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Inhibition of Tumor Promotion through Blocking Signal Transduction

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CONTENTS

ABSTRACT

Jen-Kun Lin and Shern-Fwu Lee (1995) Inhibition of tumor promotion through blocking signal transduction. *Zoological Studies* **34**(2): 67-81. Cancer chemoprevention that involves the introduction of synthetic or natural materials, especially polyphenolic compounds, into the diet is profoundly attracting the attention of scientists and clinicians worldwide. Although multi-stage carcinogenesis has been established, the promotion stage is generally recognized as the most important step which produces reactive oxygen species (ROS), activates protein kinase C (PKC) activity, elevates mRNA and protein levels of ornithine decarboxylase (ODC), and increases transcription and translation of *c-jun*/AP-1, etc., by the stimulation of promoters including 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA). All these tumor markers can be used to assess the inhibitory effects of natural polyphenolic compounds against TPA-promoting actions. The published data indicate that several phenolic compounds exhibit anti-tumor activity after parenteral application in standard initation-promotion animal models. Curcumin, the major component of *Curcuma longa*, exhibits multiple biochemical and chemopreventive actions. Recent studies indicate that curcumin has inhibitory effects on PKC, ODC, *c-jun*/AP-1 activities, and 8-hydroxydeoxyguanosine (8-OH-dG) formation induced by TPA. These findings suggest that certain polyphenolic compounds might inhibit the processes of tumor promotion through blocking cellular signal transduction.

Key words: Polyphenols, Curcumin, Protein kinase C (PKC), Anti-oxidant, Chemoprevention.

INTRODUCTION

The mechanisms of carcinogenesis involve multiple stages of biochemical and molecular alterations in target cells. The process of skin carcinogenesis involves the stepwise accumulation of genetic changes ultimately leading to malignancy (Digiovanni 1992). There are three main steps. (i) Initiation, the first step in multistage skin carcinogenesis, involves carcinogen-induced genetic

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changes; this stage appears to be rapid and irreversible. It is presumed to involve an irreversible modification of DNA, perhaps resulting in one or more mutations (reviewed by Slaga and Fischer 1983, Yuspa and Poirer 1988, Slaga 1989). (ii) Promotion, a much slower stage of carcinogenesis, is believed to involve the selective clonal expansion of initiated cells into visible clonal outgrowths (papillomas) by one or a combination of several mechanisms (reviewed by Parkinson 1985, Yuspa and Poirer 1988, Slaga 1989). These changes are believed to result from epigenetic mechanisms such as activation of the cellular receptor, protein kinase C, by some classes of tumor promoters. (iii) The process of tumor progression appears to involve the accumulation of additional genetic changes in cells comprising skin papillomas (Hennings et al. 1983, O'Connell et al. 1986, Aldaz et al. 1989, Brenner and Balmain 1990, Bianchi et al. 1990 1991).

As mentioned above, cancer is a group of diseases with multiple stages of pathological changes and is presently the leading cause of death in developed nations. While cancer is difficult to cure, chemoprevention of cancer that involves introducing synthetic or natural micronutrients into the diet of animals is profoundly attracting the attention of many scientists and clinicians worldwide.

Because the initation step of carcinogenesis is irreversible and difficult to forestall, most efforts are focused on the prevention of the tumor promotion step of carcinogenesis.

Epidemiological surveys and experimental studies provide evidence that environmental factors, including dietary substances, play a major role in the development of cancer (Doll and Peto 1981). Minor dietary constitutents have also received considerable attention in carcinogenesis and chemoprevention of cancer (Ames 1983, Wattenberg 1983, Newmark 1984). The nature of the interaction between these various minor dietary components and the final outcome, culminating in neoplasia, remains unclear. Besides naturally occurring anticarcinogenic substances, the diet may contain genotoxic agents such as polycyclic aromatic hydrocarbons, nitroso-amines etc., and therefore, it is ultimately the balance between these two groups of substances which determines the development of neoplasia.

Naturally occurring phenolic compounds of the plant kingdom are widespread in the human diet and are found in many plants traditionally used because of their vascular protective properties. A wide variety of the biological activities of these plants, including anti-inflammatory, antimicrobial, anthelminthic, anti-allergic, hepato-protective, antithrombotic, antihormonal (versus growth hormone secretion), and antineoplastic activites (Cody V et al. 1986 1988, Middleton 1992), have been attributed to the presence of flavonoids and phenolic compounds. However, some flavonoids possess cytotoxic, mutagenic and/or carcinogenic activities (Digiovanni 1992, Sahu and Washington 1992, Brown 1980, Tanaka et al. 1987, Czeczot et al. 1990).

