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Cancer Chemoprevention by Tea Polyphenols Through Modulating Signal Transduction Pathways

Jen-Kun Lin

Institute of Biochemistry, College of Medicine, National Taiwan University, Taipei, Taiwan

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The action mechanisms of several chemopreventive agents derived from herbal medicine and edible plants have become attractive issues in cancer research. Tea is the most widely consumed beverage worldwide. Recently, the cancer chemopreventive actions of tea have been intensively investigated. It have been demonstrated that the active principles of tea were attributed to their tea polyphenols. Recently, tremendous progress has been made in elucidating the molecular mechanisms of cancer chemoprevention by tea and tea polyphenols. The suppression of various tumor biomarkers including growth factor receptor tyrosine kinases, cytokine receptor kinases, PI3K, phosphatases, ras, raf, MAPK cascades, N · FB, I · B kinase, PKA, PKB, PKC, c-jun, c-fos, c-myc, cdks, cyclins, and related transducing proteins by tea polyphenols has been studied in our laboratory and others. The I · B kinase (IKK) activity in LPS-activated murine macrophages (RAW 264.7 cells) was found to be inhibited by various tea polyphenols including (-) epigallocatechin-3-gallate (EGCG), theaflavin (TF-1), theaflavin-3-gallate (TF-2) and theaflavin-3,3'-digallate (TF-3). TF-3 inhibited IKK activity in activated macrophages more strongly than did the other tea polyphenols. TF-3 inhibited both IKK1 and IKK2 activity and prevented the degradation of I · B · and I · B · in activated macrophage cells. The results suggested that the inhibition of IKK activity by TF-3 and other tea polyphenols could occur by a direct effect on IKKs or on upstream events in the signal transduction pathway. TF-3 and other tea polyphenols blocked phosphorylation of IB from the cytosolic fraction, inhibited NFB activity and inhibited increases in inducible nitric oxide synthase levels in activated macrophage. TF-3 and other tea polyphenols also inhibited strongly the activities of xanthine oxidase, cyclooxygenase, EGF-receptor tyrosine kinase and protein kinase C. These results suggest that TF-3 and other tea polyphenols may exert their cancer chemoprevention through suppressing tumor promotion and inflammation by blocking signal transduction. The mechanisms of this inhibition may be due to the blockade of the mitogenic and differentiating signals through modulating EGFR function, MAPK cascades, NFκB activation as wll as c-myc, c-jun and c-fos expression.

Key words: Tea polyphenols, Signal transduction, EGF receptor, MAPK cascade, Apoptosis, iNOS, Caspase, Xanthine oxidase

INTRODUCTION

Cancer is recognized as one of the leading causes of death in many countries and it has become along with cardiovascular and neurodegenerative diseases, the most important issue of modern preventive medicine. Tea is one of the most popular beverages worldwide. Both green tea and black tea have recently attracted attention as naturally occurring cancer preventive agent (Weisburger, 1998). Due to its characteristic aroma and taste, the tea is the most widely accepted and consumed beverage. Recent studies have provided the strong scientific basis for understanding the health promoting effects of tea to man (Nakachi, *et al.*, 2000; Lin,J. K., *et al.*, 2000; Lin, Y. L., *et al.*, 1998; Lin and Liang, 2000). The major tea beverage in the world is black tea, especially in the Western nations. Black tea leaves are produced through extensive enzymatic oxidation of polyphenols to polymerized products, such as theaflavins and thearubigins. The major theaflavins in black tea are theaflavin (TF-1), theaflavin-3-gallate (TF-2a), theaflavin-3'-gallate (TF-2b) and theaflavin-3,3'-digallate (TF-3)(Chen and Ho, 1994) (Fig. 1). It has been demonstrated that black tea could be as

Correspondence to: Dr. Jen-Kun Lin, Institute of Biochemistry College of Medicine National Taiwan University No. 1, Section 1, Jen-a Road, Taipei, Taiwan
E-mail: jklin@ha.mc.ntu.edu.tw

