoids in humans. It has been well documented that cancer of the colon, breast, prostate and lung – which are very frequent in Western industrial countries such as the United States and the European Union – are not as prevalent in countries such as India where Curcumin is frequently used as a food additive (162). This holds also true for colonic adenomas and carcinomas (163) which have both been shown to be strongly reduced in the rural populations of the Indian subcontinent with high Curcumin intake – particularly when compared to other countries. Studies of Indian immigrants in Western societies indicate that rates of cancer and other chronic diseases, such as coronary heart disease and diabetes, increase dramatically after a generation in the adopted country. It is presumed that changes in life style in particular in diet are responsible for the changing disease rates. Very little is known, however, about the role of the Indian diet in causation of cancer or its role, if any, in prevention of cancer, although more attention is being focused on certain aspects of the Indian diet, such as spices, and food additives. Of particular interest for cancer prevention is the role of Curcumin, which is a common ingredient in Indian curry spice. Moreover, many of the more than 700 plant-based drugs described in Ayurveda medicines including turmeric (Curcumin), other spices and various plant seeds have been studied for their disease prevention capabilities.

Epidemiological data from the World Health Organization (WHO) exposes, that Cancer rates in India are considerably lower than those in more developed countries such as the United States (see Table 3) (164). Data from population based cancer registries in India show that the most frequently reported cancer sites in males are lung, esophagus, stomach, and larynx. In females, cancers of the cervix, breast, ovary, and esophagus are the most commonly encountered (165). It can therefore be presumed, that lifestyle, diet and dietary factors like Curcumin play a role in the relatively low cancer incidents in India when compared to those of industrialized countries. In addition cancer cases are rising as development in India progresses, with a changing profile of burden at different cancer sites. Therefore information about the efficacy of Curcumin and other dietary components in preventing cancer initiation and progression gained from human prevention and intervention studies will be highly valuable.

7. CLINICAL STUDIES OF CURCUMIN IN TUMORS

Until now, there are only few clinical studies on the effects of Curcumin on tumors *in vivo*. Five phase I clinical studies cover colonic carcinomas (which provide the closest relation to the epidemiologic association between Curcumin consumption and cancer development); very recently, a single phase II clinical study on end-stage pancreatic carcinomas (in combination with gemcitabine standard chemotherapy) has been published.

Although the effects of Curcumin on the tumors were limited and partly restricted to a more or less small percentage of affected individuals, several cases have been documented showing beneficial outcome. This suggests that the *in vitro* and experimental *in vivo* studies (see above) may hold true – at least for some tumor types and individual cases – also for end-stage cancer cases.

A very important and major over-all observation was that all six hitherto performed clinical studies unambiguously showed that Curcumin is very well tolerated (in various formulations) up to a dosage of 8,000 mg/day with only isolated cases of slight diarrhea. This clearly confirms the absence of any significant toxicity of Curcumin.

With respect to the effects on tumor growth one has to take into account that all studies (both the phase I studies and the single phase II study) were performed on end-stage cancer patients. When summarizing the studies, the following observations could be made:

The first clinical study performed by Sharma and coworkers (27) describes a small series of 15 colon cancer patients. All patients with advanced colorectal cancer refractory to standard chemotherapies received between 440 and 2200 mg Curcuma extract for up to 4 months. Neither Curcumin nor its metabolites could be detected in blood or urine, but in feces. In order to monitor any Curcumin effect, the ingestion for 29 days was shown to result in a strong decrease in lymphocytic glutathione S-transferase activity. Radiologically stable disease was demonstrated in five patients for 2-4 months of treatment. One patient who had revealed local (non-metastasized) colon cancer had a decline in the cancer biomarker carcinoembryonic antigen (CEA) suggesting a reduction of tumor mass. This first study suggests that Curcumin can be administered safely to patients and – despite its low oral bioavailability in humans – that some patients with advanced stage colonic cancer may benefit from continuous Curcumin consumption.

In a further phase I clinical trial performed by Cheng *et al.* (28) 25 patients with pre-malignant colonic lesions (e.g. high-risk adenoma) were subjected to a daily application of 500 mg Curcumin for three months. In 7 individuals a histological improvement of the high-risk lesion was evident, frank malignancies developed during the three month treatment period in 2 patients. Unfortunately, no control data have been included so that no statistical result was obtained.

A subsequent study by Sharma and co-workers (166) controlled three biomarkers in 15 patients with histologically proven advanced colorectal cancer refractory to standard chemotherapies. Two patients had measurable disease in the colon, the other 13 displayed disease beyond the colon. In this population the enzymatic level in blood leukocytes served as markers for Cucumin effects: glutathione S-transferase activity, levels of M(1)G, and PGE(2) production induced *ex vivo* were determined. A daily dose of 3.6 g Curcumin engendered 62% and 57% decreases in inducible PGE(2) production in blood samples taken 1 hour after dose on days 1 and 29, respectively. Furthermore, however, no evidence for partial response nor reduction in tumor markers were observed, similarly in this study there was no evidence for significant differences in GST- and M(1)G-levels.

Garcea and collaborators (167) performed a further study on 12 cancer patients with confirmed colorectal tumors. A daily dosage of up to 3600 mg Curcumin prior to surgery was applied and the levels of COX-2 and M(1)G were measured. In this study, the application of Curcumin was not found to modulate the expression of COX-2 in malignant tissue, however, M(1)G levels significantly rose in malignant tissue in patients with high dose Curcumin. Although the setting of this (short-term) study was not designed to evaluate a response in terms of tumor growth, the induction of M(1)G levels suggests a direct Curcumin effect on tumor tissue.

A phase I trial with measurement of Curcumin and its metabolites in hepatic tissue and portal blood in 12 colorectal cancer patients did not reveal measurable Curcumin levels in both. However, an increase in M(1)G levels in malignant tissue was noted (168) confirming the previous observations.

Due to the success of these phase I trials recently a phase II trial was enrolled on patients with advanced pancreatic cancer analyzing the effects of a combination therapy of gemcitabine and Curcumin (42). In this study, the patients received 8 g/day of Curcumin orally in combination with gemcitabine 1,000 mg/m^2 i.v. weekly for 3 out of 4 weeks. Eleven out of 17 patients were evaluable. Time to tumor progression was 1 to 12 months (median 2 months), and overall survival was 1 to 24 months (median 6 months). One patient (9.1%) had partial response (7 months), 4 (36.4%) had stable disease (2, 3, 6 and 12 months) and 6 (54.5%) had tumor progression. Five patients discontinued Curcumin after a time period ranging from few days to 2 weeks due to intractable abdominal fullness or pain. Hematological toxicities were minimal as expected with gemcitabine, including one grade 2 neutropenia and one grade 1 thrombocytopenia. No other toxicities have been observed. The preliminary results suggest that a combination of gemcitabine and Curcumin for patients with advanced pancreatic cancer is feasible.

In summary, the few clinical studies confirm the pre-clinical observations on the anticancer activity of Curcumin holds also true for clinical situations. Nevertheless, the data indicate that only few patients have positive effects. It has, however, to be taken into account that the study populations covered advanced or end-stage cancer patients that had already received maximal conventional therapy – which failed. To this respect, it is certainly too early to draw substantial conclusions. However, all basic questions of safety, tolerability, pharmacokinetics and pharmacodynamics have clearly to be positively answered in favor of Curcumin so that subsequent clinical studies under differing conditions (e.g. other tumor types, different tumor stages etc.) can now be undertaken.

