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Review

Signal Transduction Pathways: Targets for Green and Black Tea Polyphenols

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Tea is one of the most popular beverages consumed in the world and has been demonstrated to have anti-cancer activity in animal models. Research findings suggest that the polyphenolic compounds, (-)-epigallocatechin-3-gallate found primarily in green tea, and theaflavin-3,3'-digallate, a major component of black tea, are the two most effective anti-cancer factors found in tea. Several mechanisms to explain the chemopreventive effects of tea have been presented but others and we suggest that tea components target specific cell-signaling pathways responsible for regulating cellular proliferation or apoptosis. These pathways include signal transduction pathways leading to activator protein-1 (AP-1) and/or nuclear factor kappa B (NF-κB). AP-1 and NF-κB are transcription factors that are known to be extremely important in tumor promoter-induced cell transformation and tumor promotion, and both are influenced differentially by the MAP kinase pathways. The purpose of this brief review is to present recent research data from other and our laboratory focusing on the tea-induced cellular signal transduction events associated with the MAP kinase, AP-1, and NF-κB pathways.

Keywords: Activator protein-1, Apoptosis, EGCG, Nuclear factor kappa B, Theaflavins, Tumor promotion

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Abbreviations: EGCG, (-)-epigallocatechine-3-gallate; EGF, epidermal growth factor; ERKs, extracellular signal-regulated protein kinases; JB6 Cl 41, JB6 mouse epidermal cell line Cl 41; JNKs, c-Jun N-terminal kinase; MAP, mitogen activated protein; MEK1, MAP kinase/Erk kinases; MSK, mitogen- and stress-activated protein kinase; mTOR, mammalian target of rapamycin; p70 S6-K, ribosomal p70 S6 protein kinase; PI-3K, phosphatidylinositol-3 kinase; PKC, protein kinase C; Ser, serine; Thr, threonine; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; UV, ultraviolet.

Introduction and Scope of the Review

Evidence has accumulated within the last few years indicating that green and black tea polyphenols elicit diverse biological responses resulting in potent chemopreventive effects. Tea is one of the most popular beverages consumed in the world and has been demonstrated to have anti-cancer activity in animal models. Several very recent review articles have summarized research findings regarding the anti-carcinogenic effects of tea (Ahmad and Mukhtar, 1999; Fujiki, 1999; Gupta *et al*., 1999; Mukhtar and Ahmad, 1999; Weisburger 1999b; Katiyar *et al*., 2000; Kong *et al*., 2000; Lin and Liang 2000; Trevisanato and Kim, 2000; Wang, 2000; Yang *et al*., 2000a; Ahmad and Mukhtar, 2001; Dong *et al.*, 2001; Katiyar *et al.*, 2001b; Katiyar and Elmets, 2001; Wiseman *et al.*, 2001; Gupta and Mukhtar, 2002; Guyton and Kensler, 2002; Le Marchand, 2002; Yang *et al*., 2002).

Anti-cancer Components of Tea

Most of the tea produced and consumed worldwide is black tea (78%) followed by green (20%) and oolong teas (2%). Black tea is favored in the United States, England, and other Western countries, green tea is consumed primarily in Asian and Northern African countries, and oolong tea is popular in China and Taiwan (Trevisanato and Kim 2000). A 3.5 oz "cup" of tea (~100 ml) contains around 250-350 mg tea solids (Yang, Chung *et al*., 2000a). The active compounds found in tea are the polyphenols and the green tea polyphenols are the flavanols or catechins. During the fermentation process, catechins are converted to the major black tea polyphenol components, theaflavins and thearubigins. The catechins include (-)-epigallocatechin-3-gallate (EGCG), which is the most abundant component, and (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), and (-)-epicatechin (EC) (Yang *et al*., 2000a). The theaflavins, which give black tea its characteristic color and taste, include theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate, and theaflavin-3,3'-digallate (Yang *et al.*, 2000a). Black tea also contains compounds known as thearubigens but little is known about their function.

Another component of tea that may be important in cancer prevention is caffeine. Oral administration of green tea, black tea or caffeine inhibited tumor formation in mice that were previously treated with UVB for 23 weeks to obtain a high risk for the development of tumors, but the inhibitory effect was not observed for decaffeinated teas (Lou *et al*., 1999). Adding back caffeine to the decaffeinated teas restored their inhibitory activities and caffeine alone was also effective, suggesting that caffeine is another major component of tea responsible for tea's anti-cancer effects (Lou *et al*., 1999).