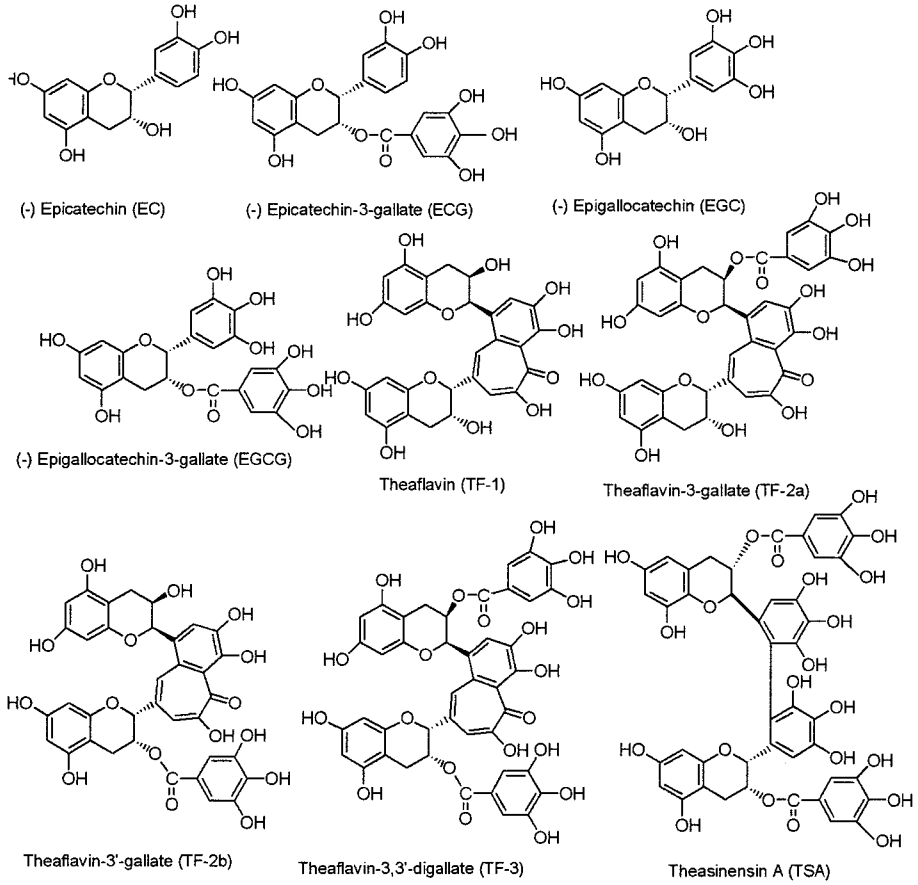

Fig. 1. Chemical Structures of Tea Polyphenols

effective as green tea in cancer chemoprevention.

The major polyphenols in green tea are catechins including catechin, gallocatechin, (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin-3-gallate, and (-)-epigallocatechin-3-gallate (EGCG) (Fig.1). EGCG is the most abundant among these catechins. In the Northern part of China, most green tea beverages were blended with jasmine flower to promote their aroma and flavor. Oolong tea is a partially fermented tea and its chemical composition of tea polyphenols is very complicated. Many intermediate oxidation products of tea polyphenols were found in oolong tea. The isolation and characterization of these oxidation products has been a challenging task for many tea chemists. The new type of tea polyphenol theasinesin A (Fig. 1) has been isolated from oolong tea. The compound was synthesized by free radical oxidation of EGCG. It is estimated that approximate 80% of tea produced in Taiwan were consumed as oolong tea. Paochong tea is quite popular, only next to oolong tea in the northern part of Taiwan. Sometimes, paochong tea has been considered as light-fermented oolong tea. The partially fermented oolong or paochong tea contains both catechins, theaflavins and possibly thearubigins. Some components in oolong or paochong tea, such as proanthocyanidins, are less well characterized and they may be important in disease prevention.

Tea polyphenols exhibit a variety of biological properties including antioxidative effects (Lin, *et al.* 2000), inhibition of extracellular mitotic signals (Lin, *et al*, 1999), inhibition of cell cycle at G1 phase (Liang, *et al* 1999), suppression of inducible nitric oxide synthase (Lin and Lin, 1997; Lin, *et al* 1999) and induction of apoptosis in cancer cells (Pan, *et al.*, 2000).

Inhibitory effects of tea polyphenols on carcinogenesis in animals.

The anticarcinogenic effects of EGCG and green tea extract on various organs including skin, glandular stomach, duodenum, colon, liver, pancreas and lung in rats and mice have been reported in several laboratories (Wang., *et al.*, 1992; Yang and Wang, 1993; Yamane, *et al.*, 1991; Lin and Liang, 2000; Xu, *et al.*, 1992). The anticarcinogenic effects of black tea extract on skin carcinogenesis and esophageal tumorigenesis in rodents were also reported (Wang, *et al.*, 1994). Sugimura and his colleagues were first to use a two-stage skin carci-

nogenesis mouse model to demonstrate that topical applicartion of EGCG inhibited tumor promotion induced by teleocidin in DMBA-initiated mouse skin (Yoshizawa *et al*, 1987). Studies by Mukhtar *et al* further showed that green tea polyphenols had a potent inhibitory effect on skin tumorigenicity in Sencar mice (Khan *et al.*, 1988).

In recent years, many studies demonstrated that topical application or oral feeding of a polyphenolic fraction from tea extract, and individual catechin derivatives , had anticarcinogenic effects in aninmal skin and other organs (Yang and Wang, 1993).

Wang *et al.*, (1992) showed that green tea polyphenols and EGCG inhibited the growth of established skin tumors induced chemically or by UV light. Oral, subcutaneous, or intraperitoneal administration of EGCG or green tea polyphenols in mice also resulted in significant suppression of the growth of implanted tumor cells (Ouguni, *et al.*, 1988; Hara *et al.*, 1989). Lu *et al.*, (1997) reported that oral administration of black tea in tumor-bearing mice inhibited proliferation and enhanced apoptosis in nonmalignant and malignant skin tumors. Landau *et al.*, (1998) demonstrated that black and green tea infusion significantly decreased the spontaneous formation of lung tumors and rhadomyosarcoma in A/J mice. The suppression of azoxymethane-induced preneoplastic lesions and inhibition of cyclooxygenase-2 activity in the colonic mucosa of rats drinking a crude green tea extract has been described (Metz, *et al.*, 2000). Recently, effects of tea polyphenols and tea pigments on the inhibition of precancerous liver lesions in rats have been reported (Gong, *et al.*, 2000). Here, tea pigments are the oxidative products of tea polyphenols, which are primarily composed of theaflavins, thearubigins and theabrownins.

