Role of chemopreventive agents in cancer therapy

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Abbreviations used: TNF-α, tumor necrosis factor-α; NF-kB, nuclear factor-kB; RANK, receptor activator of NF-kB; RANKL, receptor activator of NF-kB ligand; IkB, inhibitory subunit of IkB; IKK, IkBα kinase: TRAF, TNF-receptor associated factor; NIK, NF-kB inducing kinase; COX-2, cyclooxygenase-2; iNOS, inducible NO synthase; JNK, c-jun N-terminal kinase; MAPK, mitogen activated protein kinase; ERK, extracellular signal-regulated kinase; VEGF, vascular endothelial cell growth factor; EGFR, epidermal growth factor receptor; AP-1, activated protein-1; STAT, signal transducers and activators of transcription; JAK, janus kinase; IL-2, interleukin-2; MDR, multi drug resistance; MRP, multidrug resistance related protein; MMP, matrix metalloprotease; GRP, glucose regulated protein; TOPO-II, Topoisomerase-II, TGF-α, Transforming growth factor-α ; EGCG, (-)-epigallocatechin-3-gallate; GSH, reduced glutathione; GST-Pi, Glutathione S-transferase Pi.

Key Words: NF-κB, carcinogenesis, chemoprevention, chemotherapy, tumorigenesis, COX-2

Abstract:
Tumorigenesis or carcinogenesis is a multi-step process that is induced primarily by carcinogens leading to the development of cancer. Extensive research in the last few years has revealed that regular consumption of certain fruits and vegetables can reduce the risk of acquiring specific cancers. Phytochemicals derived from such fruits and vegetables, referred to as chemopreventive agents include genistein, resveratrol, diallyl sulfide, S-allyl cysteine, allicin, lycopene, capsaicin, curcumin, 6-gingerol, ellagic acid, ursolic acid, silymarin, anethol, catechins and eugenol. Because these agents have been shown to suppress cancer cell proliferation, inhibit growth factor signaling pathways, induce apoptosis, inhibit NF-κB, AP-1 and JAK-STAT activation pathways, inhibit angiogenesis, suppress the expression of anti-apoptotic proteins, inhibit cyclooxygenase-2, they may have untapped therapeutic value. These chemopreventive

agents also have very recently been found to reverse chemoresistance and radioresistance in patients undergoing cancer treatment. Thus, these chemopreventive agents have potential to be used as adjuncts to current cancer therapies.

1. Introduction

Tumorigenesis is a multistep process that begins with cellular transformation, progresses to hyperproliferation and culminates in the acquisition of invasive potential, angiogenic properties and establishment of metastatic lesions [1]. This process can be activated by any one of the various environmental carcinogens (such as cigarette smoke, industrial emissions, gasoline vapors), inflammatory agents (such as tumor necrosis factor [TNF] and H_2O_2), tumor promoters (such as phorbol esters and okadaic acid). This multistep process of carcinogenesis consists of three phases: tumor initiation, promotion and progression phases. Several population based studies indicate that people in South East Asian countries have a much lower risk of acquiring colon, gastrointestinal, prostate, breast and other cancers when compared to their Western counterparts [see Table1]. It is very likely that constituents of their diet such as garlic, ginger, soy, curcumin, onion, tomatoes, cruciferous vegetables, chillies and green tea play an important role in their ability to avoid these cancers. These dietary agents are believed to suppress the transformative, hyperproliferative and inflammatory processes that initiate carcinogenesis. These inhibitory influences may ultimately suppress the final steps of carcinogenesis, namely angiogenesis and metastasis. These dietary agents have been classified as chemopreventive agents since their ability to delay the onset of the carcinogenic process has been studied extensively. Because these chemopreventive agents are derived from natural sources, they are considered pharmacologically safe. The focus of the current review, although brief, is to evaluate the untapped therapeutic potential of these chemopreventive agents in the setting of several molecular targets that are currently under investigation (Fig 1).

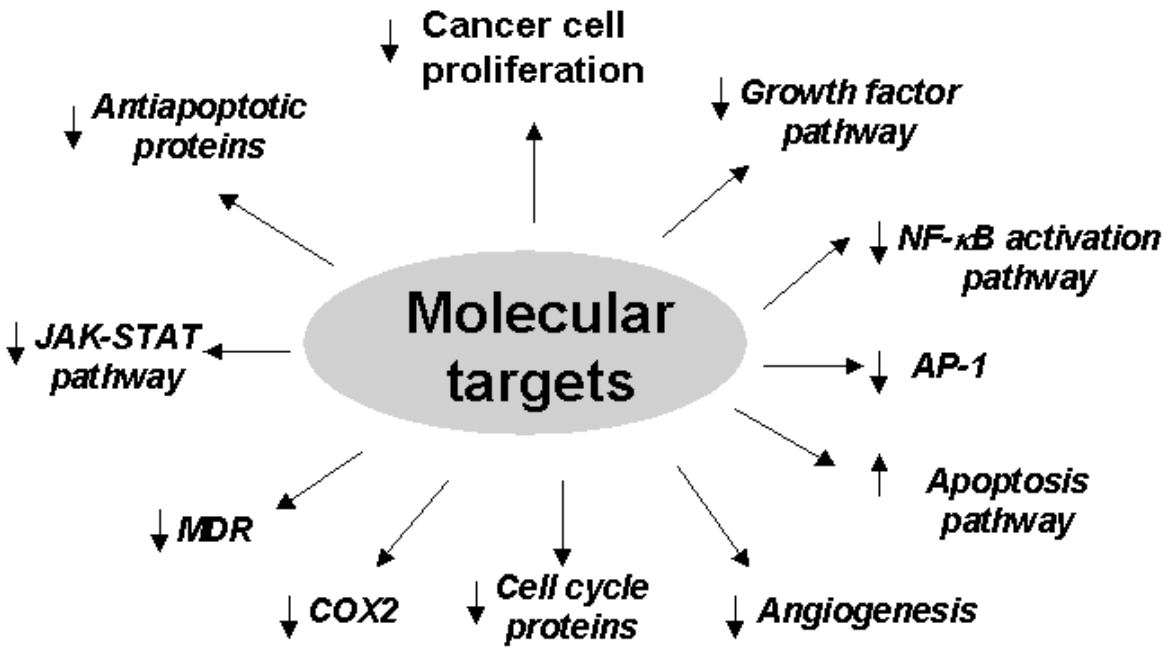

Fig. 1 . Molecular targets of chemopreventive agents in cancer.