8. PERSPECTIVES - CURCUMIN AS CHEMOPREVENTIVE AGENT AND THE IMPACT ON TUMOR PROPAGATION

Several anti-cancer effects of Curcumin on tumor cells have undoubtedly been shown in various tumor cell models. There is a considerable concordance between *in vitro* and experimental *in vivo* data (in animal models) and first clinical phase II trials in end-stage cancer patients. It appears that Curcumin indeed exerts clinically relevant anti-cancer effects, at least in some tumors, and the list of tumor types that respond to Curcumin is likely to grow further.

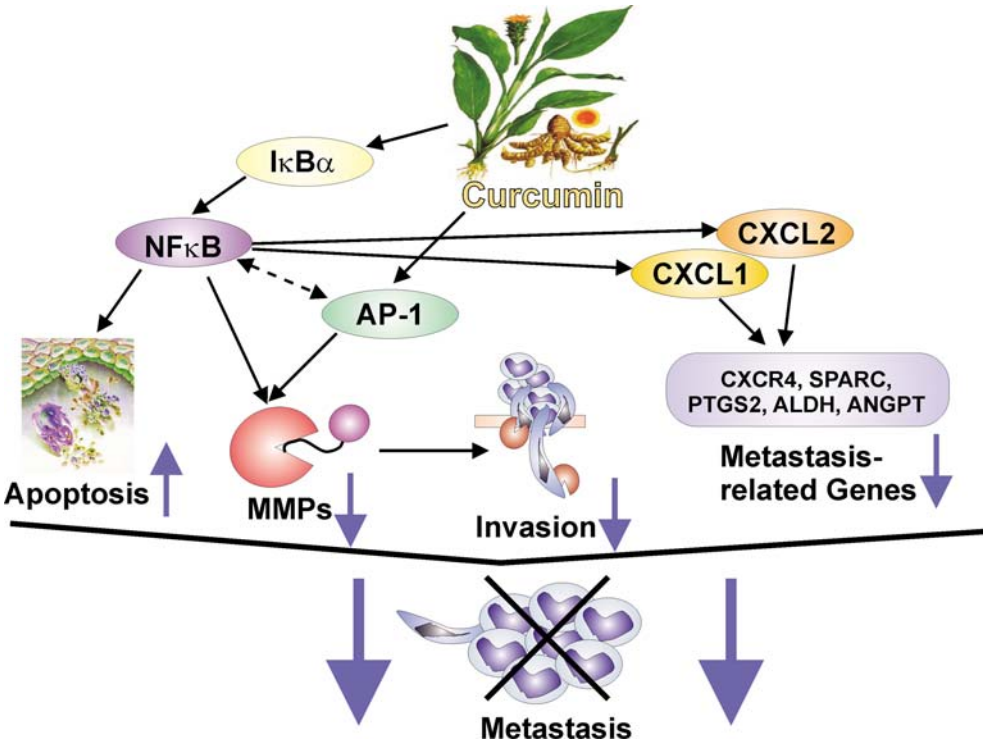

Figure 2. Molecular mechanisms of the anti-metastatic effect of Curcumin. According to our own results (13, 24), Curcumin acts on apoptosis, expression of matrix degrading enzymes, invasion, and expression of inflammation related genes leading to the inhibition of breast cancer metastasis *in vivo*.

The even bigger field that requires intense investigation covers the molecular mechanims that are affected by Curcumin. Although the main pathways that play an important role in the anti-tumoral effect of Curcumin have been clearly identified the detection of further minor pathways that are affected by the polyphenol will soon complete the picture.

On the base of our own results we have developed the following model of the molecular mechanism of Curcumin (see Figure 2). On the one hand, Curcumin acts on the tumor progression and inflammation related NFkappaB pathway by targeting its upstream inhibitor IkappaB alpha. In parallel, Curcumin can also act on the tumor-associated transcription factor AP-1. The polyphenol impairs the expression of matrix-degrading proteases like MMPs by both and each of the two pathways, which in turn leads to a diminished invasive capacity of the tumor cells. At the same time, Curcumin induces apoptosis by reducing the expression of NFkappaB dependent survival factors. We recently also showed that Curcumin impairs the expression of the pro-inflammatory cytokines CXCL1 and -2 and as a consequence that of a series of metastasis related genes via the NFkappaB pathway. Our results from *in vitro* studies reveal that Curcumin acts on four hallmarks of tumor progression: (a) apoptosis, (b) expression of matrix degrading enzymes, (c) invasion, (d) expression of inflammation related genes. The concerted action of these features leads to the inhibition of breast cancer metastasis, as we have shown for the first time in a mouse model of hematogenous.metastasis.

We therefore think that the stage is set for clinical trials that will yield the final proof of anti-cancer activities of Curcumin. It can be anticipated, that despite the overwhelming evidence from preclinical studies, not all clinical trials will end up with a success. For this reason, clinical trials with Curcumin should consider the following aspects right from the beginning:

1. Trials should be limited to cancer types with a documented role of NFkappaB and inflammation in tumor progression,
2. Where possible, the primary cancer should be available for molecular analyses,
3. Trials should monitor the bioavailability of Curcumin and its metabolites.
4. Patients should be stratified based on molecular and biochemical data in order to detect significant responses in subgroups of patients.

Further research should address the still incompletely resolved issue of bioavailability since we need to attribute the evident effects to specific molecular moieties present in the target organs. Resistance almost certainly will become an issue once Curcumin is more frequently and for longer time periods administered to patients and finally, combinations of Curcumin and chemotherapy should be addressed in a systematical manner.

Clinical research on Curcumin will face the problems linked to low cost, non patentable drugs. Yet the

evidence available makes it highly likely that Curcumin can give a major contribution to the improvement of cancer therapy and prevention. This potential should not be sacrificed to commercial interests.

9. REFERENCES

1. D. E. Brenner and A. J. Gescher: Cancer chemoprevention: lessons learned and future directions. *Br.J.Cancer*, 93(7), 735-739 (2005)

2. A. M. Samuni, E. Y. Chuang, M. C. Krishna, W. Stein, W. DeGraff, A. Russo and J. B. Mitchell: Semiquinone radical intermediate in catecholic estrogen-mediated cytotoxicity and mutagenesis: chemoprevention strategies with antioxidants. *Proc Natl Acad Sci U S A*, 100(9), 5390-5 (2003)

3. S. Yodkeeree, W. Chaiwangyen, S. Garbisa and P. Limtrakul: Curcumin, demethoxycurcumin and bisdemethoxycurcumin differentially inhibit cancer cell invasion through the down-regulation of MMPs and uPA. *J Nutr Biochem*, 20(2), 87-95 (2009)

4. S. S. Lin, K. C. Lai, S. C. Hsu, J. S. Yang, C. L. Kuo, J. P. Lin, Y. S. Ma, C. C. Wu and J. G. Chung: Curcumin inhibits the migration and invasion of human A549 lung cancer cells through the inhibition of matrix metalloproteinase-2 and -9 and Vascular Endothelial Growth Factor (VEGF) *Cancer Lett* (2009)

5. J. G. Herman, H. L. Stadelman and C. E. Roselli: Curcumin blocks CCL2-induced adhesion, motility and invasion, in part, through down-regulation of CCL2 expression and proteolytic activity. *Int J Oncol*, 34(5), 1319-27 (2009)