Taniguchi *et al.*, (1992) and Sazuka *et al.*, (1995) showed that pre-oral administration of green tea infusion or EGCG inhibited lung metastasis in mouse melanoma and Lewis lung carcinoma cells. The mechanisms of antimetastatic effect of EGCG was associated to its inhibition of cell spreading of tumor cells, suppression of matrix metalloprotease-9 (MMP-9) secretion, and serum induced tyrosine phosphorylation of focal adhesion kinase (FAK). Sazuka *et al.* (1997) also reported that the TF-3 and EGCG inhibitited MMPs secretion from culture medium of LL2-Lu3 cells.

Prevention of human cancer by tea

The preventive effects of tea on the cancer development in humans have not been so conclusive. Many studies in certain countries had reported no significant association (Stocks, 1970); in others, a positive association (La Vecchia, *et. al.*, 1992; Kinlen, *et al.*, 1988); an in still others, a negative association between tea consumption and cancer incidence was observed (Gao, *et al.*, 1994; Nakachi, *et al.*, 1998). The discrepancy among these different epidemiological studies on the association of tea drinking with cancer incidence may arise from their different study subjects and different questionnaire designs. It is worthy to note that the frequency and quantity of tea-drinking daily in a population might affect the outcome of cancer prevention in such population. There are many life-time tea-drinkers in the oriental countries such as China, Korea and Japan. They drink tea everyday even every hour during the daytime! This may be one of the reasons that more previous studies from oriental population gave definitively positive preventive effects of tea on cancer incidence (Nakachi, *et al.*, 1998; Gao, *et al.*, 1994).

A recent cohort study has showed that the slowdown in increase of cancer incidence with age observed among females who consumed more than 10 cups a day is consistent with the finding that increased consumption of green tea is associated with later onset of cancer (Imai, *et al.*, 1997).

Molecular mechanisms of cancer chemoprevention by tea polyphenols

The progress of molecular oncology has demonstrated that damage to numerous regulatory genes may result in the development of invasive and metastatic cancer. It has been established that the pathological processes of multistep carcinogenesis comprises initiation, promotion and progression (Lin and Lee, 1995). The natural history of carcinogenesis and cancer provides a strong rationale for a preventive approach to the control of this disease and leads one to consider the possibility of active pharmacological intervention to arrest or reverse the process of carcinogenesis before invasion and metastasis occur. Such intervention is called chemoprevention (Wattenberg, 1985; Morse and Stoner, 1993).

The inhibitory effects of tea against carcinogenesis have been attributed to the biologic activities of the polyphenolic fractions in tea. However, the molecular mechanisms of cancer chemoprevention by tea extract are not fully elucidated. Some recent studies in our laboratory and others are discussed herein (Table I).

Antioxidative effects

Tea polyphenols show profound antioxidative effects in various systems. Tea polyphenols are strong scavengers against superoxide, hydrogen peroxide, hydroxyl radicals, nitric oxide and peroxynitrite produced by various chemicals and biological systems. Chen and Ho (1994) extensively investigated the antioxidant properties of various tea polyphenols. Their studies showed that the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical-scavenging ability of various tea polyphenols was proportional to the number of –OH group in the catechins or theaflavins. All

Table I. Modulation of tumor promotion processes by catechins and theaflavins

Process affected	Catechins	Theaflavins	Reference
1. Xanthine oxidase activity	Inhibition (IC_{50}) EGCG, 12.5 μM	Inhibition (IC_{50}) TF-1,25.5 μM TF-3, 4.5 μM	Lin, *et al* (2000).
2. Superoxide formation in HL-60 cells	Inhibition(IC_{50}) IEGCG, 50.8μM	Inhibition (IC_{50}) TF-3,10 μM	Lin,*et al* (2000).
3. Ornithine decarboxylase (ODC) induced by TPA (Unpublished)	Inhibition 1 μM EGCG, 27.8%	Inhibition ,1 μM TF-3 48.6%	Liang, *et al.*
4. PKC induced by TPA In NIH 3T3 cells	Inhibition, 40 μM EGCG, 49%	Inhibition,40 μM TF-3, 95%	Chen, *et al.*(1999).
5. AP-1 binding in NIH 3T3 cells	Inhibition, 40 μM EGCG, 56%	Inhibition,40 μM TF-3, 90%	Chen, *et al.*(1999).
6. c-Jun expression in NIH 3T3 cells	Inhibition,20 μM EGCG, 60%	Inhibition,20 μM TF-3, 80%	Chen, *et al.*(1999).
7. EGFR binding in A431 cells	Inhibition,10 μM EGCG, 66%	Inhibition,10 μM TF-3, 90%	Liang,*et al*(1997).
8. EGFR autophosphorylation in A431 cells	Inhibition,10 μM EGCG, 80%	Inhibition,10 μM TF-3, 100%	Liang,*et al*(1997).
9. iNOS induced by LPS in	Inhibition,10 μM	Inhibition,10 μM	Lin,*et al*(1999).
10.Raf-1 expression in 30.7b Ras 12 cells	No effect, 20 μM EGCG, 0%	Inhibition,20 μM TF-3, 87%	Chung, *et al* (2001).
11.MEK-1 expression in 30.7b Ras 12 cells	Inhibition, 20 μM EGCG, 30%	Inhibition, 20 μM TF-3, 45%	Chung, *et al* (2001).
12.ERK1/2 phosphorylation In 30.7b Ras 12 cells	Inhibition,20 μM EGCG, 30%	Inhibition, 20 μM TF-3, 50%	Chung, *et al* (2001).
13.Elk-1 phosphorylation In vitro assay	Inhibition, EGCG, 29%	Inhibition TF-3, 38%	Chung, *et al* (2001).
14.JNK expression in UVB -induced keratocytes	Inhibition 20 μg/ml EGCG, 83%	Not done	Katyar, *et al* (2001).
15.PI3K expression induced by UVB in JB6 cells (continued from Table I)	Inhibition,20 μM EGCG,60%	Inhibition,20 μM TF-3, 80%	Nomura,*et al* (2001)
16.p^{70}S6-K activation in JB6 cells	Inhibition,1 μM EGCG, 40%	Inhibition, 1 μM TF-3, 30%	Nomura,*et al* (2001).
17.NFκB activation induced By LPS	Inhibition, 10 μM EGCG, 80%	Inhibition, 10 μM TF-3, 95%	Lin,Y. *et al* (1999).
18.IκB phosphorylation Induced by LPS	Inhibition, 10 μM EGCG, 60%	Inhibition,10 μM TF-3, 98%	Lin,Y.*et al*(1999).
19.Apoptosis in U-937 cells	Induction,25 μM	Induction,25 μM EGCG, 55%	Pan,*et al* (2000a). TF-3, 60%
20.Caspase cascade in U-937 cells	Activation,25 μM EGCG, 150%	Activation,25 μM TF-3, 250% TF-2, 600% TF-1, 750%	Pan,*et al* (2000a).
21.PARP cleavage in U-937 cells	Activation,25 μM EGCG, 5%	Activation,25 μM TF-3, 80%	Pan,*et al* (2000a).