Table 1. Comparison of Cancer Incidence in USA and India

Cancer	USA		India	
	Cases	Deaths	Cases	Deaths
Breast	660	160	79	41
Prostate	690	130	20	9
Colon/Rectum	530	220	30	18
Lung	660	580	3 8	37
Head & Neck SCC	140	44	153	103
Liver	41	44	12	13
Pancreas	108	103	8	8
Stomach	81	50	33	30
Melanoma	145	27	1.8	1
Testis	21	1	3	1
Bladder	202	43	15	11
Kidney	115	44	6	4
Brain, Nervous system	65	47	19	14
Thyroid	55	5	12	3
Endometrial Cancers	163	41	132	72
Ovary	76	50	20	12
Multiple myeloma	50	40	6	5
Leukemia	100	70	19	17
Non-Hodgkin lymphoma	180	90	17	15
Hodgkin's disease	20	5	7	4

Showing cases per 1 million persons calculated on the basis of current consensus: Endometrial cancers include Cervix uteri and Corpus uteri.

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2. Chemopreventive agents as inhibitors of the NF-kB activation pathway

NF-κB is a family of closely related protein dimers that bind to a common sequence motif in the DNA called the κB site [for references see 2]. The molecular identification of its p50 subunit (v-REL) as a member of the reticuloendotheliosis (REL)family of viruses provided the first evidence that NF-κB is linked to cancer. Research over the past decade has revealed that NF-κB is an inducible transcription factor for genes involved in cell survival, cell adhesion, inflammation, differentiation and growth. In most resting cells, NF-κB is sequestered in the cytoplasm by binding to the inhibitory IκB proteins which blocks the nuclear localization sequences of NF-κB. NF-κB is activated by a variety of stimuli such as carcinogens, inflammatory agents, tumor promoters including cigarette smoke, phorbol esters, okadaic acid, H_2O_2 and TNF. These stimuli promote dissociation of IκB-α through phosphorylation, ubiquitinylation and its ultimate degradation in the proteasomes. This process unmasks the nuclear localization sequence of NF-κB, facilitating its nuclear entry, binding to κB regulatory elements and activation of transcription of target genes. Many of the target genes that are activated are critical to the establishment of early and late stages of aggressive cancers such as expression of cyclin D1, apoptosis suppressor proteins such as bcl-2 and bcl-X_L and those required for metastasis and angiogenesis such as matrix mettalooproteinases (MMP) and vascular endothelial growth factor (VEGF).

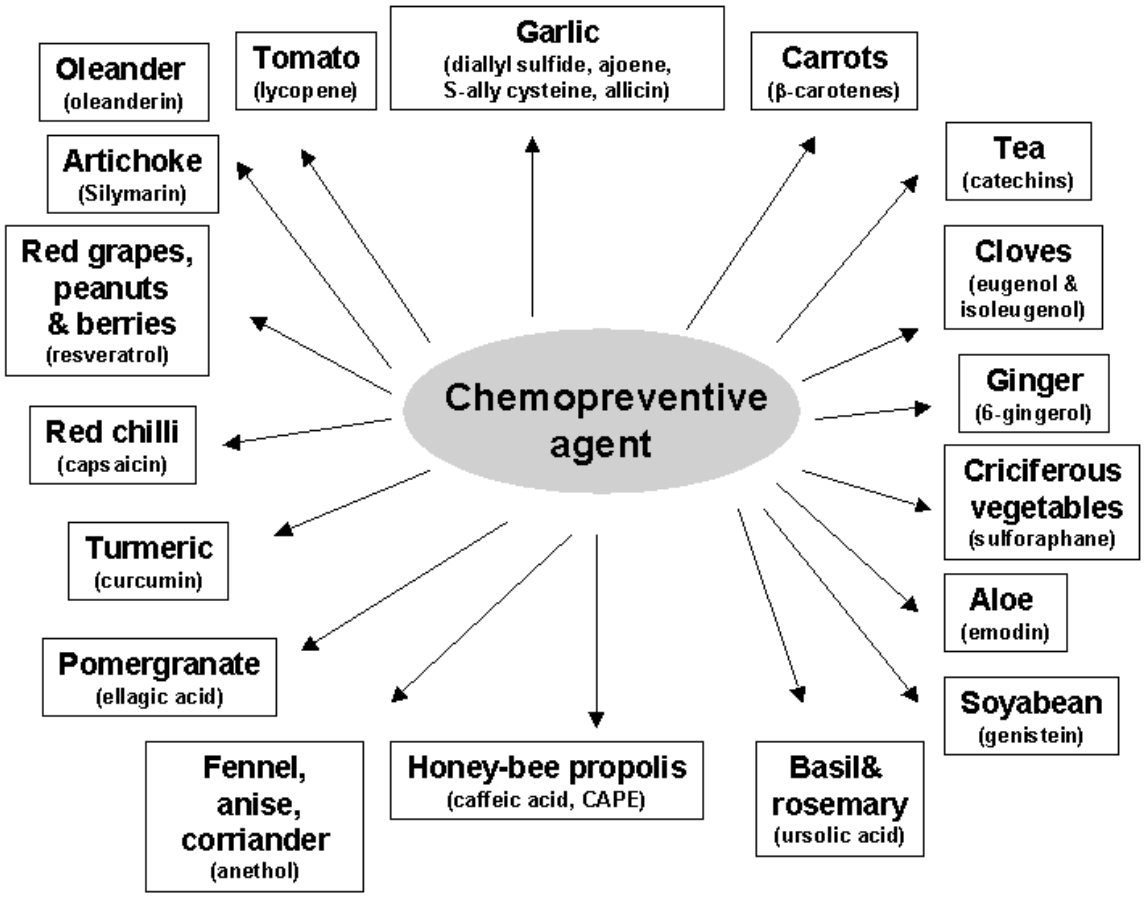

Fig. 2. Chemopreventive agents known to suppress tumorigenesis and their dietary sources.

The chemopreventive phytochemicals that are the focus of this review are shown schematically in Fig 2. Most of these chemopreventives, such as curcumin, catechins, silymarin, caffeic acid phenethyl ester (CAPE), sanguinarine, anethole, emodin, piceatannol, resveratrol, capsaicin, ursolic acid, betulinic acid, flavopiridol and oleandrin are known to block the NF-κB activation process[3-15]. Thus, although the maintenance of appropriate levels of NF-κB activity is crucial for normal cellular proliferation, constitutive NF-κB activation is involved in the enhanced growth properties as seen in several cancers [16]. Dietary intake of these safe and nontoxic chemopreventives may thus be beneficial for patients whose tumors express persistently high levels of activated NF-κB such as non-small cell lung carcinoma, thyroid, colon, breast, stomach, squamous head and neck carcinomas.