Environmental stimulators or promoters trigger cellular proliferation by a number of cascading signal transductions. There are mediated through binding to specific membrane receptors that transmit signals from the membrane and cytoplasm to the nucleus. This report describes the molecular mechanisms of signal transduction and discusses the relationship between the suppression of tumor promotion and the blocking of signal transduction.

MOLECULAR MECHANISM OF TUMOR PROMOTION BY TPA

TPA activates phosphorylation of serine/threonine

The protein kinase C (PKC) has been considered as the key enzyme for signal transduction in the processes of cell proliferation and differentiation. PKC is activated by diacylglycerol (DAG), phorbol esters and other tumor promoters in vitro as well as in vivo (Kishimoto et al. 1980, Castagna et al. 1982). In response to extracellular signals such as growth factors, hormones and neurotransmitters (Berridge 1987), activated PKC is believed to bypass a normal cellular mechanism(s) for regulating cell proliferation (Berridge and Irvine 1984, Nishizuka 1986 1989, Pandiella et al. 1989). PKC, the widely distributed protein kinase in mammalian tissues, especially brain, is cyclic nucleotide- and calmodulin-insensitive but Ca^{2+}- and phospholipid-dependent (Nishizuka 1986). TPA also has been found to increase DAG levels in several cell systems, suggesting possible activation of phospholipase C (Mufson 1984).

DAG is one of two immediate products of plasma membrane phosphatidylinostiol 4,5-bisphosphate (PIP_2) hydrolysis. DAG remains in membranes and initiates the activation of a serine- and threonine-specific protein kinase, PKC. PKC requires high levels of Ca^{2+} and a phospholipid

(particularly phosphatidylserine) for maximal activity. DAG dramatically increases the affinity of PKC for Ca^{2+}, thereby rendering the enzyme fully active without a net increase in resting Ca^{2+} concentrations (Nishizuka 1986). Several observations strongly suggest that PKC plays an important role in the cell transformation process (Fry et al. 1985, Wolfman and Macara 1987). PKC is the major receptor for tumor-promoting phorbol esters, such as TPA (Parker et al. 1984). TPA activates PKC directly by replacing the requirement for DAG (Castagna et al. 1982, Nishizuka 1988).

PKC is a lipid-activated kinase which is known to play an important role in a wide variety of fundamental cellular processes, especially in transmembrane regulation of signal transduction. It has also been found to induce many cellular responses including cell proliferation, differentiation, gene expression, and tumor promotion (Nishizuka 1988 1989). PKC also acts as a positive regulator in the transition from the quiescent to a proliferative state of aortic smooth muscle cells (Ohmi et al. 1990).

PKC phosphorylates certain cytoplasmic proteins on serine and threonine residues; their possible role in the mitogenic pathway, however, remains unclear. Despite the fact that PKC is activated in response to certain growth factors and tumor promoters, this enzyme seems to be dispensable for growth stimulation. One of the major actions of PKC appears to be the phosphorylation of cell surface receptors, a function which, in general, appears to serve as a feedback or down-regulatory mechanism, due to an increased rate of PKC degradation (Rozengurt et al. 1983, Mizuguchi et al. 1988).

Among the substrates for PKC are a number of growth factor receptors. The EGF receptor is phosphorylated at Thr-654, which causes a decrease in both the ligand binding capacity and in the tyrosine kinase activity (Cochet et al. 1984, Hunter et al. 1984, Davies and Czeck 1985). Phosphorylation of the insulin receptor by protein kinase C also causes a decrease in the tyrosine kinase activity (Bollag et al. 1986). The implications of these observations are not clear; they may indicate the existence of a negative feedback control mechanism.

Growth stimulation is accompanied by a cytoplasmic alkalinization (Pouysségur et al. 1986) which is probably mediated by PKC-induced phosphorylation of the Na^+/H^+ carrier protein, leading to an efflux of H^+ ions and an influx of Na^+ ions. Cytoplasmic alkalinization may serve a permissive role in mitogenicity, since cells do not grow if the cytoplasm has an acidic pH (Pouysségur et al. 1984).