the theaflavins showed the same capacity to inhibit the production of superoxide. Green tea, black tea and EGCG were shown to block the production of oxygen free radicals derived from IQ in the presence of NADPH-cytochrome p-450 reductase (Hasaniya *et al.*,(1997). Recent study shows that catechins of green tea are highly active in reducing the amount of oxidative damage sustained by DNA through · OH radical attack. Catechins when compared with other classes of flavanoids (Anderson, *et al.*, 2000) are found to be very active in reducing the amount of strand breakage and residual base damage by a mechanism other than direct scavenging of · OH radicals before they react with DNA. Pulse radiolysis data support the mechanism of electron

transfer (or H-transfer) from catechins to radical sites on DNA (Anderson, *et al.*, 2001). These results support an antioxidant role of catechins in their direct interaction with DNA radicals.

Peroxynitrite is a cytotoxic species generated by the reaction between superoxide and nitric oxide. Catechin polyphenols could also decrease the peroxynitrite-induced nitration of tyrosine and protect apolipoprotein B-100 of LDL from peroxynitrite- induced modification of critical amino acids, which contribute to its surface charge (Pannala *et al.*, 1997).

Recently, several studies have found that black tea and green tea offered protection against oxidative damage to red blood cells induced by a variety of inducers, such as hydrogen peroxide, primaquine, 2,2'-azo-bis (2-amidinopropane) dihydrochloride (AAPH)., phenylhydrazine, copper-ascorbic acid and the xanthine/xanthine oxidase system (Grinberg *et al.*, 1997). Recently, we found that oral feeding of green tea leaves to rats resulted in enhanced SOD activity in serum and catalase activity in liver and an increased concentration of glutathione in the liver (Lin *et al.*,1998).

The inhibitory effects of tea polyphenols on xanthine oxidase (XO) were investigated (Lin *et al.*, 2000). Theaflavins and EGCG inhibit XO to produce uric acid and also act as scavengers of superoxide. TF-3 acts as a competitive inhibitor and is the most potent inhibitor of XO among these compounds. TF-3 also inhibited the superoxide production in HL-60 cells. Therefore, the antioxidative activity of tea polyphenols is due not only to their ability to scavenge superoxides but also to their ability to block XO and relative oxidative signal transducers (Lin *et al.*, 2000).

Recent studies have shown that EGCG has a neuroprotective effect against hippocampal neuronal damage following global ischemia in the gerbils (Lee, *et al.*, 2000). Tea catechins protected the cultured newborn-mouse cerebral nerve cells from death induced by glucose oxicase. Intracisternal injection of (-)-epicatechin improved the memory impairment induced by intracisternal glucose oxicase, and iv injection of (+)-catechin or (-)-epicatechin improved that induced by the cerebral ischemia (Matsuoka, *et al*, 1995). These findings suggest that tea catechins ameliorate the injuries or impairments induced by active oxygens through scavenging intracellular oxygens.

The neuroprotective property of green tea and EGCG in N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine(MPTP)-mice model of Parkinsons disease has been described (Levites, *et al.* 2001). MPTP neurotoxin caused dopamine neuron loss in substantia nigra concomitant with a depletion in striatal dopamine and tyrosine hydroxylase protein levels. These adverse effects could be suppressed by the green tea extract or EGCG.

Inhibition of tumor proliferation and mitogenic signal transduction

Recent development in molecular biology has demonstrated the pivotal role of mitogenic signal transduction in controlling the tumor proliferation. The induction of ornithine decarboxylase (ODC), PKC, protein kinase activities, and oxidative stress by TPA is believed to be closely related to the tumor promotion activity of this compound (Lin and Lee, 1995). Topical application of green tea polyphenols to mouse skin was found to inhibit TPA-caused induction of ODC activity in a dose-dependent manner (Agarwal *et al.*, 1992). Our studies demonstrated that EGCG and TF-3 inhibited TPA-induced transformation, PKC activation and AP-1 binding activities in mouse fibroblast cells (Lee and Lin, 1997; Chen *et al.*, 1999).