3. Chemopreventive agents as inhibitors of the AP-1 activation pathway:

Activated protein-1 (AP-1) is another transcription factor that regulates the expression of several genes that are involved in cell differentiation and proliferation. Functional activation of the AP-1 transcription complex is implicated in tumor promotion as well as malignant transformation. This complex consists of either homo or heterodimers of the members of the JUN and FOS family of proteins [17]. This AP-1 mediated transcription of several target genes can also be activated by a complex network of signaling pathways that involves external signals such as growth factors, mitogen activated protein kinases (MAPK), extracellular-signal regulated protein kinases (ERK) and JUN-terminal kinases (JNK). Some of the target genes that are activated by AP-1 transcription complex mirror those activated by NF-κB and include Cyclin D1, bcl-2 , bcl-X_L, VEGF, MMP and urokinase plasminogen activator (uPA). Expression of genes such as MMP and uPA especially promotes angiogenesis and invasive growth of cancer cells. Most importantly, AP-1 can also promote the transition of tumor cells from an epithelial to mesenchymal morphology which is one of the early steps in tumor metastasis. These oncogenic properties of AP-1 are primarily dictated by the dimer composition of the AP-1 family proteins and their post-transcriptional and translational modifications.

Several phytochemicals such as curcumin, capsaicin, resveratrol and green tea catechins have been shown to suppress the AP-1 activation process [10,15]. An AP-1 blockade has been shown to interfere with the transmission of proliferative signals induced by peptide growth factors as well as steroid growth factors such as estrogens [17]. These results suggest that chemopreventive agents specifically targeting AP-1 or its activating kinases could be promising agents for the treatment of several cancers.

4. Chemopreventive agents as inhibitors of cell proliferation and initiators of apoptosis

During the past 8 years, several reports were published which showed that activation of NF-kB promotes cell survival and proliferation and down regulation of NF-kB sensitizes the cells to apoptosis. How NF-kB promotes these proliferation and cell survival mechanisms has become increasingly clear. Expression of several genes including bcl-2, bcl-X_L, cIAP, survivin, cyclin D1, TRAF1, TRAF2 have been reported to be up-regulated by NF-kB [2]. The proteins coded by these genes function primarily by blocking the apoptosis pathway. Several studies have demonstrated that NF-kB activation promotes cell survival and proliferation mechanisms and that suppression of NF-kB leads to an abrogation of these mechanisms. Similarly, c-JUN is primarily a positive regulator of cell proliferation since c-JUN deficient fibroblasts have a marked proliferation defect *in vitro* and *in vivo*. c-JUN protein, once fully activated by JNK kinases induces the transcription of positive regulators of cell cycle progression such as cyclin

D1 and represses the negative regulators such as the tumor suppressor p53 and the cyclin dependent kinase inhibitor p16 (INK4A). Moreover, activated and oncogenic AP-1 can antagonize apoptosis in several tumors. Several phytochemicals that are known to inhibit the NF-kB or the AP-1 activation process, most notably curcumin, green tea, 6-gingerol and resveratrol can cause a significant suppression of cell proliferation and sensitizes cells for apoptosis [15,18]. Most notably, phytochemicals such as curcumin is also known to down regulate the expression of apoptosis suppressor proteins, such as bcl-2 and bcl-X_L in several cancer cell lines.

5. Chemopreventive agents as inhibitors of growth factor activation pathway

The potent cell proliferation signals generated by various growth factor receptors such as the epidermal growth factor (EGF)-receptor, insulin-like growth factor (IGF)-1 receptor and VEGF-receptor networks constitute the basis for receptor driven tumorigenicity in the progression of several cancers [1]. Consequences of these abnormal growth factor receptor signaling pathways include, besides increased cell proliferation, suppression of apoptotic signals, especially under anchorage independent conditions and an increase in the tumor's invasive behavior contributing to metastatic spread and the growth of new blood vessels. Several chemopreventive phytochemicals including curcumin, genistein, resveratrol and catechins have been recently shown to be powerful inhibitors of several growth factor receptors, including EGFR. Some of these phytochemicals, such as curcumin also possess the capacity to inhibit the ligand stimulated activation of the EGF-Receptor indicating that they have the potential to break the autocrine loops that are established in several advanced cancers [19]. The inhibitory actions of these phytochemicals also have several other potential advantages to patients with late stage cancers. A blockade of EGFR, for example, may predispose the cancer cells to apoptosis. Moreover, inhibition of EGFR disables the protein's capacity to provide the cancer cell the matrix independent survival support it needs for expansion and the acquisition of invasive potential. Third, these chemopreventive chemicals function by inhibiting other tyrosine kinases such as *c-src* which are involved in the coupling of activation of the G-protein coupled receptor to the transactivation of EGF-Receptor which occurs extensively in established cancers. Finally, most of these phytochemicals also inhibit by a similar mechanism, the HER2/*neu* receptor which is overexpressed in breast, prostate, ovarian and lung cancers. Curcumin was earlier shown to not only inhibit the tyrosine kinase activity of this receptor but also deplete the protein itself, by interfering with the function of the ATP dependent GRP94 chaperone protein which is involved in the maintenance of the properly folded state of the receptor [20]. Most of these phytochemicals, moreover, by inhibiting HER2/neu can also interfere with the cross talk between the receptor and the estrogen receptor (ER) pathways in these cancers. Thus, they may be beneficial in treating the hormone resistant breast cancer patients by restoring hormone responsiveness.