TPA induces oxidative stress

TPA with promoting activity produces modifications of several biosynthetic products and enzyme activities. It causes an increase in phospholipid turnover (Suss et al. 1971, Balmain and Hecker 1974) and in prostaglandin accumulation (Fürstenberger and Marks 1980, Verma et al. 1980). Tumor promoter treatments lead to a decrease in the glucocorticoid receptor (Davidson and Slaga 1982 1983, Warren et al. 1991), a decrease in epidermal histidase (Colburn et al. 1975) and histidine decarboxylase (Watanabe et al. 1981), modification of epidermal keratins and keratin expression (Balmain 1976, Nelson and Slaga 1982, Schweizer and Winter 1982, Roop et al. 1983), increased synthesis and phosphorylation of histones (Raineri et al. 1973 1978, Link and Marks 1981), and a large induction of ornithine decarboxylase (ODC) (O'Brien et al. 1975), the rate-limiting enzyme in polyamine biosynthesis. As a result of the induction of epidermal ODC, the levels of putrescine and, especially, spermidine, in the epidermis become elevated (O'Brien 1976, Kruszewski and DiGiovanni 1988). The elevated ratio of spermidine: spermine that occurs after TPA treatment is tightly linked to the DNA synthesis response induced by this promoter (Astrup and Paulsen 1981, Kruszewski and DiGiovanni 1988).

Evidence is emerging that generation of free radicals may be involved in the skin tumor-promoting actions of several classes of promoting agents (Cerutti 1985, Kensler and Taffe 1986), including the phorbol esters. For example, TPA stimulates production of O_2^- and possibly other free radicals by polymorphonuclear leukocytes (PMNs) (Repine et al. 1974, Troll et al. 1982), by activating the ubiquitous membrane-bound NADPH oxidase system (Segal and Abo 1993). Fischer et al. (1986) demonstrated the production of O_2^- in isolated epidermal cells by active, but not by inactive, phorbol ester analogs using a chemiluminescence assay and also the suppression of O_2^- by antipromoters (Fischer and Adams 1985).

More direct evidence for the involvement of free radicals in tumor promotion comes from studies with free radical-generating compounds such as the organic peroxides and anthrones. Benzoyl peroxide and other organic peroxides are effective skin tumor promoters in sensitive mouse stocks

and strains (Slaga et al. 1981 1983). Benzoyl peroxide and TPA can cause differential oxidative stress (Durán and Rey 1991). In addition, structure-activity studies for tumor-promoting activity with anthrone derivatives strongly suggest that oxidation at C_{10} of the molecule, with subsequent generation of free radical intermediates, is crucial for their tumor promoting actions (DiGiovanni et al. 1987 1988).

A number of additional studies have also demonstrated that free radical generating systems such as xanthine/xanthine oxidase can mimic the effects of phorbol esters on enhancing cell transformation in cultured C3H10T1/2 (Zimmerman and Cerutti 1984), mouse epidermal JB6 cells (Cerutti 1987) and SENCAR mouse skin (Pence and Reiners 1987, Reiners et al. 1987). Antioxidants are effective inhibitors of chemical carcinogenesis and skin tumor promotion, which further supports a role for free radicals in tumor promotion (reviewed in Slaga and DiGiovanni 1984, Kensler and Taffe 1986). TPA, benzoyl peroxide and anthralin also have been shown to decrease the activities of SOD and catalase in mouse epidermis, shortly after their application (Solanki et al. 1981, Slaga et al. 1983, Reiners et al. 1991a,b).

Perchellet et al. (1986) have demonstrated that a wide variety of tumor promoters decrease the ratio of reduced (GSH)/oxidized (GSSG) glutathione in mouse epidermal cells treated with a variety of promoting agents. TPA was also found to stimulate a rapid, transient increase in GSH-peroxidase followed by a prolonged depression in the activity of this enzyme (Perchellet et al. 1986 1987). These changes presumably reflect the induction of a prooxidant state in the epidermal cells by TPA and other types of tumor promoters. More recent studies by Perchellet and coworkers (Perchellet et al. 1988, Perchellet and Perchellet 1989) have demonstrated that tumor promoters can induce the production of hydroperoxides in mouse epidermal homogenates. Taken together, all these studies support the role for active oxygen species in tumor promotion by certain types of compounds (Witz 1991).

The exact biochemical and molecular mechanism(s) whereby certain free radical intermediates might lead to the process of tumor promotion remain unknown. Both genetic as well as epigenetic mechanisms have been postulated (reviewed in Cerutti 1985 1991, Kensler and Taffe 1986, Perchellet and Perchellet 1988). Cerutti (1985 1991) has proposed that the induction of a prooxidant state leads to altered gene expression through activation of poly (ADP-ribose) synthetase and subsequent ADP-ribosylation of chromosomal proteins. The activation of poly (ADP-ribose) synthetase is proposed to result from oxidant-induced DNA strand breaks and increased levels of oxidized pyridine nucleotides.