Tea polyphenols are known to inhibit a wide variety of enzymatic activities associated with cell proliferation and tumor progression. We have investigated the effects of the major tea polyphenols EGCG on the proliferation of human epidermoid cell line A431 (Liang *et al.*, 1997). Using a tritiated thymidine incorporation assay, EGCG could significantly inhibit the DNA synthesis of A431 cells. In in vitro assay, EGCG strongly inhibited the protein tyrosine kinase activities of EGF-R, PDGF-R and FGF-R and exhibited an IC50 value of 0.5-1 · g/ml. But EGCG scarcely inhibited the protein kinase activities of $pp60^{src}$, PKC and PKA (IC50 > 10 · g/ml).

In an in vivo assay, EGCG could reduce the autophosphorylation level of EGF-R by EGF. Phosphoa-mino acid analysis of the EGF-R revealed that EGCG inhibited the EGF-stimulated increase in phosphotyrosine level in A431 cells. In addition, we showed that EGCG blocked EGF binding to its receptor. These results suggested that the inhibition of proliferation and suppression of the EGF signaling by EGF might mainly mediate dose-dependent blocking of ligand binding to its receptor, and subsequently through inhibition of EGF-R kinase activity (Liang *et al.*, 1997).

Inhibition of MAPK signaling

Exposure of normal human epidermal keratinocytes (NHEK) to UVB radiation induces intracellular release of hydrogen peroxide (oxidative stress) and phosphorylation of MAPK cell signaling pathways. Pretreatment of NHEK with EGCG inhibits UVB-induced hydrogen peroxide production and its mediated phosphorylation of MAPK signaling pathways (Katiyar, *et al.*, 2001). Treatment of EGCG (20 μg/ml of media) to NHEK before UVB (30 mJ/cm^2) exposure inhibited UVB-induced hydrogen peroxide production (66-80%) concomitant with the inhibition of UVB-induced phosphorylation of ERK1/2 (57-80%), JNK (53-83%) and p38 (50-70%) proteins. These findings

demonstrate that EGCG has the potential to inhibit UVB-induced oxidative stress-mediated phosphorylation of MAPK signaling pathways, suggesting that EGCG could be useful in attenuation of oxidative stress-mediated and MAPK-caused skin disorders in humans (Katiyar, *et al.*, 2001).

It has been demonstrated that tea polyphenols inhibited PKC, MAPK and AP-1 activities in NIH 3T3 cells (Chen, *et al.*, 1999) and mouse epidermal JB6 cells and the corresponding H-ras-transformed cell line 30.7b Ras 12 (Chung, *et al.*, 2001). The cells were incubated with EGCG or TF-3 (20 μM) for different times and the cell lysate was analyzed by immunoblotting. EGCG treatment decreased the levels of phospho-Erk1/2 time dependently by 30% at 30 min, and 60% at 60 min, (from the presented data indicated that EGCG could slightly increased the phosphor-Erk by 10% at 15 min); TF-3 lowered their levels by 50% at 15 min, 40% at 40 min and 30% at 60 min. TF-3 effectively decreased total Raf-1 protein levels most likely through lysosomal degradation. On the contrary, EGCG did not affect protein levels or the activity of Raf-1 significantly, but decreased its association with MEK 1 as determined by co-immunoprecipitation. In addition, EGCG anf TF-3 (10 μM) inhibited the phosphorylation of Elk-1 by isolated phosphor-Erk1/2 in vitro (Chung, *et al.*, 2001).

Inhibition of PI3K pathway

The inhibitory effects of tea polyphenols on UVB-induced phosphatidylinositol- 3-kinase (PI3K) activation has been demonstrated in mouse apidermal JB6 C1 41 cells (Nomura, *et al.*, 2001). Pretreatment of cells with EGCG and TF-3 inhibited UVB-induced PI3K activation. Furthermore, UVB-induced activation of Akt and ribosomal p70S6 kinase (p^{70}S6-K), PI3K downstream effectors, were also attenuated by these tea polyphenols. In addition to LY294002, a PI3K inhibitor, pretreatment with MAP-ERK kinase 1 inhibitor, U0126, or a specific p38 kinase inhibitor, SB202190, blocked UVB-induced activation of both Akt and p^{70}S6-K. It is worthy to note that UVB-induced p^{70}S6-K activation was directly blocked by the addition of EGCG or TF-3, whereas these polyphenols showed only a weak inhibition on UVB-induced Akt activation (Nomura, *et al.*, 2001).

Inhibition of cell cycle progression

It is generally accepted that the cell proliferation is intimately associated with cell cycle progression. EGCG potently inhibit cell proliferation and suppresses tumor growth both in vitro and in vivo, but little is known regarding the cell cycle regulatory proteins mediating these effects. We have investigated the effects of EGCG and other catechins on the cell cycle progression (Liang *et al.*, 1999). DNA flow cytometric analysis indicated that EGCG blocked cell cycle progression at G1 phase in asynchronous MCF-7 cells. Over a 24 h exposure to EGCG, the Rb protein changed from hyper- to hypophosphorylated form and G1 arrest developed. The protein expression of cyclin D1, and E reduced slightly under the same conditions. Immunocomplex kinase experiments showed that EGCG inhibited the activities of cyclin-dependent kinase 2 (Cdk-2) and 4 (Cdk-4) in a dose-dependent manner in the cell free system. As the cells were exposed to EGCG (30 · M) over 24 h a gradual loss of both Cdk2 and Cdk4 kinase activities occurred. EGCG also induced the expression of the Cdk inhibitor p21 and this effect correlated with the increase in p53 levels. The level of p21 mRNA also increased under the same conditions. In addition, EGCG also increased the expression of the Cdk inhibitor p27 protein within 6 h after EGCG treatment. These results suggest that EGCG either exerts its growth-inhibitory effects through modulation of the activities of several key G1 regulatory proteins such as Cdk2 and cdk4 or mediates the induction of Cdk inhibitors p21 and p27. A dual combination of these two pathways is also possible.