6. Chemopreventive agents as inhibitors of the JAK-STAT pathway

Even though cancer arises by several genetic or epigenetic mechanisms contributing to a number of abnormal oncogenic signaling pathways, all seem to converge on a very limited number of nuclear transcription factors that function as final effectors, starting specific gene expression patterns for a particular cancer. They are the canonical STAT (signal transducers and activators of transcription)family of proteins [21]. They can be activated by phosphorylation through JAK kinases or cytokine receptors, G-protein coupled receptors or growth factor receptors such as EGFR or by platelet derived growth factor receptor that have

intrinsic tyrosine kinase activity, or by intracellular non-receptor tyrosine kinase recruitment. Of the seven STAT proteins known so far, constitutive activation of STAT3 and STAT5 have been implicated in human cancers such as multiple myeloma, lymphomas, leukemias and several solid tumors which makes them logical targets for cancer therapy. These STAT proteins contribute to cell survival and growth by preventing apoptosis through increased expression of anti-apoptotic proteins such as bcl-2 and bcl-X_L. Recently, STAT 3 has been shown to be a direct activator of the VEGF gene, responsible for increased angiogenesis. More importantly, the increased expression of STAT3 and STAT5 transcription factors are crucially involved in the processes by which the tumor evades immunological surveillance by increasing the expression of immune-suppressing factors, decreasing the expression of pro-inflammatory cytokines which are responsible for the matutation of the dendritic cells (DC) [25]. Several cancer chemopreventive phytochemicals have been shown to inhibit the JAK-STAT mediated signaling processes in cancer situations such as multiple myeloma [22]. Some of these phytochemicals also have been shown to inhibit cytokine such as (IL)-6-mediated STAT 3 phosphorylation in multiple myeloma cells, showing that they can effectively interfere with the constitutive and abnormal signaling processes that occur in these cancers. The activation of *src*-homology domain (SH2) containing protein tyrosine phosphatases such as SHP-2, a negative regulator of JAK activity was recently shown to be one of the mechanisms by which curcumin could negatively influence STAT signaling [23].

7. Chemopreventive agents as inhibitors of multi-drug resistance

The use of chemotherapy to treat cancer invariably results in the development of broad resistance to a wide variety of drugs with different chemical structures and mechanisms of action. This form of resistance is mediated primarily by classical ATP-driven drug efflux pumps such as the P-Glycoproteins and the MRP family of proteins. The multidrug resitance (MDR) related P-Glycoprotein is inherently expressed at high levels in cancers derived from epithelial tissues such as kidney, prostate and colon. Because of this, a classic blockade of these transporter proteins with agents that were characterized earlier such as verapamil, PSC-833 has significant toxic effects. Recent reports on the reversal of this classic MDR process by chemopreventive compounds such as curcumin and genistein have provided encouraging results. In the multidrug resistant human cervical carcinoma cells (KB-V1), curcumin was shown to down regulate the expression of P-glycoprotein at the protein as well as at the RNA levels [24]. Treatment of these cells increased their sensitivity to vinblastine, which was consistent with the increased accumulation of the rhodamine (Rh123) dye. In addition, curcumin inhibited the verapamil stimulated ATP-ase activity and the photo-affinity labeling of P-Glycoprotein with iodinated prazocin analog in a concentration dependent manner, demonstrating that curcumin interacted directly with the transporter. It is very likely that curcumin is able to achieve this in its capacity to function as a general ATP-competitor. Similar studies done with green tea components (EGCG) indicated that these compounds also interacted with drug efflux pumps such as MRP-1 and MRP-2 in a slightly different capacity, in that they might actually be substrates for these pumps [25]. Thus, these agents may contribute to an increased intracellular accumulation of cytotoxic drugs by saturating or titrating out the pumps. These studies suggest that these agents actually could be used as safe and non-toxic MDR reversing agents. Recent reports from several laboratories suggest that agents such as curcumin may interfere with the drug resistance processes mediated by Topoisomerase-II (TOPO-II) poisons, either by inhibiting the associated heat shock proteins such as GRP78 and GRP94 or by inhibiting the intracellular

proteasomal function [26]. Thus, these phytochemicals have the capacity to act at multiple sites and levels to interfere with the classic as well as the other modes of generation of resistance generated by microenvironmental stress conditions.

8. Chemopreventive agents as inhibitors of COX-2

Numerous preclinical studies point to the importance of regulation of cyclo-oxygenase-2 (COX-2) expression in the prevention and, most importantly, in the treatment of several malignancies. This enzyme is overexpressed in practically every pre-malignant and malignant conditions involving the colon, liver, pancreas, breast, lung, bladder, skin, stomach, head and neck and esophagus [27]. Overexpression of this enzyme is a consequence of deregulation of transcriptional and post-transcriptional control. Several growth factors, cytokines, oncogenes, tumor promoters stimulate COX-2 transcription . Expression of COX-2 is increased in HER2/neu expressing breast carcinomas owing to enhanced *ras* signaling. Depending upon the stimulus and the cell type, different transcription factors including AP-1, NF-IL-6, NF-kB can stimulate COX-2 transcription [27]. Wild type p53 protein expression can suppress COX-2 transcription while the mutant p53 protein can not. Consistent with this observation, increased COX-2 levels are seen in several epithelial cancers that express mutant p53. Taken together, these findings suggest that the balance between the activation of the oncogenes and the inactivation of the tumor suppressor genes and expression of several pro-inflammatory cytokines can modulate the expression of COX-2 in tumors. Complicating matters further is the fact that conventional cancer therapies such as radiation and chemotherapy can induce COX-2 and prostaglandin biosynthesis. Thus, inhibition of this enhanced COX-2 activity in tumors clearly has a therapeutic potential.

Curcumin was one of the first chemopreventive phytochemicals shown to possess significant COX-2 inhibiting activity through the suppression of NF-κB. Thus , non-toxic compounds such as curcumin will be useful in the treatment of several cancers targeting angiogenesis since COX-2 expression stimulates angiogenesis [28]. Since COX-2 derived prostaglandins stimulate aromatase activity in an organ specific manner, an independent source of estradiol generation in breast cancer patients undergoing anti-estrogen therapies can be blocked by curcumin and other chemopreventives that have significant COX-2 inhibitory activity. COX-2 inhibitors will be particularly useful in the treatment of advanced breast cancers through inhibition not only of HER-2/neu activity but also of aromatase activity [27]. Curcumin is also perfectly suited for the treatment of cancers such as colon cancer since it is also capable of inhibiting the NF-κB activation process. Preclinical studies showed that curcumin achieves this effect by inhibiting the upstream activator complex consisting of NF-kB-inducing kinase (NIK) and IKBα kinase (IKK) enzymes [29]. Other chemopreventive agents, such as genistein and catechins may work through down regulation of EGF-R and HER-2/*neu* activity, resulting in a reduced expression of COX-2. Resveratrol has also been shown to down-regulate COX-2 expression [30]. Our laboratory recently showed the suppression of 7,12 Dimethyl-Benz(a)anthracene (DMBA)-induced mammary carcinogenesis in rats by resveratrol and this correlated with inhibition of NF-kB, COX-2 and MMP-9 [31].