Because of the reactivity of unsaturated and sulfur-containing molecules with free radicals, proteins containing such functional groups will be susceptible to free radical-mediated amino acid modification (Pryor 1976, Freeman and Crapo 1982). A variety of cellular proteins and/or enzymatic pathways could thus be changed leading to altered phenotypic characteristics of a cell (reviewed by Freeman and Crapo 1982, Kensler and Taffe 1986, Cerutti 1991). In this regard, anthralin is known to inhibit glucose-6-phosphate dehydrogenase (G6PDH) in vitro as well as in epidermal preparations of human skin (Hammar 1970, Cavey et al. 1982). The ability of anthralin to inhibit G6PDH in vitro correlates with its ability to undergo oxidation to 1,8-dihydroxyanthraquinone and the anthralin dimer (Cavey et al. 1982).

PKC may be regulated to a certain extent by direct oxidation. In this regard, mild oxidation of the regulatory domain of PKC may eliminate the requirement for Ca^{2+} and phospholipid for its activation (Gopalakrishna and Anderson 1989). Furthermore, H_2O_2 has been reported to alter the distribution of PKC in JB6 cells (Larsson and Cerutti 1989), and benzoyl peroxide to alter PKC distribution in mouse epidermis (Donnelly et al. 1987). The activities of other proteins may also be regulated to a certain extent directly by redox reactions including: *c-fos* (Abate et al. 1990, Amstad et al. 1992); *c-jun* (Abate et al. 1990); a tyrosine kinase located in the endoplasmic reticulum (Bauskin et al. 1991); GSSG reductase and Mg^{2+}-dependent, Na^+/K^+-stimulated ATPase (Thor and Orrenius 1980); and possibly many others.

During oxidative stress, most cells suffer from compromised energy homeostasis due to uncoupling of oxidative phosphorylation, decreased levels of GSH, and decreased levels of NADPH as a result of its utilization by the GSH-peroxidase redox cycle leading to the subsequent release of intracellular Ca^{2+} stores (Trump and Berezesky 1987, Reed 1990). The resulting increase in intracellular Ca^{2+} concentrations could lead to the activation of a cascade of biochemical pathways (reviewed in Trump and Berezesky 1987, Reed 1990). It is interesting to note that PKC and Ca^{2+} are believed to act synergistically in stimulating various cellular responses (Nishizuka 1983, Berridge and Irvine 1984). In addition, release of intracellular Ca^{2+}

has been postulated to account for the phosphorylation of the ribosomal protein, S6, in cells treated with H_2O_2 (Larsson and Cerutti 1988) through an intermediate Ca^{2+}/calmodulin-sensitive kinase (Larsson and Cerutti 1988, Cerutti 1991). The reported down-regulation of epidermal PKC by anthrone tumor promoters (Imamoto et al. 1991) could result from the activation of Ca^{2+}-dependent proteases (Mellgren 1987) through a similar mechanism.

Despite the fact that tumor promoters are not mutagenic in different tested systems (Lankas et al. 1977, Thomson et al. 1980) and do not bind covalently to DNA, there is cumulative evidence that TPA induces alterations at the genetic level which could result in toxicity and/or alterations in gene expression. It has been shown that TPA induces replication of endogenous and integrated viral genomes (Zur-Hausen et al. 1979, Imbra and Karin 1986, Fischer et al. 1987), enhances sister chromatid exchanges in hamster fibroblasts (Kinsella and Radman 1978) and induces DNA single-strand breaks in human leukocytes (Birnboim 1982) and mouse keratinocytes (Dutton and Bowden 1985, Hartley et al. 1985). Moreover, TPA treatment induces and/or enhances numerical and structural chromosomal aberrations in different system, such as yeast (Parry et al. 1981), human leukocytes (Emerit and Cerutti 1981, Callen and Ford 1983) and mouse keratinocytes (Dzarlieva and Fusening 1982, Fusenig and Dzarlieva 1982, Dzarlieva-Petrusevska and Fusening 1985). Recently it has been reported that TPA also induced cytogenetic changes in cultures of primary mouse keratinocytes (Petrusevska et al. 1988).

Additionally, recent reports indicate that oxidized DNA bases were found in TPA-induced granulocytes or mouse skin (Floyd et al. 1986, Wei and Frenkel 1991), and human female breast cancer (Malins and Haimanot 1991). 8-Hydroxydeoxyguanosine (8-OH-dG), which is a hydroxyl radical adduct of dG at the C-8 position (Steenken 1989) and may be an important factor in carcinogenesis (Floyd 1990a,b), causes misreading of DNA polymerase (Kuchino et al. 1987) when present in the DNA template. A change of DNA bases induced by TPA is one of the events required for tumor promotion (Frenkel 1989).