Anti-inflammation and anti-tumor effects through *suppressing iNOS*

The small molecule nitric oxide (NO) has been shown to exhibit several profound physiological and pharmacological functions in the target cells. NO plays an important role in inflammation and multistep carcinogenesis. We have investigated the effects of tea polyphenols on the induction of NO synthase (iNOS) in thioglycolate-elicited and lipopoly-saccharide (LPS)-activated peritoneal macrophages (Lin and Lin, 1997; Lin *et al.*, 1999). Gallic acid, EGC, EGCG, TF-1, TF-2, and TF-3 were found to inhibit nitrite production, iNOS protein and mRNA in activated macrophages.Western blot, reverse transcription poly-merase chain reaction, and Northern blot analyses demonstrated that significantly reduced 130-kDa protein and 4.5 kb mRNA levels of iNOS were expressed in LPS-activated macrophages with EGCG or theaflavins com-pared with those without tea polyphenols. Electrophoretic mobility shift assay indicated that EGCG blocked the activation of nuclear factor-B, a transcription factor necessary for iNOS induction. EGCG and theaflavins also blocked the disappearance of inhibitor IB from cytosolic fraction. These results suggest that EGCG and theaflavins decrease the activity and protein levels of iNOS by reducing the expression of iNOS mRNA and the reduction could occur through prevention of the binding of NF-B to the iNOS promoter, thereby inhibiting the induction of iNOS transcription. (Lin and Lin, 1997).

Suppression of NF · B activation through down-regulating I · B kinase

Recently, we have investigated the inhibition of I · B kinase (IKK) activity in LPS activated murine macrophages (RAW 264.7 cell line) by various polyphenols including EGCG and theaflavins (Pan *et al.*, 2000). TF-3 inhibited IKK activity more strongly than did the other polyphenols. TF-3 strongly inhibited both IKK1 and IKK2 activity and prevented the degradation of I · B and I · B in activated macrophage cells. The results suggested that the inhibition of IKK activity by TF-3 and other tea polyphenols could occur by direct effect on IKKs or on up-stream events in the signal trnsduction pathway. Furthermore, TF-3 blocked phosphorylation of I · B from the cytosolic fraction, inhibited NF · B activity, and inhibited increases in iNOS levels in activated macro-phages. These results suggest that TF-3 and other poly-phenols may exert their anti-inflammatory and cancer chemoprevention actions by suppressing the activation of NF · B through inhibition of IKK activity (Pan *et al.*, 2000).

In the JB6 mouse epidermal cell line, the tumor promoter TPA causes cell transformation at high frequency, marked induced NFκB activation. EGCG and TF-3 inhibited TPA-induced NFκB activity in a concentration-dependent manner. These tea polyphenols blocked TPA-induced phosphorylation of IκBα at Ser 32 in the same concentration range. Moreover, the NFκB sequence-specific DNA binding activity induced by TPA was also inhibited by these polyphenols (Nomura, *et al.*,2000). These results confirmed that inhibition of NFκB activation is also important in evaluation of the antitumor promotion effects of tea polyphenols.

Induction of apoptosis in cancer cells by tea polyphenols

Apoptosis is induced by a variety of stimuli, such as genotoxic compounds, tumor necrosis factor, Fas ligand and various environmental stresses. Despite the diversity of apoptosis-inducing agents, numerous experiments indicate that signals leading to the activation of members of the intracellular cysteine protease family, for instance, the caspase, may play a pivotal role in the initiation and execution of apoptosis induced by various stimuli (Faleiro *et al.* 1997).

We have examined the growth inhibitory effects of theasinensin A (from oolong tea), EGCG and theaflavins in human cancer cells. (Pan, *et al.*, 2000).

Theasinensin A TF-1 and TF-2 displayed strong growth inhibitory effects against human histolytic lymphoma U937 (IC50, 12M) but were less effective against human acute T-cell leukemia Jurkat, whereas TF-3 and EGCG had lower activities. The molecular mechanisms of tea poly-phenols induced apoptosis as determined by annexin V apoptosis assay, DNA fragmentation, and caspase activation were further investigated. Loss of membrane potential and ROS generation were also detected by flow cytometry. Treatment with tea polyphenols caused rapid induction of caspase-3, but not caspase-1, activity and stimulated proteolytic cleavage of poly(ADP-ribose)-polymerase (PARP). Pretreatment with a potent caspase-3 inhibitor, Z-Asp-Glu-Dal-Asp-fluoromethyl ketone, inhibited theasinensin A-induced DNA fragmemtation. Furthermore, it was found that theasinensin A induced loss of mitochondrial potential, elevation of ROS production, release of mitochondrial cytochrome c into the cytosol, and subsequently induction of caspase-9 activity. Further experimental results indicate that theasinensin A is effective in inducing DFF-45 (an inhibitor binding to Dnase) degradation which allows caspase-activated Dnase to enter the nucleus and degrade chromosomal DNA.