6. X. Z. Cai, J. Wang, X. D. Li, G. L. Wang, F. N. Liu, M. S. Cheng and F. Li: Curcumin suppresses proliferation and invasion in human gastric cancer cells by downregulation of PAK1 activity and cyclin D1 expression. *Cancer Biol Ther*, 8(14) (2009)

7. M. Narasimhan and S. Ammanamanchi: Curcumin blocks RON tyrosine kinase-mediated invasion of breast carcinoma cells. *Cancer Res*, 68(13), 5185-92 (2008)

8. A. B. Kunnumakkara, P. Anand and B. B. Aggarwal: Curcumin inhibits proliferation, invasion, angiogenesis and metastasis of different cancers through interaction with multiple cell signaling proteins. *Cancer Lett.*, 269(2), 199-225 (2008)

9. H. I. Kim, H. Huang, S. Cheepala, S. Huang and J. Chung: Curcumin inhibition of integrin (alpha6beta4)-dependent breast cancer cell motility and invasion. *Cancer Prev Res (Phila Pa)*, 1(5), 385-91 (2008)

10. H. W. Chen, J. Y. Lee, J. Y. Huang, C. C. Wang, W. J. Chen, S. F. Su, C. W. Huang, C. C. Ho, J. J. Chen, M. F. Tsai, S. L. Yu and P. C. Yang: Curcumin inhibits lung cancer cell invasion and metastasis through the tumor suppressor HLJ1. *Cancer Res*, 68(18), 7428-38 (2008)

11. M. S. Kim, H. J. Kang and A. Moon: Inhibition of invasion and induction of apoptosis by curcumin in H-ras-transformed MCF10A human breast epithelial cells. *Arch.Pharm.Res.*, 24(4), 349-354 (2001)

12. L. I. Lin, Y. F. Ke, Y. C. Ko and J. K. Lin: Curcumin inhibits SK-Hep-1 hepatocellular carcinoma cell invasion in vitro and suppresses matrix metalloproteinase-9 secretion. *Oncology*, 55(4), 349-353 (1998)

13. B. Bachmeier, A. G. Nerlich, C. M. Iancu, M. Cilli, E. Schleicher, R. Vene, R. Dell'Eva, M. Jochum, A. Albini and U. Pfeffer: The chemopreventive polyphenol Curcumin prevents hematogenous breast cancer metastases in immunodeficient mice. *Cell Physiol Biochem*, 19(1-4), 137-152 (2007)

14. A. Ejaz, D. Wu, P. Kwan and M. Meydani: Curcumin inhibits adipogenesis in 3T3-L1 adipocytes and angiogenesis and obesity in C57/BL mice. *J Nutr*, 139(5), 919-25 (2009)

15. S. Shankar, S. Ganapathy, Q. Chen and R. K. Srivastava: Curcumin sensitizes TRAIL-resistant xenografts: molecular mechanisms of apoptosis, metastasis and angiogenesis. *Mol Cancer*, 7, 16 (2008)

16. D. G. Binion, M. F. Otterson and P. Rafiee: Curcumin inhibits VEGF-mediated angiogenesis in human intestinal microvascular endothelial cells through COX-2 and MAPK inhibition. *Gut*, 57(11), 1509-17 (2008)

17. S. Shankar, Q. Chen, K. Sarva, I. Siddiqui and R. K. Srivastava: Curcumin enhances the apoptosis-inducing potential of TRAIL in prostate cancer cells: molecular mechanisms of apoptosis, migration and angiogenesis. *J Mol Signal*, 2, 10 (2007)

18. Y. G. Lin, A. B. Kunnumakkara, A. Nair, W. M. Merritt, L. Y. Han, G. N. Armaiz-Pena, A. A. Kamat, W. A. Spannuth, D. M. Gershenson, S. K. Lutgendorf, B. B. Aggarwal and A. K. Sood: Curcumin inhibits tumor growth and angiogenesis in ovarian carcinoma by targeting the nuclear factor-kappaB pathway. *Clin Cancer Res*, 13(11), 3423-30 (2007)

19. L. Li, B. Ahmed, K. Mehta and R. Kurzrock: Liposomal curcumin with and without oxaliplatin: effects on cell growth, apoptosis, and angiogenesis in colorectal cancer. *Mol Cancer Ther*, 6(4), 1276-82 (2007)

20. A. B. Kunnumakkara, S. Guha, S. Krishnan, P. Diagaradjane, J. Gelovani and B. B. Aggarwal: Curcumin potentiates antitumor activity of gemcitabine in an orthotopic model of pancreatic cancer through suppression of proliferation, angiogenesis, and inhibition of nuclear factor-kappaB-regulated gene products. *Cancer Res.*, 67(8), 3853-3861 (2007)

21. S. S. Bhandarkar and J. L. Arbiser: Curcumin as an inhibitor of angiogenesis. *Adv Exp Med Biol*, 595, 185-95 (2007)

22. M. K. Bae, S. H. Kim, J. W. Jeong, Y. M. Lee, H. S. Kim, S. R. Kim, I. Yun, S. K. Bae and K. W. Kim: Curcumin inhibits hypoxia-induced angiogenesis via down-regulation of HIF-1. *Oncol Rep*, 15(6), 1557-62 (2006)

23. Y. Ohashi, Y. Tsuchiya, K. Koizumi, H. Sakurai and I. Saiki: Prevention of intrahepatic metastasis by curcumin in an orthotopic implantation model. *Oncology*, 65(3), 250-258 (2003)

24. B. E. Bachmeier, I. V. Mohrenz, V. Mirisola, E. Schleicher, F. Romeo, C. Hohneke, M. Jochum, A. G. Nerlich and U. Pfeffer: Curcumin downregulates the inflammatory cytokines CXCL1 and -2 in breast cancer cells via NFkappaB. *Carcinogenesis*, 29(4), 779-789 (2008)

25. H. H. Tonnesen: Solubility, chemical and photochemical stability of curcumin in surfactant solutions. Studies of curcumin and curcuminoids, XXVIII. *Pharmazie*, 57(12), 820-4 (2002)

26. H. H. Tonnesen and J. Karlsen: Studies on curcumin and curcuminoids. VI. Kinetics of curcumin degradation in aqueous solution. *Z Lebensm Unters Forsch*, 180(5), 402-4 (1985)

27. R. A. Sharma, H. R. McLelland, K. A. Hill, C. R. Ireson, S. A. Euden, M. M. Manson, M. Pirmohamed, L. J. Marnett, A. J. Gescher and W. P. Steward: Pharmacodynamic and pharmacokinetic study of oral Curcuma extract in patients with colorectal cancer. *Clin.Cancer Res.*, 7(7), 1894-1900 (2001)

28. A. L. Cheng, C. H. Hsu, J. K. Lin, M. M. Hsu, Y. F. Ho, T. S. Shen, J. 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 lesions. *Anticancer Res.*, 21(4B), 2895-2900 (2001)

29. S. I. Hoehle, E. Pfeiffer and M. Metzler: Glucuronidation of curcuminoids by human microsomal and recombinant UDP-glucuronosyltransferases. *Mol Nutr Food Res*, 51(8), 932-8 (2007)

30. S. I. Hoehle, E. Pfeiffer, A. M. Solyom and M. Metzler: Metabolism of curcuminoids in tissue slices and subcellular fractions from rat liver. *J Agric Food Chem*, 54(3), 756-64 (2006)

31. J. S. Dempe, E. Pfeiffer, A. S. Grimm and M. Metzler: Metabolism of curcumin and induction of mitotic catastrophe in human cancer cells. *Mol Nutr Food Res*, 52(9), 1074-81 (2008)