TPA activates early response genes

To understand the mechanisms by which growth factors or stimulators modulate cell growth, it is necessary to define the complex series of events that ensue following mitogen receptor binding. Among these events are the rapid generation of second messengers in the cytosol and plasma membrane. In turn, a set of immediate-early response genes are activated, whose induction does not require new protein synthesis (Lau and Nathans 1985 1987).

An important subset of these genes encodes transcriptional factors. These include: *c-fos* (Verma and Graham 1987), *fra-1* (Cohen and Curran 1988), and *fos B* (Zerial et al. 1989); three distinct genes (members of the EGR family) designated *EGR-1* (Christy et al. 1988), *EGR-2* (Chavrier et al. 1988, Joseph et al. 1988), and *EGR-3*, all of which encode proteins with Cys2-His2 zinc finger motifs; a member of the steroid and thyroid hormone receptor family (Hazel et al. 1988, Milbrandt 1988); and *c-jun* (Bohmann et al. 1987, Lamph et al. 1988, Ryder and Nathans 1988, Ryseck et al. 1988) and its closely related genes (Nakabeppu et al. 1988).

The importance of these genes is that, by virtue of their structure and induction kinetics, they are likely to play broad roles as "third messengers" by coupling early biochemical processes to long-term changes in gene expression that are required to modulate cell growth. Furthermore, these gene products also participate as parts of a regulatory cascade in other cellular processes such as in differentiation and proliferation (Verma and Graham 1987, Sukhatme et al. 1988). A major challenge ahead is to define the mechanisms by which specific intracellular second messengers affect expression of one or more members of this subgroup of "immediate-early" genes and to indentify target genes and/or proteins with which products of these genes interact.

The addition of TPA to cells has been shown to induce the transcriptional activation of several proto-oncogenes, including *c-fos* (Greenberg and Ziff 1984, Kruijer et al. 1984), *c-myc* (Kelly et al. 1983), *c-sis* (Colamenici et al. 1986) and *c-jun* (Bohmann et al. 1987, Lee et al. 1987).

C-fos and *c-myc* are implicated in transregulation and may complex with cellular transcription factors (Sassone-Corsi et al. 1988). In particular, *c-jun*, known to be a member of the AP-1 family of transcription factors (Rauscher et al. 1988) can be directly enhanced by *fos* proteins to bind to the AP-1 site by means of heterodimer formation of *fos* and *jun* proteins facilitated by a "leucine zipper" sequence (Kouzarides and Ziff 1988, Schuermann et al. 1989).

When cells are stimulated, protein products of the early responsed genes act as transcription

factors and thereby regulate the expression of a variety of genes by way of specific regulatory domains (Nose et al. 1991). Activation of the early response genes does not require protein synthesis and is usually of a transient nature. The *c-fos* and *c-jun* protein products form conjugates that participate in the activator protein (AP-1) transcription factor complex present in promoter regions of numerous genes (Angel 1987). The early response gene *c-myc* is induced by activated oxygen modulates cell proliferation and differentiation (Crawford et al. 1988, Dang et al. 1989).

Elevated expression of gene transcription induced by TPA is among the events required for tumor promotion (Bernstein and Colburn 1989). Analysis of these genes reveals the highly conserved motif 5'-TGASTCAG-3' (where S is cytosine or guanine), conferring TPA inducibility (Angel et al. 1987, Lee et al. 1987). This TPA-responsive element, referred to as TRE, also serves as the binding site for the AP-1 family of transcription factors. The proto-oncogene product *c-jun*/AP-1 represents one member of the AP-1 proteins. Enhanced binding of *c-jun*/AP-1 to the TRE in TPA-stimulated cells has been associated with the increased transcription of different responsive genes (Chiu et al. 1988, Angel et al. 1988, Bohmann and Tjian 1989). On the other hand, glucocorticoid hormones inhibit phorbol ester tumor promotion and inflammation and drastically decrease collagenase gene expression (Jonat et al. 1990, Yang-Yen et al. 1990, Schüle et al. 1990). A TRE site within the collagenase promoter was found responsible for mediating the inducibility by TPA and inflammatory agents. The mechanism of interference is from repression of *c-jun*/AP-1 activity by the glucocorticoid receptor. Therefore, activation of *c-jun*/AP-1 is probably crucial in transmitting the tumor-promoting signals from the extracellular environment to the nuclear transcriptional machinery.