Recent studies on apoptosis and cell cycle arrest in cancer cells by in vivo metabolites of teas have been described (Zhang, *et al.*, 2000). The tea extracts from green, oolong and black teas, the rat sera obtained after oral intubation of the tea extracts, and the tea polyphenolic compounds, EGCG, EGC, ECG and theaflavins were used in the related tests. The extracts, the sera from the treated rats and the polyphenolic compounds significantly inhibited the proliferation of a rat hepatoma cell line (AH109A) and murine B16 melanoma cells but not normal rat mesothelial (M) cells. These results suggest that the induction of apoptosis by theasinensin A and other tea polyphenols may provide a pivotal mechanism for their cancer chemopreventive function (Pan *et al.*,2000). A commentary on the cancer chemoprevention by tea polyphenols through mitotic signal transduction blockade has been critically elaborated by Lin *et al* (1999) and Lin and Liang (2000).

Hypolipidemic effect of green tea leaves through induction of antioxidant and phase II enzymes

In a recent study, we have investigated the effect of green tea leaves on the levels of cholesterol, lipid, antioxidant and phase II enzymes in Wistar rats by long-term oral feeding. The results indicate that oral feeding of green tea leaves can result in the reduction of total cholesterol, triglyceride, and LDL-cholesterol and the enhancement of the activities of superoxide dismutatse (SOD) in serum and phaseII enzyme, glutathione S-transferase (GST) and catalase in the rat liver (Lin, *et al.* 1998). At the 15th week, the average body weights of the green tea leaves-fed group and basal diet group were 449 and 510 g, respectively. The oral feeding of green tea leaves resulted in a significant 12% decrease in the body weight as compared to the control group ($p < 0.05$).

The dose of green tea laves used in the present study

did not reduce diet and water consumption throughout the feeding regimen. The survival ratios of both tea-fed and basal diet-fed groups are 100% (12/12) during the course of experiment (Lin, *et al*, 1998).

Many previous studies have focused on the anticarcinogenic and antimutagenic effects of tea. Furthermore, the mechanisms contributing to the anticarcinogenic and antimutagenic effects of tea may involve the anti-oxidative activity (Lin, *et al*, 1996), induction of phase II enzymes (Lee, *et al* 1995), blocking the formation of ultimate carcinogens, inhibition of the covalent binding of carcinogen to DNA and inhibition of DNA synthesis and cell proliferation (Liang, *et al.*, 1997; Lin, *et al.*, 1999). In addition, the level of antioxidant defense enzymes such as SOD and catalase are also known to be lower in transformed cells and/or tumors (Sun, 1990). Our present results show that oral feeding of green tea leaves to rats results in the reduction of triglyceride, total cholesterol and LDL-cholesterol and enhancement of activities of SOD in serum, and phase II enzymes GST and catalase in liver. The significance of these results can be implicated in relation to the hypolipidemic effect, body weight reduction and the cancer chemoprevention of green tea leaves against the induction of tumors (Lin, *et al.*, 1999).

General remark on the mechanisms of cancer chemoprevention by tea polyphenols

It is encouraging that the action mechanisms of cancer chemoprevention by tea polyphenols have been intensively investigated recently (Lin, *et al.*, 1999; Lin and Liang, 2000; Nomura, *et al.*, 2001 and Chung, *et al.*, 2001). Different target enzymes or proteins at different cellular compartments have been affected by tea polyphenols. EGFR tyrosine kinase on the plasma membrane is significantly inhibited by EGCG and TF-3 (Liang, *et al.*, 1997; 1999). The membrane-bound xanthine oxidase is inhibited by theaflavins (TF-1, TF-2, and TF-3) and EGCG (Lin, *et al.*, 2000). Several cytosolic enzymes and transducing proteins such as PI3K, Akt, raf, MEK, ERK, JNK, p38 kinase, and IKK have been shown to be inhibited by EGCG and theaflavins (Nomura, *et al.*, 2001; Chung, *et al.*, 2001; Pan, *et al.*, 2000b). On the other hand, the cytosolic caspase 3 and 9 were activated by tea polyphenols (Pan, *et al.*, 2000b).

Cytochrome c was found to be liberated from mitochondrial membrane by the oolong tea polyphenol theasinesin A (Pan, *et al.*, 2000a). Some cell cycle regulating proteins in the nucleus such as Rb, CDK2, and CDK4 were inhibited by tea polyphenols (Liang, *et al.*, 1999), on the other hand, the CDK inhibitors p21 and p27 were elvated by the tea polyphenols. All these enzymes and transducing proteins are topologically arranged into a signal transducing network from cell membrane, through cytosolic compartment and finally reach nuclear genome the site of gene expression.

Extracellular stimulus will evoke a appropriate signal that transduces along the transducers (proteins or enzymes) from membrane to nucleus and turn on the appropriate gene expression. These signal transduction pathways are strictly controlled by endogenous and exogenous factors such as hormones, growth factors, cytokines, and exogenous stimuli. Based on the recent investigation, these signal transduction pathways could be modulated by tea polyphenols (Lin and Lee, 1995; Lin, *et al.*, 1999; Lin and Lin, 1997).

Since Stanley Cohens initial discovery of the EGF in 1962, it was predicted that a therapeutic target for cancer could emerge from a better understanding of this signal pathway (Carpenter and Cohen, 1979). The first drug approved for cancer therapy come from this signaling pathway was an antibody targeted for the Her-2/neu receptor. However, a role for EGFR kinase inhibitors in cancer chemoprevention was known to be dysregulated in many different types of cancer. The precise role of the EGFR in neoplastic transformation continues to be an area of intensive research. Obviously, activation of the EGFR-associated tyrosine kinase activity stimulates multiple signaling pathways, which culminates in increased cell division, altered cell motility and a number of other biologic responses. We have demonstrated that the EGFR activation was significantly inhibited by tea polyphenols (Liang, *et al.*, 1997; 1999).