32. E. Pfeiffer, S. I. Hoehle, S. G. Walch, A. Riess, A. M. Solyom and M. Metzler: Curcuminoids form reactive glucuronides in vitro. *J Agric Food Chem*, 55(2), 538-44 (2007)

33. M. H. Pan, T. M. Huang and J. K. Lin: Biotransformation of curcumin through reduction and glucuronidation in mice. *Drug Metab Dispos.*, 27(4), 486-494 (1999)

34. F. C. Campbell and G. P. Collett: Chemopreventive properties of curcumin. *Future.Oncol.*, 1(3), 405-414 (2005)

35. S. K. Vareed, M. Kakarala, M. T. Ruffin, J. A. Crowell, D. P. Normolle, Z. Djuric and D. E. Brenner: Pharmacokinetics of curcumin conjugate metabolites in healthy human subjects. *Cancer Epidemiol.Biomarkers Prev.*, 17(6), 1411-1417 (2008)

36. L. Baum, S. K. Cheung, V. C. Mok, L. C. Lam, V. P. Leung, E. Hui, C. C. Ng, M. Chow, P. C. Ho, S. Lam, J. Woo, H. F. Chiu, W. Goggins, B. Zee, A. Wong, H. Mok, W. K. Cheng, C. Fong, J. S. Lee, M. H. Chan, S. S. Szeto, V. W. Lui, J. Tsoh, T. C. Kwok, I. H. Chan and C. W. Lam: Curcumin effects on blood lipid profile in a 6-month human study. *Pharmacol.Res.*, 56(6), 509-514 (2007)

37. H. H. Tonnesen: Solubility and stability of curcumin in solutions containing alginate and other viscosity modifying macromolecules. Studies of curcumin and curcuminoids. XXX. *Pharmazie*, 61(8), 696-700 (2006)

38. G. Shoba, D. Joy, T. Joseph, M. Majeed, R. Rajendran and P. S. Srinivas: Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. *Planta Med*, 64(4), 353-6 (1998)

39. W. Tiyaboonchai, W. Tungpradit and P. Plianbangchang: Formulation and characterization of curcuminoids loaded solid lipid nanoparticles. *Int J Pharm*, 337(1-2), 299-306 (2007)

40. S. Bisht, G. Feldmann, S. Soni, R. Ravi, C. Karikar and A. Maitra: Polymeric nanoparticle-encapsulated curcumin ("nanocurcumin"): a novel strategy for human cancer therapy. *J Nanobiotechnology*, 5, 3 (2007)

41. J. Shaikh, D. D. Ankola, V. Beniwal, D. Singh and M. N. Kumar: Nanoparticle encapsulation improves oral bioavailability of curcumin by at least 9-fold when compared to curcumin administered with piperine as absorption enhancer. *Eur J Pharm Sci*, 37(3-4), 223-30 (2009)

42. N. Dhillon, B. B. Aggarwal, R. A. Newman, R. A. Wolff, A. B. Kunnumakkara, J. L. Abbruzzese, C. S. Ng, V. Badmaev and R. Kurzrock: Phase II trial of curcumin in patients with advanced pancreatic cancer. *Clin.Cancer Res.*, 14(14), 4491-4499 (2008)

43. K. Shimoi and T. Nakayama: Glucuronidase deconjugation in inflammation. *Methods Enzymol.*, 400, 263-272 (2005)

44. K. Shimoi, H. Okada, M. Furugori, T. Goda, S. Takase, M. Suzuki, Y. Hara, H. Yamamoto and N. Kinae: Intestinal absorption of luteolin and luteolin 7-O-beta-glucoside in rats and humans. *FEBS Lett*, 438(3), 220-4 (1998)

45. M. L. Kuo, T. S. Huang and J. K. Lin: Curcumin, an antioxidant and anti-tumor promoter, induces apoptosis in human leukemia cells. *Biochim.Biophys.Acta*, 1317(2), 95-100 (1996)

46. H. Chen, Z. S. Zhang, Y. L. Zhang and D. Y. Zhou: Curcumin inhibits cell proliferation by interfering with the cell cycle and inducing apoptosis in colon carcinoma cells. *Anticancer Res.*, 19(5A), 3675-3680 (1999)

47. K. Mehta, P. Pantazis, T. McQueen and B. B. Aggarwal: Antiproliferative effect of curcumin (diferuloylmethane) against human breast tumor cell lines. *Anticancer Drugs*, 8(5), 470-481 (1997)

48. A. Khar, A. M. Ali, B. V. Pardhasaradhi, Z. Begum and R. Anjum: Antitumor activity of curcumin is mediated through the induction of apoptosis in AK-5 tumor cells. *FEBS Lett.*, 445(1), 165-168 (1999)

49. M. T. Huang, Y. R. Lou, J. G. Xie, W. Ma, Y. P. Lu, P. Yen, B. T. Zhu, H. Newmark and C. T. Ho: Effect of dietary curcumin and dibenzoylmethane on formation of 7,12-dimethylbenz[a]anthracene-induced mammary tumors and lymphomas/leukemias in Sencar mice. *Carcinogenesis*, 19(9), 1697-1700 (1998)

50. P. Limtrakul, S. Lipigorngoson, O. Namwong, A. Apisariyakul and F. W. Dunn: Inhibitory effect of dietary curcumin on skin carcinogenesis in mice. *Cancer Lett.*, 116(2), 197-203 (1997)

51. T. Kawamori, R. Lubet, V. E. Steele, G. J. Kelloff, R. B. Kaskey, C. V. Rao and B. S. Reddy: Chemopreventive effect of curcumin, a naturally occurring anti-inflammatory agent, during the promotion/progression stages of colon cancer. *Cancer Res.*, 59(3), 597-601 (1999)

52. 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)

53. M. H. Pan, W. 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)

54. R. Rashmi, T. R. Santhosh Kumar and D. Karunagaran: Human colon cancer cells differ in their sensitivity to curcumin-induced apoptosis and heat shock protects them by inhibiting the release of apoptosis-inducing factor and caspases. *FEBS Lett.*, 538(1-3), 19-24 (2003)

55. M. S. Squires, E. A. Hudson, 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 phosphotidylinositol-3-kinase/protein kinase B (PI3K/PKB) pathways to induction of apoptosis by curcumin in breast cells. *Biochem.Pharmacol.*, 65(3), 361-376 (2003)

56. C. Park, G. Y. Kim, G. D. Kim, B. T. Choi, Y. M. Park and Y. H. Choi: Induction of G2/M arrest and inhibition of cyclooxygenase-2 activity by curcumin in human bladder cancer T24 cells. *Oncol.Rep.*, 15(5), 1225-1231 (2006)

57. L. M. Howells, A. Mitra and M. M. Manson: Comparison of oxaliplatin- and curcumin-mediated antiproliferative effects in colorectal cell lines. *Int.J.Cancer*, 121(1), 175-183 (2007)

58. S. Shishodia, H. M. Amin, R. Lai and B. B. Aggarwal: Curcumin (diferuloylmethane) inhibits constitutive NF-kappaB activation, induces G1/S arrest, suppresses proliferation, and induces apoptosis in mantle cell lymphoma. *Biochem Pharmacol.*, 70(5), 700-713 (2005)