ANTI-CARCINOGENESIS OF SEVERAL NATURAL POLYPHENOLIC COMPOUNDS

Research in recent years has strengthened the association between reduced cancer rates and consumption of vegetable or natural polyphenolic compounds or flavonoids. The relatively nontoxic flavonoids are distributed ubiquitously in the leaves and stems of vascular plants. Several of these have been shown to inhibit various TPA-induced phenomena, such as the increased activity of epidermal ornithine decarboxylase (ODC) (Kato et al. 1983, Nakadate et al. 1984), protein kinase C (PKC), and protein phosphorylation (Gschwendt et al. 1983, Fujiki et al. 1984); all of which are believed to represent nonspecific markers of tumor promotion.

Green tea is the second (next to water) most popular and commonly consumed beverage in the world, especially in eastern countries. In prior studies, the polyphenolic fraction isolated from green tea (GTP) exerted antigenotoxic effects in various mutagenic test systems (Wang et al. 1989a). Topical application or oral feeding of GTP in drinking water protected against polycyclic aromatic hydrocarbon-induced skin tumor initiation and complete carcinogenesis in SENCAR, BALB/c and A/J mice (Khan et al. 1988, Wang et al. 1989b 1992b), UVB radiation-induced photocarcinogenesis in SKH-1 hairless mice (Wang et al. 1991a 1992c), and topical application to mouse skin inhibited TPA-induced epidermal ODC activity in a dose-dependent manner (Cheng et al. 1989, Agarwal et al. 1992). In addition, GTP suppressed the increase of 8-hydroxydeoxyguanosine (8-OH-dG) levels in A/J mouse lung DNA (Xu et al. 1992). Among several other naturally occurring polyphenols studied, (−)-epigallocatechin gallate (EGCG), a major polyphenolic catechin, exerted the maximum inhibition (Agarwal et al. 1992, Xu et al. 1992).

Glycyrrhizin (GL) and 18α- and 18β-glycyrrhetinic acids (18α-GA and 18β-GA) are the major polyphenolic constituents present in licorice. 18α-GA and 18β-GA exhibited inhibitory effects against mutagenicity in *Salmonella typhimurium* and skin tumor-initiating and skin tumor-promoting activities in SENCAR mice (Wang et al. 1991b). The chronic oral feeding of GL in drinking water resulted in significant protection against skin tumor-initiating activity of DMBA in SENCAR mice (Agarwal et al. 1991).

The extract of licorice showed protective effects against benzo[a]pyrene- and N-nitrosodiethylamine-induced lung and forestomach tumorigenesis in A/J mice (Wang et al. 1992a). In addition, GA inhibited the specific binding of TPA to mouse epidermal membrane fractions in a dose- and time-dependent manner, but GL had no inhibitory effect (Kitagawa et al. 1986). The inhibitory effect of GA on TPA binding to the membrane receptor may play a role in its in vivo antitumor-promoting activity.

Curcumin, the major yellow pigment and active principle of turmeric, obtained from powdered rhizomes of the plant *Curcuma longa* Linn., is commonly used as a coloring agent in food, drugs

and cosmetics. It exhibited antioxidant, antimutagenic, and anticarcinogenic activity and was a potent inhibitor of TPA-induced tumor promotion in mouse skin (Huang et al. 1992, Polasa et al. 1992, Nagabhushan et al. 1992). Several vitamins, their precursors or derivatives have shown antioxidant or oxygen-radical absorbance capacity in vivo and in vitro (Cao et al. 1993, Palozza and Krinsky 1992). Collectively, many natural polyphenolic compounds have the ability to scavenge oxygen radicals to inhibit transformation by activation of TPA.

CURCUMIN INHIBITS TUMOR PROMOTION THROUGH BLOCKING SIGNAL TRANSDUCTION

Spices and condiments are widely used in Indian cuisine. Turmeric (*Curcuma longa* Linn.) is a spice regularly used in India and China for its coloring, flavoring and medicinal properties. Curcumin has been isolated and identified as the major active component and yellow pigment in turmeric, and this substance has also been used as a spice and food preservative agent (Bacon 1979, Govindarajan 1980). But when given orally, most of the curcumin is excreted via the feces so it may be that curcumin is poorly absorbed by the gastrointestinal tract. Curcumin is a compound that shows promise as a chemopreventive agent.

Previous pharmacological studies have demonstrated the following properties of curcumin from *Curcuma longa* (Ammon and Wahl 1990): anti-inflammatory activity, antibacterial effects, antifungal effects, anticoagulant activity and, anti-fertility action. Administration of 0.1mg/day of alcohol extract to immature male rats for ten days resulted in significant decrease in testes weight and testosterone concentration. Rats fed with curcumin and cholesterol in their diet had only one-half to one-third of the serum and liver cholesterol levels compared to the control group receiving cholesterol alone. In another study the effect of extract of *Curcuma longa* caused an increase of HDL-cholesterol/total cholesterol ratio (Ammon and Wahl 1990).