9. Chemopreventive agents as inhibitors of angiogenesis

Angiogenesis, the regulated formation of new blood vessels from existing ones, is the basis of several physiological processes such as embryonic development, placenta formation and wound healing. It is one of the best examples of how a tumor can take control of these processes

and deregulate them to its own advantage. The normal and orderly formation of new blood vessels consists of the endothelial cell receiving the stimulatory signal and secretion of MMP and heparanase, which cause the dissolution of the extracellular matrix (ECM). The tight junction between the endothelial cells is then altered and the cells project through the newly created space where the newly formed endothelial cells organize into fresh capillary tubes, allowing the sprouting vessel to grow toward the source of fresh blood supply [32]. When the tumor tries to grow new blood vessels, most of these normal physiological rules governing new blood vessel growth are subverted. Blood vessels newly formed by tumors often feature incomplete basement membranes and the microvasculature is often chaotic, following convoluted paths without organization. These vessels also have disproportionate endothelial cell to pericyte ratio and abnormal pericyte coverage. The new blood vessels formed are hyperpermeable because of an imbalance of pro and anti-angiogenic factors and are often leaky [32]. Moreover, tumor cells themselves try to mimic the properties of endothelial cells and form loose vasculogenic mesh work by processes such as vessel cooption and vasculogenic mimicry [33]. Thus , interference with the mechanisms of angiogenic switch, vessel cooption and vasculogenic mimicry will be of great therapeutic value in several advanced cancers.

While several inhibitors of angiogenesis are in clinical trials, it is very important to note here that several safe chemopreventive phytochemicals are already known to target these pathways. These include curcumin, resveratrol and catechins [34-37]. First, curcumin can interfere with the activity of MMP- 2 and 9, reducing the degradation of ECM which forms the basis of angiogenic switch [37]. Thus, it can also interfere with the release of angiogenic and other growth factors that are stored in the ECM. By inhibiting several growth factor receptors such as EGF-R and VEGF-R, it can also significantly impact upon the mechanisms of angiogenic switch and vessel cooption that are necessary for the sprouting growth of new blood vessels in the tumor [38]. By interfering with the non-receptor tyrosine kinases such as *src* and FAK, agents such as curcumin, genistein and green tea components can interfere with the downstream PI-3 Kinase signaling responsible for the induction of the angiogenic target genes such as COX-2, VEGF, IL-8 and the MMPs [39,40]. By inhibiting another member of the MMP family, namely MMP-2, curcumin may have a negatively impact upon the MMP-2 mediated degradation of the lamin-5 isoform which is implicated in the formation of loose and primitive looking meshwork formed by aggressive cancers such as melanoma and prostate cancers. This plasticity of the cancer cells mimicking the endothelial cells is mainly brought out by the capacity of the cancer cells to express endothelium associated genes such as VE-Cadherin, *src*, FAK and PI-3 Kinases, all of which are good targets for these chemopreventive agents. Recent findings showed that curcumin can also inhibit another member of the MMP family, aminopeptidase N (APN) which is implicated in the angiogenic switch process [41]. Most notably, curcumin and to a lesser extent genistein can also interfere with the expression of VEGF by processes other than hypoxia, such as transforming growth factor (TGF)-β release, COX-2 overexpression, hydrogen peroxide release from bone cells, constitutive and aberrant EGF-R and *src* signaling and most importantly, by aberrant NF-kB signaling in established cancers. Chemopreventive phytochemicals such as curcumin, genistein and green tea components are also known to interfere with the endothelial cell function by inhibiting specific integrin engagement and usage.

10. Chemopreventive agents as inhibitors of cell cycling

Even though there are literally hundreds of cancer type s with global changes in the gene expression, a very small number of crucial alterations are shared by perhaps all the tumors. These common alterations are related to those that disrupt the normal cell cycle control checkpoints. The reinoblastoma (Rb) and the tumor suppressor p53 proteins that are crucial for these controls are usually lost in several cancers. The central role of the G1 to S and the G2 to M transition and the corresponding checkpoints in the cancer development have been well established [42]. Formation and regulation of the enzyme complexes with the D-type cyclins and their partners and the B-type cyclins with their associating proteins are particularly well characterized as is the control of Rb function by phosphorylation.

Several chemopreventive phytochemicals such as curcumin, resveratrol and catechins have been shown to interfere with these cell cycle regulatory pathways qualifing them as potential therapeutic agents [43,44]. In multiple myeloma cells, for example, an inhibition of NF-kB activity produced a parallel down regulation of one of its target proteins, cyclin D1 [45]. This resulted in a decrease in the formation of cyclinD1-cdk4 holoenzyme complex, resulting in suppression of proliferation and induction of apoptosis. In another study, curcumin induced G0/G1 and /or G2/M phase cell cycle arrest, upregulated cdk inhibitors such as p21Cip1, p27Kip1 and down regulated cyclin B1 and cdc2 [46]. As a consequence of these cell cycle inhibitory activities and its down regulation of apoptosis suppressor proteins such as bcl-2 and bcl-X_L , curcumin treatment also abrogates cell survival mechanisms, especially those mediated by the transcription factors such as AP-1, STATs and NF-kB. In a similar vein, the green tea component EGCG causes cell cycle dysregulation and promotes apoptosis via a dose and time dependent upregulation of p21 /Waf1, p27/Kip1, INK4a/p16, and down regulation of proteins such as CyclinD1, CyclinE, cdk2, cdk4 [47]. Some of the properties of EGCG mirror those of curcumin by inhibiting the cyclin-cdk complexes operative in the G1-S transition, causing cell cycle arrest and ultimately leading to apoptotic cell death. Resveratrol on the other hand , has been shown to increase the DNA synthesis and cause a significant accumulation of cells in the S-phase at lower concentrations while it inhibits DNA synthesis at higher concentrations. Other reports have strongly suggested that resveratrol can also cause G2 phase arrest by inactivating p34 (CDC2) and CDK7 protein kinase activity. This cell cycle signaling conflict may partly explain resveratrol's apoptosis inducing and anti-proliferative effects [48].