59. S. Balasubramanian and R. L. Eckert: Keratinocyte proliferation, differentiation, and apoptosis--differential mechanisms of regulation by curcumin, EGCG and apigenin. *Toxicol.Appl.Pharmacol.*, 224(3), 214-219 (2007)

60. R. K. Srivastava, Q. Chen, I. Siddiqui, K. Sarva and S. Shankar: Linkage of curcumin-induced cell cycle arrest and apoptosis by cyclin-dependent kinase inhibitor p21(/WAF1/CIP1) *Cell Cycle*, 6(23), 2953-61 (2007)

61. D. Deeb, Y. X. Xu, H. Jiang, X. Gao, N. Janakiraman, R. A. Chapman and S. C. Gautam: Curcumin (diferuloyl-methane) enhances tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis in LNCaP prostate cancer cells. *Mol.Cancer Ther.*, 2(1), 95-103 (2003)

62. S. Shankar and R. K. Srivastava: Bax and Bak genes are essential for maximum apoptotic response by curcumin, a polyphenolic compound and cancer chemopreventive agent derived from turmeric, Curcuma longa. *Carcinogenesis*, 28(6), 1277-86 (2007)

63. S. Shankar and R. K. Srivastava: Involvement of Bcl-2 family members, phosphatidylinositol 3'-kinase/AKT and mitochondrial p53 in curcumin (diferulolylmethane)-induced apoptosis in prostate cancer. *Int J Oncol*, 30(4), 905-18 (2007)

64. Y. Sugiyama, S. Kawakishi and T. Osawa: Involvement of the beta-diketone moiety in the antioxidative mechanism of tetrahydrocurcumin. *Biochem Pharmacol.*, 52(4), 519-525 (1996)

65. I. Rahman, S. K. Biswas and P. A. Kirkham: Regulation of inflammation and redox signaling by dietary polyphenols. *Biochem Pharmacol*, 72(11), 1439-52 (2006)

66. A. C. Reddy and B. R. Lokesh: Studies on the inhibitory effects of curcumin and eugenol on the formation of reactive oxygen species and the oxidation of ferrous iron. *Mol.Cell Biochem*, 137(1), 1-8 (1994)

67. M. K. Unnikrishnan and M. N. Rao: Inhibition of nitrite induced oxidation of hemoglobin by curcuminoids. *Pharmazie*, 50(7), 490-492 (1995)

68. Sreejayan and M. N. Rao: Nitric oxide scavenging by curcuminoids. *J.Pharm.Pharmacol.*, 49(1), 105-107 (1997)

69. A. C. Reddy and B. R. Lokesh: Studies on spice principles as antioxidants in the inhibition of lipid peroxidation of rat liver microsomes. *Mol.Cell Biochem*, 111(1-2), 117-124 (1992)

70. R. Motterlini, 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 Radic.Biol.Med.*, 28(8), 1303-1312 (2000)

71. R. K. Maheshwari, A. K. Singh, J. Gaddipati and R. C. Srimal: Multiple biological activities of curcumin: a short review. *Life Sci.*, 78(18), 2081-2087 (2006)

72. P. A. Baeuerle and D. Baltimore: NF-kappa B: ten years after. *Cell*, 87(1), 13-20 (1996)

73. L. Li, B. B. Aggarwal, S. Shishodia, J. Abbruzzese and R. Kurzrock: Nuclear factor-kappaB and IkappaB kinase are constitutively active in human pancreatic cells, and their down-regulation by curcumin (diferuloylmethane) is associated with the suppression of proliferation and the induction of apoptosis. *Cancer*, 101(10), 2351-2362 (2004)

74. S. Singh and B. B. Aggarwal: Activation of transcription factor NF-kappa B is suppressed by curcumin (diferuloylmethane) [corrected]. *J.Biol.Chem.*, 270(42), 24995-25000 (1995)

75. L. Vermeulen, G. De Wilde, S. Notebaert, W. Vanden Berghe and G. Haegeman: Regulation of the transcriptional activity of the nuclear factor-kappaB p65 subunit. *Biochem.Pharmacol.*, 64(5-6), 963-970 (2002)

76. P. Viatour, M. P. Merville, V. Bours and A. Chariot: Phosphorylation of NF-kappaB and IkappaB proteins: implications in cancer and inflammation. *Trends Biochem.Sci.*, 30(1), 43-52 (2005)

77. C. Y. Wang, M. W. Mayo and A. S. Baldwin, Jr.: TNF- and cancer therapy-induced apoptosis: potentiation by inhibition of NF-kappaB. *Science*, 274(5288), 784-787 (1996)

78. A. A. Beg and D. Baltimore: An essential role for NF-kappaB in preventing TNF-alpha-induced cell death. *Science*, 274(5288), 782-784 (1996)

79. A. Garg and B. B. Aggarwal: Nuclear transcription factor-kappaB as a target for cancer drug development. *Leukemia*, 16(6), 1053-1068 (2002)

80. J. T. Wu and J. G. Kral: The NF-kappaB/IkappaB signaling system: a molecular target in breast cancer therapy. *J.Surg.Res.*, 123(1), 158-169 (2005)

81. A. C. Bharti, N. Donato, S. Singh and B. B. Aggarwal: Curcumin (diferuloylmethane) down-regulates the constitutive activation of nuclear factor-kappa B and IkappaBalpha kinase in human multiple myeloma cells, leading to suppression of proliferation and induction of apoptosis. *Blood*, 101(3), 1053-1062 (2003)

82. W. G. Stetler-Stevenson and A. E. Yu: Proteases in invasion: matrix metalloproteinases. *Semin.Cancer Biol.*, 11(2), 143-152 (2001)

83. Z. Wang, Y. Zhang, S. Banerjee, Y. Li and F. H. Sarkar: Notch-1 down-regulation by curcumin is associated with the inhibition of cell growth and the induction of apoptosis in pancreatic cancer cells. *Cancer*, 106(11), 2503-2513 (2006)

84. P. J. Morin, A. B. Sparks, V. Korinek, N. Barker, H. Clevers, B. Vogelstein and K. W. Kinzler: Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. *Science*, 275(5307), 1787-90 (1997)

85. H. Fujie, K. Moriya, Y. Shintani, T. Tsutsumi, T. Takayama, M. Makuuchi, S. Kimura and K. Koike: Frequent beta-catenin aberration in human hepatocellular carcinoma. *Hepatol Res*, 20(1), 39-51 (2001)

86. D. K. Woo, H. S. Kim, H. S. Lee, Y. H. Kang, H. K. Yang and W. H. Kim: Altered expression and mutation of beta-catenin gene in gastric carcinomas and cell lines. *Int J Cancer*, 95(2), 108-13 (2001)

87. R. Yoshida, N. Kimura, Y. Harada and N. Ohuchi: The loss of E-cadherin, alpha- and beta-catenin expression is associated with metastasis and poor prognosis in invasive breast cancer. *Int J Oncol*, 18(3), 513-20 (2001)

88. W. Kildal, B. Risberg, V. M. Abeler, G. B. Kristensen, J. Sudbo, J. M. Nesland and H. E. Danielsen: beta-catenin expression, DNA ploidy and clinicopathological features in ovarian cancer: a study in 253 patients. *Eur J Cancer*, 41(8), 1127-34 (2005)

89. C. H. Park, E. R. Hahm, S. Park, H. K. Kim and C. H. Yang: The inhibitory mechanism of curcumin and its derivative against beta-catenin/Tcf signaling. *FEBS Lett*, 579(13), 2965-71 (2005)