Curcumin has also shown many biochemical and pharmacological activities. These include antithrombotic (Srivastava et al. 1985) and antimutagenic effects in vitro (Nagabhushan et al. 1987), anti-oxidant properties by its capacity to inhibit lipid peroxidation in rat brain homogenate (Sharma 1976), anti-mutagenic effects in vivo against carcinogens such as benzo[a]pyrene, methylcholanthrene (Polasa et al. 1991), 4-nitroquinoline 1-oxide, and cigarette smoke (Camoirano et al. 1994), anti-cancer and anti-TPA-induced ODC (ornithine decarboxylase) activity in animals (Huang et al. 1988), and cell cultures (Kuttan et al. 1985). In addition, curcumin has even shown protection to DNA against TDS (Twigs-dry leaves smoke condensate) and TPA as clastogenic agents (Shalini and Srinivas 1990). It showed significant anti-initiation (Mehta and Moon 1991, Huang et al. 1992), and anti-promotion (Huang et al. 1988, Han 1994) activities in animal systems.

Curcumin has also been shown to inhibit nitrite-induced methemoglobin formation (Unnikrishnan and Rao 1992), resist mutagenicity of benzo[a]pyrene-induced genotoxicity and carcinogenicity (Azuine et al. 1992), significantly reduce the level of benzo[a]pyrene-DNA adducts (Mukundan et al. 1993, Lahiri and Bhide 1993), and inhibit chemical carcinogenesis in mouse skin (Azuine and Bhide 1992), forestomach (Nagabhushan and Bhide 1987, Azuine and Bhide 1992) and mammary glands (Nagabhushan and Bhide 1987) as well as hamster buccal pouch and rat mammary glands (Azuine et al. 1992).

Curcumin inhibited $\Delta 5$ desaturase, which catalizes the desaturation of dihomo-γ-linolenic acid to arachidonic acid (Shimizu et al. 1992). It showed significant chemopreventive (i.e., anti-cancer) effects in rodents (Boone et al. 1992), reduced the urinary excretion of mutagens in smokers (Polasa et al. 1992) and inhibited type 1 human immunodeficiency virus long terminal repeat-directed gene expression and virus replication (Li et al. 1993).

Regarding molecular mechanisms of anti-promotion, curcumin inhibited TPA-induced PKC activity in the particulate fraction (Liu et al. 1993), inhibited TPA- induced increases in epidermal ornithine decarboxylase (ODC) activity by inhibiting the synthesis of and/or enhancing the breakdown of ODC mRNA (Lu et al. 1993), inhibited TPA-induced tumor promotion by functioning as an hydroxyl free radical scavenger to prevent 8-hydroxydeoxyguanosine (8-OH-dG) formation within the DNA molecule (Shih and Lin 1993), and has shown it can down-regulate transcriptional activation of the *c-jun* gene by TPA and can block the increase in TRE binding activity of the *c-jun*/Ap-1 protein (Huang et al. 1991).

In addition, curcumin markedly inhibited the monocyte chemo-attractant JE gene expression in osteoblastic MC3T3-E1 cells and inhibited monocyte chemotactic activity induced by cytokine (Hanazawa et al. 1993).

DISCUSSION

Although the mechanisms of TPA-dependent promotion and the progression of integumentary papilloma to squamous cell carcinoma (SCC) are completely unknown, reactive oxygen species (ROS), superoxide anion, hydrogen peroxide and hydroxyl radical have been circumstantially implicated in these processes. Regulation of the activity of superoxide-generating enzymes (xanthine oxidase (XO) and NADPH oxidase) and antioxidant enzymes (i.e. superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX)) during the promotion and progression stages of induction by TPA are involved in ROS metabolism. Regulation may also be affected by pretreatment with polyphenolic compounds prior to TPA. Whether or not the cytosolic component of NADPH oxidase, p47, is phosphorylated and tumor marker, ODC, is elevated during treatment with natural polyphenolic compounds deserve further investigation.

In addition, the most important characteristic of the first-stage tumor promoters seems to be an induction of heritable DNA damage and thus oxidation of DNA bases is capable of causing transformation of cells (Frenkel and Chrzan 1987, Frenkel 1989). Further, two thymidine derivatives, 5-hydroxymethy1-2'-deoxyuridine (HMdU) and thymidine glycol (dTG), a guanosine derivative, 8-OH-dG and an adenine derivative, 8-hydroxyadenine, have been identified by GC-MASS in DNA that was exposed to TPA (Frenkel and Chrzan 1987, Frenkel 1989).