11. Chemopreventive agents as chemosensitizers and radiosensitizers

Recent reports point out that these safe and non-toxic cancer chempreventive phytochemicals can function as sensitizers, augmenting the effectiveness of cancer chemotherapy and radiotherapy. This sensitization is thought to occur at various levels. First, by directly competing with the ATP binding site of the MDR or MRP drug efflux pumps, curcumin can inhibit the pump and increase the intracellular concentrations of the chemotherapeutic drugs such as vinblastine or vincristine. Second, by functioning as efflux substrates for pumps such as MDR or MRP, chemopreventives such as genistein and green tea components (EGCG) can saturate and hence titrate out the pumps, increasing the amount of the chemotherapeutic drug within the cell. This type of competition with the MDR or MRP substrates in effect sensitizes the cancer cell for a better cell kill by chemotherapeutic agents. Third, curcumin can interfere with the functioning of pumps such as the MRP which require a steady supply of reduced glutathione (GSH), since it is known to be an inhibitor of GSH synthetase. This type of inhibition might enhance the sensitivity of these cancer cells

overexpressing MRP to chemotherapeutic agents such as vincristine, arsenicals and platinum derivatives by impairing their efflux [49].

One other clinical strategy that is currently being pursued is to target c-JUN expression to reduce intracellular GSH levels. Stable increases in the c-JUN expression are associated with the AP-1 mediated increase in the GSH synthetase levels [50]. Since curcumin targets the same elements, it would be a strong inhibitor, reducing the intracellular GSH at the transcriptional level [51]. Expression of glutathione S-Transferase Pi (GST-Pi) is also correlated with the resistance of cancer cells to chemotherapeutic agents. In a recent study, curcumin efficiently inhibited the TNF- and phorbol ester induced AP-1 and NF-κB transcription factor binding to the sites located on the GST-Pi gene promoter in K562 leukemia cells [51]. This process efficiently reduced the GST-Pi levels causing an interference with drug resistance and ultimate apoptosis. Chemopreventive agents such as curcumin also can sensitize cancer cells to other traditional chemotherapeutic agents such as etoposide and camptothecin in another capacity. Topo II poisons stabilize the cleavable complexes, an intermediate product of the TopoII catalyzed reaction. Accumulation of these cleavable complexes is thought to lead to cell death. Conversely, a decrease in the number of cleavable complexes could confer drug resistance.

Proteasome inhibition has recently been found to decrease this inducible resistance by inhibiting the TopoII depletion by hypoxia or glucose starvation. More over, the observation that proteasomal inhibitors such as lactacystin significantly enhanced the anti-tumor activity of etoposide in xenografts *in vivo* strongly suggested that the TopoII depletion occurred through a proteasomal mechanism [52]. With this rationale, several proteasomal inhibitors such as PS-341 are currently showing promise in phase II clinical trials . It is worth noting that curcumin has recently been shown to inhibit cellular proteasome activity in a concentration dependent manner with a parallel increase in the accumulation of ubiquitinated proteins. This agent may be able to inhibit the proteasomes by inhibiting the ubiquitin isopeptidase activity, as shown in recent studies [53]. This mode of proteasome mediated sensitization of cancer cells to drugs such as etoposide and camptothecin by curcumin will be therapeutically beneficial for patients with several different types of cancer. This expectation is based on inhibition of degradation of TopoII by the proteasomes, resulting in more DNA cleavable complexes.

Most of the chemotherapeutic agents and gamma irradiation commonly administered to cancer patients have been found to activate NF-κB. The activation of NF-κB can lead to resistance to apoptosis. Activation of these survival processes occurs in parallel to the induction of the apoptosis by the activation of several caspases by the same agents. In this respect, co-administration of chemopreventive agents such as curcumin will activate the apoptotic pathways in these cancer cells while at the same time down regulating the cell survival pathways, mediated by PI-3 Kinase and AKT proteins. This is generally accomplished without activating the anti-apoptotic pathways which in effect alters the bcl-2 : bax ratio, contributing to the sensitization effect. This explains, in a nutshell, the sensitizing or potentiating effect of these chemopreventives to achieve a better target cell kill than what can be achieved by chemotherapy or radiotherapy alone. Other mechanisms by which curcumin and other chemopreventive agents may enhance the cytotoxicity of chemo and radiotherapies include the induction of p21 (WAF-1/CIP1). Recently, resveratrol was found to mediate chemosensitization through downregulation of survivin, a cell survival gene[54]. Similarly, curcumin was found to induce radiosensitization of prostate cancer cells through the suppression of NF-κB activation[55]. In general, these chemopreventive agents bring out significant sensitization effects by overcoming therapy-induced prosurvival gene expression in several cancers.

12. Conclusions

This minireview presents evidence that chemopreventive agents can be used not just to prevent cancer but also to treat cancer. Because of their pharmacological safety, most chemopreventive agents can be used in combination with chemotherapeutic agents to enhance the effect at lower doses and thus minimize chemotherapy-induced toxicity. Because cancer is primarily a disease of old age, less toxic therapy is a major priority. This review reveals that molecular targets of chemopreventive agents are similar to those currently being used for the treatment of cancer. Tumor cells use multiple cell survival pathways to prevail, and thus agents that can suppress multiple pathways have great potential for the treatment of cancer.

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References

[1] W.C.Hahn, R.A.Weinberg. Rules for making human tumor cells. New. Engl. J. Med. 347(2002) 1593-1603.

[2] A. Garg, B.B Aggarwal . Nuclear transcription factor-kB as a target for cancer Drug Development. Leukemia 16(2002) 1053-1068.

[3] S.Singh, B.B.Aggarwal. Activation of transcription factor NF-kB is suppressed by curcumin (Diferuloylmethane). J.Biol.Chem.270(1995) 24995-25000.

[4] S.Singh, K.Natarajan, B.B. Aggarwal. Capsaicin (8-methyl-N-vanillyl-6-noneamide) is a potent inhibitor of the transcription factor NF-kB activation by diverse agents. J.Immunol 157(1996) 4412-4420.

[5] K.Natarajan, S.Singh, T.R.Burke, Jr, D.Grunberger, B.B.Aggarwal. Caffeic acid phenethyl ester (CAPE) is a potent and specific inhibitor of activation of nuclear transcription factor NF-kB. Proc.Natl.Acad.Sci.USA.93(1996) 9090-9095.

[6] M.Chaturvedi, A.Kumar, B.Darnay, G.B.N.Chainy, S.Agarwal, B.B.Aggarwal. Sanguinarine (pseudochelerythrine) is a potent inhibitor of NF-kB activation, IkBa phophorylation and degradation. J.Biol.Chem. 272(1997) 30129-30134.