90. A. S. Jaiswal, B. P. Marlow, N. Gupta and S. Narayan: Beta-catenin-mediated transactivation and cell-cell adhesion pathways are important in curcumin (diferuylmethane)-induced growth arrest and apoptosis in colon cancer cells. *Oncogene*, 21(55), 8414-27 (2002)

91. V. P. Sukhatme: Early transcriptional events in cell growth: the Egr family. *J Am Soc Nephrol*, 1(6), 859-66 (1990)

92. S. B. McMahon and J. G. Monroe: The role of early growth response gene 1 (egr-1) in regulation of the immune response. *J Leukoc Biol*, 60(2), 159-66 (1996)

93. C. Liu, E. Adamson and D. Mercola: Transcription factor EGR-1 suppresses the growth and transformation of human HT-1080 fibrosarcoma cells by induction of transforming growth factor beta 1. *Proc Natl Acad Sci U S A*, 93(21), 11831-6 (1996)

94. R. P. Huang, Y. Fan, I. de Belle, C. Niemeyer, M. M. Gottardis, D. Mercola and E. D. Adamson: Decreased Egr-1 expression in human, mouse and rat mammary cells and tissues correlates with tumor formation. *Int J Cancer*, 72(1), 102-9 (1997)

95. B. H. Choi, C. G. Kim, Y. S. Bae, Y. Lim, Y. H. Lee and S. Y. Shin: p21 Waf1/Cip1 expression by curcumin in U-87MG human glioma cells: role of early growth response-1 expression. *Cancer Res*, 68(5), 1369-77 (2008)

96. U. R. Pendurthi and L. V. Rao: Suppression of transcription factor Egr-1 by curcumin. *Thromb Res*, 97(4), 179-89 (2000)

97. A. Chen, J. Xu and A. C. Johnson: Curcumin inhibits human colon cancer cell growth by suppressing gene expression of epidermal growth factor receptor through reducing the activity of the transcription factor Egr-1. *Oncogene*, 25(2), 278-287 (2006)

98. U. Pfeffer, N. Ferrari, R. Dell'Eva, S. Indraccolo, M. Morini, D. M. Noonan and A. Albini: Molecular mechanisms of action of angiopreventive anti-oxidants on endothelial cells: Microarray gene expression analyses. *Mutat.Res.* (2005)

99. B. B. Aggarwal, S. Banerjee, U. Bharadwaj, B. Sung, S. Shishodia and G. Sethi: Curcumin induces the degradation of cyclin E expression through ubiquitin-dependent pathway and up-regulates cyclin-dependent kinase inhibitors p21 and p27 in multiple human tumor cell lines. *Biochem Pharmacol*, 73(7), 1024-32 (2007)

100. C. M. Croce and G. A. Calin: miRNAs, cancer, and stem cell division. *Cell*, 122(1), 6-7 (2005)

101. A. Esquela-Kerscher and F. J. Slack: Oncomirs - microRNAs with a role in cancer. *Nat.Rev.Cancer*, 6(4), 259-269 (2006)

102. P. S. Meltzer: Cancer genomics: small RNAs with big impacts. *Nature*, 435(7043), 745-746 (2005)

103. M. Sun, Z. Estrov, Y. Ji, K. R. Coombes, D. H. Harris and R. Kurzrock: Curcumin (diferuloylmethane) alters the expression profiles of microRNAs in human pancreatic cancer cells. *Mol.Cancer Ther.*, 7(3), 464-473 (2008)

104. B. E. Bachmeier, P. Boukamp, R. Lichtinghagen, N. E. Fusenig and E. Fink: Matrix metalloproteinases-2,-3,-7,-9 and-10, but not MMP-11, are differentially expressed in normal, benign tumorigenic and malignant human keratinocyte cell lines. *Biol.Chem.*, 381(5-6), 497-507 (2000)

105. B. E. Bachmeier, A. G. Nerlich, P. Boukamp, R. Lichtinghagen, H. Tschesche, H. Fritz and E. Fink: Human keratinocyte cell lines differ in the expression of the collagenolytic matrix metalloproteinases-1,-8, and -13 and of TIMP-1. *Biol.Chem.*, 381(5-6), 509-516 (2000)

106. L. C. Meade-Tollin, P. Boukamp, N. E. Fusenig, C. P. Bowen, T. C. Tsang and G. T. Bowden: Differential expression of matrix metalloproteinases in activated c-ras-Ha-transfected immortalized human keratinocytes. *Br.J.Cancer*, 77(5), 724-730 (1998)

107. B. E. Bachmeier, A. G. Nerlich, R. Lichtinghagen and C. P. Sommerhoff: Matrix metalloproteinases (MMPs) in breast cancer cell lines of different tumorigenicity. *Anticancer Res.*, 21(6A), 3821-3828 (2001)

108. U. Benbow, M. P. Schoenermark, K. A. Orndorff, A. L. Givan and C. E. Brinckerhoff: Human breast cancer cells activate procollagenase-1 and invade type I collagen: invasion is inhibited by all-trans retinoic acid. *Clin.Exp.Metastasis*, 17(3), 231-238 (1999)

109. M. Balduyck, F. Zerimech, V. Gouyer, R. Lemaire, B. Hemon, G. Grard, C. Thiebaut, V. Lemaire, E. Dacquembronne, T. Duhem, A. Lebrun, M. J. Dejonghe and G. Huet: Specific expression of matrix metalloproteinases 1, 3, 9 and 13 associated with invasiveness of breast cancer cells in vitro. *Clin.Exp.Metastasis*, 18(2), 171-178 (2000)

110. M. Egeblad and Z. Werb: New functions for the matrix metalloproteinases in cancer progression. *Nat.Rev.Cancer*, 2(3), 161-174 (2002)

111. S. Philip, A. Bulbule and G. C. Kundu: Osteopontin stimulates tumor growth and activation of promatrix metalloproteinase-2 through nuclear factor-kappa B-mediated induction of membrane type 1 matrix metalloproteinase in murine melanoma cells. *J.Biol.Chem.*, 276(48), 44926-44935 (2001)

112. Y. P. Han, T. L. Tuan, H. Wu, M. Hughes and W. L. Garner: TNF-alpha stimulates activation of pro-MMP2 in human skin through NF-(kappa)B mediated induction of MT1-MMP. *J.Cell Sci.*, 114(Pt 1), 131-139 (2001)

113. S. Swarnakar, K. Ganguly, P. Kundu, A. Banerjee, P. Maity and A. V. Sharma: Curcumin regulates expression and activity of matrix metalloproteinases 9 and 2 during prevention and healing of indomethacin-induced gastric ulcer. *J.Biol.Chem.*, 280(10), 9409-9415 (2005)

114. M. Shakibaei, T. John, G. Schulze-Tanzil, I. Lehmann and A. Mobasheri: Suppression of NF-kappaB activation

by curcumin leads to inhibition of expression of cyclo-oxygenase-2 and matrix metalloproteinase-9 in human articular chondrocytes: Implications for the treatment of osteoarthritis. *Biochem Pharmacol.*, 73(9), 1434-1445 (2007)

115. A. Mitra, J. Chakrabarti, A. Banerji, A. Chatterjee and B. R. Das: Curcumin, a potential inhibitor of MMP-2 in human laryngeal squamous carcinoma cells HEp2. *J.Environ.Pathol.Toxicol.Oncol.*, 25(4), 679-690 (2006)