During the last few years, the number of molecules which have been identified as involved in signal transduction has increased tremendously and include phosphatidylinositol (PI), PKC, protein tyrosine kinase (PTK), receptor protein tyrosine kinase (RPTK), and several growth factors such as EGF, PDGF, and CSF. Many oncogene and tumor supressor gene products also belong to this category. There is considerable evidence that these signal molecules may operate as coordinated and interacting systems that communicate with and control each other by what is called "cross-talk". Presumably, disruption of information flow along these pathways would alter normal cell growth. Because many oncogene and tumor supressor gene products function in signal transduction mechanisms, these particular loci offer genuine hope for selective blocking of cancer cell growth. This would eventually provide a molecular mechanism for cancer chemoprevention in the future. A tentative molecular mechanism of anti-promotion through blocking signal transduction is illustrated in Fig. 1.

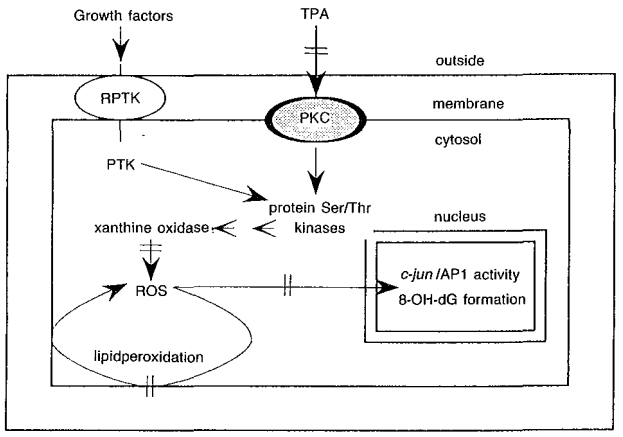

Fig. 1. Mechanisms of anti-promotion by curcumin. RPTK: receptor protein tyrosine kinase, PTK: protein tyrosine kinase, TPA: 12-*O*-tetradecanoylphorbol-13-acetate, PKC: protein kinase C, ROS: reactive oxygen species, AP1: activator protein, 8-OH-dG: 8-hydrodeoxyguanosine, ‡: blocked sites by curcumin.

According to our studies, formation of several oncogenes (*c-jun, c-fos*) was induced by the TPA-biomimetic material H_2O_2, with the hydroxyl radical being the active inducer. It has also been demonstrated that many PTKs may take part in the induction (unpublished observation). Hence, in the future we will try to identify the expression of oncogenes and PTK activity after pretreatment with polyphenolic compounds. A two-stage (initiation and promotion) model of chemical transformation of mouse embryonic fibroblasts is being used to elucidate the inhibition of tumor promotion in vitro. The antioxidant activity of these polyphenolic compounds will be further assessed. These studies will hopefully tell us which compounds have anti-tumor activity and can be used in cancer chemoprevention.

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經由阻斷訊息傳導來抑制腫瘤之促進作用

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將合成的或天然物應用在癌症之化學預防上，尤其是食物中的多酚類化合物更是引起全世界科學家及臨床學者的矚目。雖然多步驟致癌作用機制已被確立，但是在促進作用這個階段中一般被認爲是造成腫瘤其中最重要的步驟，這是因爲在此階段中若是像細胞受到巴豆酚(TPA)這種促進劑刺激時，便會有產生反應性或活化性的氧物質、活化蛋白激酶C的活性、提升鳥胺酸脫羧酶的信使核糖核酸及蛋白量、以及增加*c-jun*/AP-1的轉錄及轉譯作用等等現象。我們可利用這些腫瘤標記來評估天然多酚類化合物對抗巴豆酚(TPA)促進作用的抑制效果。在已發表的資料中指出在標準的啟始作用及促進作用的動物模式下，數種多酚類化合物具有抗腫瘤生成的作用；像薑黃中最主要的成份薑黃素便是具有許多種生化上及化學預防上的作用。最近的研究報告更指出薑黃素具有抑制蛋白激酶C，鳥胺酸脫羧酶，*c-jun*/AP-1的活性及抑制8-hydroxydeoxyguanosine之生成。基於這些發現，便提出一些多酚類化合物可能經由抑制細胞的訊息傳遞來抑制腫瘤的促進作用。

關鍵詞：多酚類，薑黃素，蛋白激酶C，抗氧化劑，化學預防。

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