[7] K.Kumar, S.Dhawan, B.B.Aggarwal. Emodin (3-methyl-1,6,8-trihydroxy anthraquinone) inhibits the TNF-induced NF-kB activation, IkB degradation and expression of cell surface adhesion proteins in human vascular endothelial cells. Oncogene 17(1998) 913-918.

[8] S.K.Manna, A.Mukhopadhyay, N.T.Van, B.B.Aggarwal. Silymarin suppresses TNF induced activation of nuclear transcription factor-kB, c-Jun N-terminal kinase and apoptosis. J.Immunol. 163(1999) 6800-6809.

[9] S.K.Manna, N.K.Sah, R.A.Newman, A.Cisneros, B.B.Aggarwal. Oleandrin suppresses the activation of nucler transcription factor-kB, activator protein-1 and c-Jun N-terminal kinase. Cancer.Res. 60(2000) 3838-3847.

[10] S.K.Manna, A. Mukhopadhyay, B.B.Aggarwal. Resveratrol suppresses TNF-induced activation of nuclear transcription factors NF-kB, Activator protein-1 and apoptosis:

Potential role of reactive oxygen intermediates and lipid peroxidation. J.Immunol. 164(2000) 6509-6519.

[11] K.Ashikawa, S.Majumdar, S.Banerjee, A.C.Bharti, S.Shishodia, B.B.Aggarwal. Piceatannol inhibits TNF-induced NF-kB activation and NF-kB mediated gene expression through suppression of IkappaBalpha kinase and p65 phosphorylation. J.Immunol. 169(2002)6490-6497.

[12] S.Shishodia, S.Majumdar, S.Banerjee, B.B.Aggarwal. Ursolic acid inhibits nuclear factor-kB activation induced by carcinogenic agents through suppression of IkBα Kinase and p65 phosphorylation: Correlation with down regulation of COX-2, MMP-9 and cyclinD1. Cancer Res 63(2003) 4375-4383.

[13] Y.Takada, B.B.Aggarwal. Betulinic acid suppresses carcinogen induced nuclear factor –kB activation through inhibition of Ikba kinase and p65 phosphorylation: Abrogation of Cyclooxygenase-2 and Matrix metalloproteinase-9. J.Immunol.171(2003) 3278-3286.

[14] Y.Takada, B.B.Aggarwal. Flavopiridol inhibits NF-kB activation induced by various carcinogens and inflammatory agents through inhibition of Ikappa Balpha kinase and p65 phosphorylation: Abrogation of CyclinD1, cyclooxygenase-2 and matrix metalloproteinase-9. J.Biol.Chem 279 (2004)4750-4759.

[15] Y.J.Surh. Cancer chemoprevention with dietary phytochemicals. Nat.Rev.Cancer 3(2003)768-780.

[16] A.C.Bharti, B.B.Aggarwal. Nuclear factor-kappa B and cancer: its role in prevention and therapy. Biochem. Pharmacol 64(2002) 883-888.

[17] R.Eferl, E.F.Wagner. AP-1: A double edged sword in tumorigenesis. Nat.Rev.Cancer 3(2003) 859-868.

[18] A.M.Bode, W.Y.Ma, Y.J.Surh, Z.Dong. Inhibition of epidermal growth factor induced cell transformation and AP-1 activation by [6]-gingerol. Cancer. Res 61(2001)850-853.

[19] L.Korutla, J.Y.Cheung, J.Mendelsohn, R.Kumar. Inhibition of ligand induced activation of epidermal growth factor receptor tyrosine phosphorylation by curcumin. Carcinogenesis 16(1995) 1741-1745.

[20] R.L.Hong, W.H.Spohn, M-C Hung. Curcumin inhibits the tyrosine kinase activity of p185neu and also depletes p185neu. Clin.Cancer.Res 5(1999) 1884-1891.

[21] H.Yu, R.Jove. The STATs of cancer. New molecular targets come of age. Nat.Rev. Cancer 4(2004) 97-103.

[22] A.C.Bharti, N.Donato, B.B.Aggarwal. Curcumin (diferuloyl methane) inhibits constitutive and IL-6 inducible STAT3 phosphorylation in multiple myeloma cells. J.Immunol. 171(2003) 3863-3871.

[23] S.Y.Kim, E.J.Park, E.H.Joe, I.Jou. Curcumin suppresses Janus Kinase-STAT inflammatory signaling through activation of src homology-2 domain containing tyrosine phosphatase 2 in brain microglia. J.Immunol. 171(2003) 6072-6079.

[24] S.Anuchapreeda, P.Leechanachai, M.M.Smith, S.V.Ambudkar, P.N.Limtrakul. Modulation of P-Glycoprotein expression and function by curcumin in multi-drug resistant human KB cells. Biochem.Pharmacol 64 (2002) 573-582.

[25] J.Hong, J.D.Lambert, S.H.Lee, P.J.Sinko, C.S.Yang. Involvement of multidrug resistance associated proteins in regulating the cellular levels of (-)-epigallocatechin-3- gallate and its methyl metabolites. Biochem.Biophys.Res.Commun. 310(2003) 222-227.