116. C. C. Su, G. W. Chen, J. G. Lin, L. T. Wu and J. G. Chung: Curcumin inhibits cell migration of human colon cancer colo 205 cells through the inhibition of nuclear factor kappa B /p65 and down-regulates cyclooxygenase-2 and matrix metalloproteinase-2 expressions. *Anticancer Res.*, 26(2A), 1281-1288 (2006)

117. J. H. Hong, K. S. Ahn, E. Bae, S. S. Jeon and H. Y. Choi: The effects of curcumin on the invasiveness of prostate cancer in vitro and in vivo. *Prostate Cancer Prostatic.Dis.*, 9(2), 147-152 (2006)

118. A. Banerji, J. Chakrabarti, A. Mitra and A. Chatterjee: Effect of curcumin on gelatinase A (MMP-2) activity in B16F10 melanoma cells. *Cancer Lett.*, 211(2), 235-242 (2004)

119. S. Yodkeeree, S. Garbisa and P. Limtrakul: Tetrahydrocurcumin inhibits HT1080 cell migration and invasion via downregulation of MMPs and uPA. *Acta Pharmacol.Sin.*, 29(7), 853-860 (2008)

120. M. S. Woo, S. H. Jung, S. Y. Kim, J. W. Hyun, K. H. Ko, W. K. Kim and H. S. Kim: Curcumin suppresses phorbol ester-induced matrix metalloproteinase-9 expression by inhibiting the PKC to MAPK signaling pathways in human astroglioma cells. *Biochem Biophys.Res.Commun.*, 335(4), 1017-1025 (2005)

121. S. Aggarwal, Y. Takada, S. Singh, J. N. Myers and B. B. Aggarwal: Inhibition of growth and survival of human head and neck squamous cell carcinoma cells by curcumin via modulation of nuclear factor-kappaB signaling. *Int.J.Cancer*, 111(5), 679-692 (2004)

122. Z. M. Shao, Z. Z. Shen, C. H. Liu, M. R. Sartippour, V. L. Go, D. Heber and M. Nguyen: Curcumin exerts multiple suppressive effects on human breast carcinoma cells. *Int.J.Cancer*, 98(2), 234-240 (2002)

123. M. P. Vincenti: The matrix metalloproteinase (MMP) and tissue inhibitor of metalloproteinase (TIMP) genes. Transcriptional and posttranscriptional regulation, signal transduction and cell-type-specific expression. *Methods Mol.Biol.*, 151, 121-148 (2001)

124. J. Westermarck and V. M. Kahari: Regulation of matrix metalloproteinase expression in tumor invasion. *FASEB J.*, 13(8), 781-792 (1999)

125. M. Bond, A. J. Chase, A. H. Baker and A. C. Newby: Inhibition of transcription factor NF-kappaB reduces matrix metalloproteinase-1, -3 and -9 production by vascular smooth muscle cells. *Cardiovasc.Res.*, 50(3), 556-565 (2001)

126. M. P. Vincenti, C. I. Coon and C. E. Brinckerhoff: Nuclear factor kappaB/p50 activates an element in the distal matrix metalloproteinase 1 promoter in interleukin-1beta-stimulated synovial fibroblasts. *Arthritis Rheum.*, 41(11), 1987-1994 (1998)

127. L. G. Menon, R. Kuttan and G. Kuttan: Anti-metastatic activity of curcumin and catechin. *Cancer Lett.*, 141(1-2), 159-165 (1999)

128. J. F. Santibanez, M. Quintanilla and J. Martinez: Genistein and curcumin block TGF-beta 1-induced u-PA expression and migratory and invasive phenotype in mouse epidermal keratinocytes. *Nutr.Cancer*, 37(1), 49-54 (2000)

129. E. R. Hahm, Y. S. Gho, S. Park, C. Park, K. W. Kim and C. H. Yang: Synthetic curcumin analogs inhibit activator protein-1 transcription and tumor-induced angiogenesis. *Biochem.Biophys.Res.Commun.*, 321(2), 337-344 (2004)

130. S. Narayan: Curcumin, a multi-functional chemopreventive agent, blocks growth of colon cancer cells by targeting beta-catenin-mediated transactivation and cell-cell adhesion pathways. *J.Mol.Histol.*, 35(3), 301-307 (2004)

131. S. Philip and G. C. Kundu: Osteopontin induces nuclear factor kappa B-mediated promatrix metalloproteinase-2 activation through I kappa B alpha /IKK signaling pathways, and curcumin (diferulolylmethane) down-regulates these pathways. *J.Biol.Chem.*, 278(16), 14487-14497 (2003)

132. S. Philip, A. Bulbule and G. C. Kundu: Matrix metalloproteinase-2: mechanism and regulation of NF-kappaB-mediated activation and its role in cell motility and ECM-invasion. *Glycoconj.J.*, 21(8-9), 429-441 (2004)

133. B. B. Aggarwal, S. Shishodia, Y. Takada, S. Banerjee, R. A. Newman, C. E. Bueso-Ramos and J. E. Price: Curcumin suppresses the paclitaxel-induced nuclear factor-kappaB pathway in breast cancer cells and inhibits lung metastasis of human breast cancer in nude mice. *Clin.Cancer Res.*, 11(20), 7490-7498 (2005)

134. S. Somasundaram, N. A. Edmund, D. T. Moore, G. W. Small, Y. Y. Shi and R. Z. Orlowski: Dietary curcumin inhibits chemotherapy-induced apoptosis in models of human breast cancer. *Cancer Res.*, 62(13), 3868-3875 (2002)

135. A. C. Bharti, N. Donato and B. B. Aggarwal: Curcumin (diferuloylmethane) inhibits constitutive and IL-6-inducible STAT3 phosphorylation in human multiple myeloma cells. *J.Immunol.*, 171(7), 3863-3871 (2003)

136. T. C. Hour, J. Chen, C. Y. Huang, J. Y. Guan, S. H. Lu and Y. S. Pu: Curcumin enhances cytotoxicity of chemotherapeutic agents in prostate cancer cells by inducing p21(WAF1/CIP1) and C/EBPbeta expressions and

suppressing NF-kappaB activation. *Prostate*, 51(3), 211-218 (2002)

137. D. Chendil, R. S. Ranga, D. Meigooni, S. Sathishkumar and M. M. Ahmed: Curcumin confers radiosensitizing effect in prostate cancer cell line PC-3. *Oncogene*, 23(8), 1599-1607 (2004)

138. K. C. Thresiamma, J. George and R. Kuttan: Protective effect of curcumin, ellagic acid and bixin on radiation induced toxicity. *Indian J.Exp.Biol.*, 34(9), 845-847 (1996)

139. K. C. Thresiamma, J. George and R. Kuttan: Protective effect of curcumin, ellagic acid and bixin on radiation induced genotoxicity. *J.Exp.Clin.Cancer Res.*, 17(4), 431-434 (1998)

140. H. Inano and M. Onoda: Radioprotective action of curcumin extracted from Curcuma longa LINN: inhibitory effect on formation of urinary 8-hydroxy-2'-deoxyguanosine, tumorigenesis, but not mortality, induced by gamma-ray irradiation. *Int.J.Radiat.Oncol.Biol.Phys.*, 53(3), 735-743 (2002)

141. N. Venkatesan, D. Punithavathi and V. Arumugam: Curcumin prevents adriamycin nephrotoxicity in rats. *Br.J.Pharmacol.*, 129(2), 231-234 (2000)