Several lines of evidence have demonstrated that signal transduction events leading to the activation of the MAPK pathways including ERK, JNK, and P38, NFκB pathway and JAK-STAT pathway can result in cell proliferative, survival, differentiating and apoptotic responses (Fig. 2). As illustrating in Fig. 2, it is to emphasize the action mechanism that lead to the inhibition of survival gene expression (c-jun, c-fos, c-myc, etc) and activation of apoptotic signal pathways (caspase 8 and caspase 9 cascades). We have tried to illustrate two important signaling events, namely the MAPK, NIK (NFκB inducing kinase) and caspase cascades (ICE/ced 3 family proteases) pathways. Most tea polyphenols could suppress the MAPK and NIK pathways (Pan, *et al.*, 2000b; Chung, *et al.*, 2001); but activate the caspase cascade pathways (Pan, *et al.*, 2000a). The combination of these two effects will potentiately lead to apoptotic response in the target cells.

Most tea polyphenols with cancer chemopreventive activities are antioxidants (Chen and Ho, 1994;Lin, *et al.*, 2000). It should be emphasized that in addition to act as ROS scavengers, these compounds can act through multiple mechanisms to modulate the functions of receptors, effectors, protein kinases, protein phos-

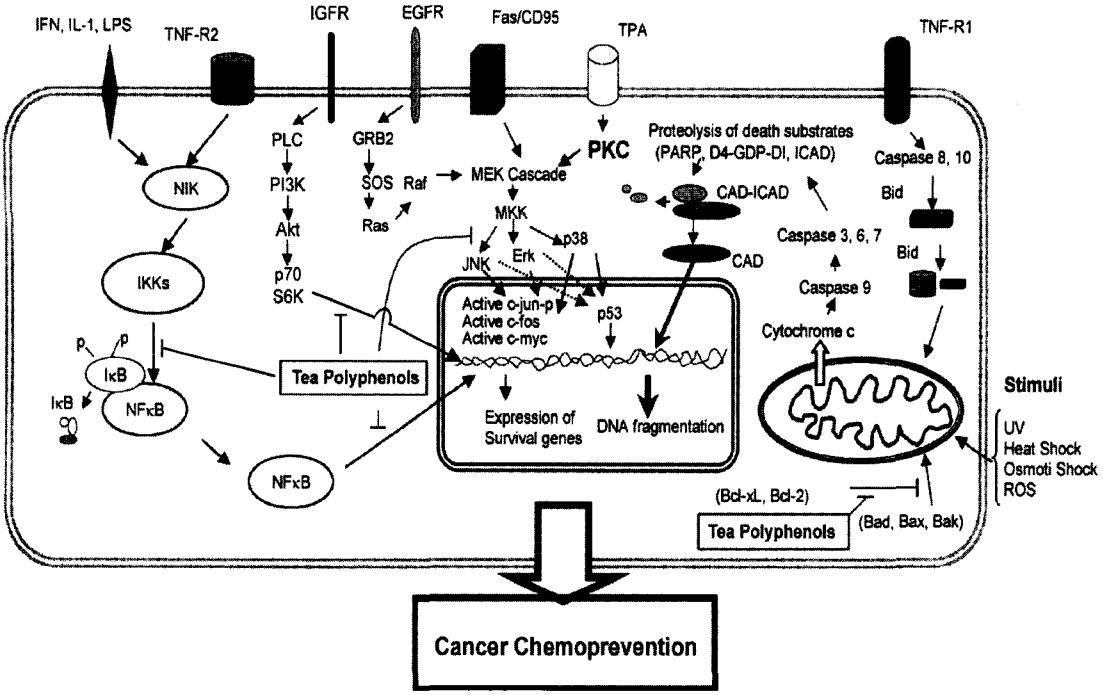

Fig. 2. Cancer Chemoprevention through Modulation of Signal Transduction Pathway by Tea Polyphenols Cancer chemoprevention can be achieved by modulating signal transduction pathways. Cell proliferation and differentiation are strictly regulated by a programmed networks of intracellular signal transduction pathways through various transducers including intrinsic factors such as receptor proteins, PTK, PKA, PKB, PKC, MAPK NFκB, AP-1, c-jun, c-fos, c-myc, iNOS, ROS and others and extrinsic factors such as cytokines, growth factors, tumor promoters, TNF, LPS and others. The illegitimate regulation or hyperfunction of these signalings may lead to the induction of carcinogenesis, inflammation or apoptosis. The tea polyphenols were found to suppress the hyperfunction of these signalings in various systems that may block the processes of carcinogenesis and lead to cancer chemoprevention.

phatases and protein substrates in the mitogenic and differentiating signaling that link to the process of tumor promotion.

In summary, cancer chemopreventive agents can inhibit the tumor growth through arresting cell cycle and inducing cellular apoptosis. During the past few years, experimental results from our laboratory and others have demonstrated that cancer chemoprevention by tea polyphenols can be achieved by signal transduction blockade (Lin, *et al.*, 1999). Discovering novel the-rapeutics and chemopreventive agents with clinical utility continues to be the focus of biochemical and pharmacological scientists working in the signal transduction therapy. Developing compounds designed to manipulate kinase pathways and signal events through both inhibitory and stimulatory methods for treating cancer and other diseases has become promising trends for pharmaceutical research.

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