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

[27] K.Subbaramaiah, A.J.Dannenberg. Cyclooxygenase-2 : A molecular target for chemoprevention and treatment. Trends Pharmacol Sci. 24(2003) 96-102.
[28] M.A.Iniguez, A.Rodriguez, O.V.Volpert, M.Fresno, J.M.Redondo. Cyclooxygenase-2: A therapeutic target for angiogenesis. Trends.Mol.Med 9(2003) 73-78.
[29] S.M.Plummer, A.Kaptein, S.Farro, L.Howells. Inhibition of cyclooxygenase-2 expression in colon cells by the chemopreventive agent curcumin involves inhibition of NF-kB activation via the NIK/IKK signaling complex. Oncogene 18(1999) 6013-6020.
[30] K.Subbaramaiah, W.J.Chung, P.Michaluart, N.Telang, T.Tanabe, H.Inoue, M.Jang, J.M.Pezzuto, A.J.Dannenberg. Resveratrol inhibits cyclooxygenase-2 transcription and activity in phorbol ester-treated human mammary epithelial cells. J.Biol.Chem. 273(1998) 21875-21882.
[31] S. Banerjee, C.Bueso-Ramos, B.B.Aggarwal. Suppression of 7,12 Dimethyl-Benz(a)anthracene –induced mammary carcinogenesis in rats by resveratrol: role of nuclear factor-kB, cyclooxygenase-2 and matrix metalloproteinase-9. Cancer. Res. 62(2002) 4945-4954.
[32] J.Folkman. Fundamental concepts of the angiogenic process. Curr.Mol.Med. 3(2003) 643-651.
[33] M.J.C.Hendrix, E.A.Seftor, A.R.Hess, R.E.B.Seftor. Vasculogenic mimicry and tumor cell plasticity: lessons from melanoma. Nat.Rev.Cancer 3(2003) 411-421.
[34] J.L.Arbiser, N. Klauber, R.Rohan, R.van Leeuwen, M.T.Huang, Fisher, C, E. Flynn, H.R.Byers. Curcumin is an *in vivo* inhibitor of angiogenesis. Mol.Med. 4(1998) 376-383.
[35] E.Brakenhielm, R.Cao, Y.Cao. Suppression of angiogenesis, tumor growth and wound healing by resveratrol, a natural compound in red wine and grapes. FASEB.J. 15(2001) 1798-1800.
[36] S.Lamy, D.Gingras, R.Beliveau. Green tea catechins inhibit vascular endothelial growth factor receptor phosphorylation. Cancer.Res 62(2002) 381-385.
[37] H.W.Chen, S.L.Yu, J.J.Chen, H.N.Li, Y.C.Lin, P.C.Yao, H.Y Chou, C.T.Chien, W.J.Chen, Y.T.Lee, P.C.Yang. Anti-invasive gene expression profile of curcumin in lung adenocarcinoma based on a high throughput microarray analysis. Mol.Pharmacol 65(2004) 99-110.
[38] T.Dorai, Y-C Cao, B.Dorai, R.Buttyan, A.E.Katz. Therapeutic potential of curcumin in prostate cancer-III. Curcumin inhibits proliferation, induces apoptosis and inhibits angiogenesis of LNCaP prostate cancer cells *in vivo*. Prostate 47(2001) 293-303.
[39] S.Reddy, B.B.Aggarwal. Curcumin is a non-competitive and selective inhibitor of phosphorylase kinase. FEBS.Lett 341(1994)19-22.
[40] T.H.Leu, S.L.Su, Y.C.Chuang, M.C.Maa. Direct inhibitory effect of curcumin on src and focal adhesion kinase activity. Biochem Pharmacol 66(2003) 2323-2331.
[41] J.S.Shim, J.H.Kim, H.Y.Cho, Y.N.Yum, S.H.Kim, H.J.Park, B.S.Shim, S.H.Choi, H.J.Kwon. Irreversible inhibition of CD13/AminopeptidaseN by the anti-angiogenic agent curcumin. Chem Biol 10 (2003) 695-704.
[42] C.J.Sherr. Cancer cell cycles revisited.. Cancer Res 60(2000) 3689-3695.
[43] A. Mukhopadhyay, S.banerjee, L.J.Stafford, C.X.Xia, M.Liu, B.B.Aggarwal. Curcumin induced suppression of cell proliferation correlates with down regulation of cyclin D1 expression and CDK4-mediated retinoblastoma protein phosphorylation. Oncogene. 21(2002) 8852-8861.
[44] Z.Estrov, S.Shishodia, S.Faderl, D.Harris, Q.Van, H.M.Kantarjian, M.Talpaz,

B.B.Aggarwal. Resveratrol blocks interleukin-1[beta]-induced activation of the nuclear transcription factor NF-[kappa]B, inhibits proliferation, causes S-phase arrest and induces apoptosis of acute myeloid leukemia cells. Bllod. 102(2003) 987-995.

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

[46] M.J.Park, E.H.Kim, I.C Park, H.C.Lee, S.H.Woo, J.Y.Lee, Y.J.Hong, C.H.Rhee, S.H.Choi, B.S.Shim, S.H.Lee, S.I.Hong. Curcumin inhibits cell cycle progression of immortalized human umbilical vein endothelial cells by upregulating cyclin dependent kinase inhibitors p21WAF1/CIP1, p27Kip1 and p 53. Int.J.Oncol 21(2002) 379-383.

[47] S.Gupta, T.Hussain, H.Mukhtar. Molecular pathway for (-)- epigallocatechin-3-gallate induced cell cycle arrest and apoptosis of human prostate carcinoma cells. Arch.Biochem.Biophys. 410(2003) 177-185.

[48] N.Kuwajerwala, E.C.Fuentes, S.Gautam, M.Menon, E.R.Barrack, G.P.Reddy. Resveratrol induces prostate cancer cell entry into S-phase and inhibits DNA synthesis. Cancer Res. 62(2002) 2488-2492.

[49] A.Harbottle, A.K.Daly, K.Atherton, F.C.Campbell. Role of Glutathione –S-Transferase Pi, P-Glycoprotein and multidrug associated protein-1 in acquired doxorubicin resistance. Int.J.Cancer 92(2001) 777-783.

[50] M.L.Iersel, J.P.Plomen, I.Struik, C.van Amersfoort, A.E Keyzer, J.G.Schefferlie, P.J.van Bladeren. Inhibition of Glutathione-S-Transferase activity in human melanoma cells by alpha and beta unsaturated carbonyl derivatives: effects of acrolein, cinnamaldehyde, citral, crotonaldehyde, curcumin, ethacrynic acid and trans-2 hexanol. Chem.Biol.Interact 102(1996) 117-132.

[51] A.Duvoix, F.Morceau, S.Delhalle, M.Schmitz, M.Schnekenburger, M.M.Galteau, M.Dicato, M.Diedrich. Induction of apoptosis by curcumin:mediation by glutathione S-transferase P1-1 inhibition. Biochem. Pharmacol 66(2003) 1475-1483.

[52] Y.Ogiso, A.Tomida, S.Lei, S.Omura, T.Tsuruo. Proteasomal inhibition circumvents solid tumor resistance to topoisomerase II directed drugs. Cancer Res 60 (2000) 2429-2434.

[53] N.R.Jana, P.Dikshit, A.Goswami, N.Nukina. Inhibition of proteasomal function by curcumin induces apoptosis through mitochondrial pathway. J.Biol.Chem 279(2004) 11680-11685.

[54] S.Fulda, K.M.Debatin. Sensitization for tumor necrosis factor-related apoptosis inducing ligand induced apoptosis by the chemopreventive agent resveratrol. Cancer.Res 64(2004) 337-346.

[55] D.Chendil, R.S.Ranga, D.Meigooni, S.Sathishkumar, M.M.Ahmed. Curcumin confers radiosensitizing effect in prostate cancer cell line PC-3. Oncogene. 23(2004)1599-1607.