142. N. Venkatesan: Curcumin attenuation of acute adriamycin myocardial toxicity in rats. *Br.J.Pharmacol.*, 124(3), 425-427 (1998)

143. L. G. Menon, R. Kuttan and G. Kuttan: Inhibition of lung metastasis in mice induced by B16F10 melanoma cells by polyphenolic compounds. *Cancer Lett.*, 95(1-2), 221-225 (1995)

144. K. Ichiki, N. Mitani, Y. Doki, H. Hara, T. Misaki and I. Saiki: Regulation of activator protein-1 activity in the mediastinal lymph node metastasis of lung cancer. *Clin.Exp.Metastasis*, 18(7), 539-545 (2000)

145. N. Frank, J. Knauft, F. Amelung, J. Nair, H. Wesch and H. Bartsch: No prevention of liver and kidney tumors in Long-Evans Cinnamon rats by dietary curcumin, but inhibition at other sites and of metastases. *Mutat Res*, 523-524, 127-35 (2003)

146. S. J. Youngs, S. A. Ali, D. D. Taub and R. C. Rees: Chemokines induce migrational responses in human breast carcinoma cell lines. *Int.J.Cancer*, 71(2), 257-266 (1997)

147. E. Loukinova, G. Dong, I. Enamorado-Ayalya, G. R. Thomas, Z. Chen, H. Schreiber and C. Van Waes: Growth regulated oncogene-alpha expression by murine squamous cell carcinoma promotes tumor growth, metastasis, leukocyte infiltration and angiogenesis by a host CXC receptor-2 dependent mechanism. *Oncogene*, 19(31), 3477-3486 (2000)

148. A. J. Minn, G. P. Gupta, P. M. Siegel, P. D. Bos, W. Shu, D. D. Giri, A. Viale, A. B. Olshen, W. L. Gerald and J. Massague: Genes that mediate breast cancer metastasis to lung. *Nature*, 436(7050), 518-524 (2005)

149. F. Balkwill and A. Mantovani: Inflammation and cancer: back to Virchow? *Lancet*, 357(9255), 539-545 (2001)

150. A. Mantovani, P. Allavena, A. Sica and F. Balkwill: Cancer-related inflammation. *Nature*, 454(7203), 436-44 (2008)

151. G. G. Mackenzie, N. Queisser, M. L. Wolfson, C. G. Fraga, A. M. Adamo and P. I. Oteiza: Curcumin induces cell-arrest and apoptosis in association with the inhibition of constitutively active NF-kappaB and STAT3 pathways in Hodgkin's lymphoma cells. *Int J Cancer*, 123(1), 56-65 (2008)

152. H. Choi, Y. S. Chun, S. W. Kim, M. S. Kim and J. W. Park: Curcumin inhibits hypoxia-inducible factor-1 by degrading aryl hydrocarbon receptor nuclear translocator: a mechanism of tumor growth inhibition. *Mol Pharmacol*, 70(5), 1664-71 (2006)

153. H. Lu, W. Ouyang and C. Huang: Inflammation, a key event in cancer development. *Mol.Cancer Res.*, 4(4), 221-233 (2006)

154. P. Anand, C. Sundaram, S. Jhurani, A. B. Kunnumakkara and B. B. Aggarwal: Curcumin and cancer: an "old-age" disease with an "age-old" solution. *Cancer Lett.*, 267(1), 133-164 (2008)

155. J. W. Cho, K. S. Lee and C. W. Kim: Curcumin attenuates the expression of IL-1beta, IL-6, and TNF-alpha as well as cyclin E in TNF-alpha-treated HaCaT cells; NF-kappaB and MAPKs as potential upstream targets. *Int.J.Mol.Med.*, 19(3), 469-474 (2007)

156. D. Ranjan, C. Chen, T. D. Johnston, H. Jeon and M. Nagabhushan: Curcumin inhibits mitogen stimulated lymphocyte proliferation, NFkappaB activation, and IL-2 signaling. *J.Surg.Res.*, 121(2), 171-177 (2004)

157. T. Kobayashi, S. Hashimoto and T. Horie: Curcumin inhibition of Dermatophagoides farinea-induced interleukin-5 (IL-5) and granulocyte macrophage-colony stimulating factor (GM-CSF) production by lymphocytes from bronchial asthmatics. *Biochem Pharmacol.*, 54(7), 819-824 (1997)

158. A. J. Fahey, R. R. Adrian and C. S. Constantinescu: Curcumin modulation of IFN-beta and IL-12 signalling and cytokine induction in human T cells. *J.Cell Mol.Med.*, 11(5), 1129-1137 (2007)

159. A. Grandjean-Laquerriere, F. Antonicelli, S. C. Gangloff, M. Guenounou and R. Le Naour: UVB-induced IL-18 production in human keratinocyte cell line NCTC 2544 through NF-kappaB activation. *Cytokine*, 37(1), 76-83 (2007)

160. J. A. Burger and A. Peled: CXCR4 antagonists: targeting the microenvironment in leukemia and other cancers. *Leukemia*, 23(1), 43-52 (2009)

161. A. Albini, V. Mirisola and U. Pfeffer: Metastasis signatures: genes regulating tumor-microenvironment interactions predict metastatic behavior. *Cancer Metastasis Rev*, 27(1), 75-83 (2008)

162. B. B. Aggarwal, A. Kumar and A. C. Bharti: Anticancer potential of curcumin: preclinical and clinical studies. *Anticancer Res.*, 23(1A), 363-398 (2003)

163. K. M. Mohandas and D. C. Desai: Epidemiology of digestive tract cancers in India. V. Large and small bowel. *Indian J.Gastroenterol.*, 18(3), 118-121 (1999)

164. D. M. Parkin, F. Bray, J. Ferlay and P. Pisani: Estimating the world cancer burden: Globocan 2000. *Int J Cancer*, 94(2), 153-6 (2001)

165. V. Gajalakshmi, R. Swaminathan and V. Shanta: An Independent Survey to Assess Completeness of Registration: Population Based Cancer Registry, Chennai, India. *Asian Pac J Cancer Prev*, 2(3), 179-183 (2001)

166. R. A. Sharma, S. A. Euden, S. L. Platton, D. N. Cooke, A. Shafayat, H. R. Hewitt, T. H. Marczylo, B. Morgan, D. Hemingway, S. M. Plummer, M. Pirmohamed, A. J. Gescher and W. P. Steward: Phase I clinical trial of oral curcumin: biomarkers of systemic activity and compliance. *Clin Cancer Res*, 10(20), 6847-54 (2004)

167. G. Garcea, D. P. Berry, D. J. Jones, R. Singh, A. R. Dennison, P. B. Farmer, R. A. Sharma, W. P. Steward and A. J. Gescher: Consumption of the putative chemopreventive agent curcumin by cancer patients: assessment of curcumin levels in the colorectum and their pharmacodynamic consequences. *Cancer Epidemiol Biomarkers Prev*, 14(1), 120-5 (2005)

168. J. J. Johnson and H. Mukhtar: Curcumin for chemoprevention of colon cancer. *Cancer Lett*, 255(2), 170-81 (2007)

169. T. H. Leu and M. C. Maa: The molecular mechanisms for the antitumorigenic effect of curcumin. *Curr.Med.Chem.Anti.-Canc.Agents*, 2(3), 357-370 (2002)

170. R. Sinha, D. E. Anderson, S. S. McDonald and P. Greenwald: Cancer risk and diet in India. *J Postgrad Med*, 49(3), 222-8 (2003)

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