Possible Mechanisms for Anti-cancer Activities of Tea

Tea consumption appears to decrease cancer risk but its mode of action is unclear. The mechanisms responsible for the anti-cancer actions of tea are not well understood but are being intensively investigated. Recent research findings indicate that tea polyphenols can protect against the multistages of cancer initiation, promotion, and progression. A number of mechanisms explaining tea's anti-cancer actions have been presented including results suggesting that the gallate structure of theaflavins is important for growth inhibition of tumor cell lines by these compounds (Yang *et al*., 2000b). One of the most well known actions of tea-associated polyphenolic compounds is their potent antioxidant activity, which has been suggested to be important in alleviating cancer-associated oxidative stresses (Ahmad and Mukhtar, 1999; Benzie *et al*., 1999; Brown, 1999; Katiyar *et al*., 1999; Kondo *et al*., 1999; Lin *et al*., 1999; Weisburger, 1999b; Weisburger, 1999a; Chen *et al*., 2000a; Ichihashi *et al*., 2000; Johnson and Loo, 2000; Jovanovic and Simic, 2000; Katiyar, Ahmad *et al*., 2000; Langley-Evans, 2000; Lin *et al*., 2000; Liu *et al*., 2000; Shi *et al*., 2000; Trevisanato and Kim, 2000; Ahmad and Mukhtar, 2001; Katiyar *et al*., 2001a; Katiyar, Bergamo *et al*., 2001b; Katiyar and Elmets, 2001; Owuor and Kong, 2002; Zhang *et al*., 2002). Results of one recent study in which normal human epidermal keratinocytes (NHEK) were pretreated with EGCG prior to UVB exposure, indicated that EGCG inhibited UVB-induced hydrogen peroxide (H_2O_2) production and H_2O_2-mediated phosphorylation of mitogen-activated protein kinase (MAPK) signaling pathways (Katiyar *et al*., 2001a). Kennedy, *et al.* studied the relationship between cellular sulfhydryl (SH) groups and the cytotoxicity of green tea polyphenols in Ehrlich ascites tumor cells (Kennedy *et al*., 1999). They found that green tea extract and EGC significantly decreased glutathione, protein-sulfhydryl levels and cell viability suggesting that sulfhydryl groups could be a novel target for green tea in cancer cells (Kennedy, Matsumoto *et al*., 1999). Further studies indicated that the effects on cell viability were associated with activation of p38 kinase and JNKs but not ERKs (Kennedy *et al*., 2001). A more recent study by the same group showed that EGC caused cell cycle arrest and decreased phosphorylation of the retinoblastoma (Rb) protein in a cellular thiol-dependent manner (Kennedy *et al*., 2002). All of these data suggest that the antioxidant properties of tea polyphenols are somehow involved in the anti-cancer effects of tea. However, at least one group reported recently that the anti-cancer action of EGCG on cell surface oxidase activity is not mediated through the antioxidant action of EGCG (Cutter *et al*., 2001). In addition, the precise targets and mechanism of the antioxidant activity are still elusive.

Tea components may also produce anti-cancer effects through mediation of carcinogen metabolizing or detoxification enzymes (Yu *et al*., 1997; Brown, 1999; Maliakal *et al*., 2001; Rushmore and Kong, 2002; Weisburger and Chung, 2002) or by inhibition of carcinogen-induced mutagenesis (Kuroda and Hara, 1999; Muto *et al*., 1999; Mure and Rossman, 2001; von Pressentin *et al*., 2001; Amantana *et al*., 2002). Accumulating evidence also suggests that tea compounds may act by inducing cell cycle arrest and apoptosis (Ahmad *et al*., 2000a; Bertolini *et al*., 2000; Gupta *et al*., 2000; Islam *et al*., 2000; Li *et al*., 2000; Tan *et al*., 2000; Chung *et al*., 2001b; Hayakawa *et al*., 2001a; Hayakawa *et al*., 2001b; Jin *et al*., 2001; Sakagami *et al*., 2001; Tan *et al*., 2002). However, others and we suggest that tea components target specific signal transduction pathways leading to AP-1 or NF-κB, which are transcription factors that have been shown to play a key role in carcinogenesis. The role of tea in signal transduction has also been reviewed briefly elsewhere (Agarwal, 2000; Bode and Dong, 2000; Kong *et al*., 2000; Manson *et al*., 2000; Dong, Nomura *et al*., 2001). The purpose of this review is to present recent research data from others and our laboratory focusing on the tea-induced cellular signal transduction events associated with MAP kinase, AP-1, and NF-κB pathways.

Experimental Tools Used to Study Tea and Signal Transduction

The JB6 mouse epidermal cell system of clonal genetic variants that are promotion-sensitive (P^+) or promotion-resistant (P^-) are used extensively to study genetic susceptibility to transformation, promotion and progression at the molecular level. These variants (P^+, P^-, Tx or transformed) are a series of cell lines representing stages of preneoplastic-to-neoplastic progression. P^- variants gain P^+ phenotype upon transfection with mutated p53 (Sun *et al*., 1993; Huang *et al*., 1997b). Following treatment with 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA), epidermal growth factor (EGF) or other tumor promoters with c-Jun overexpression, the P^+ cells gain Tx phenotype irreversibly (Colburn *et al*., 1979; Colburn *et al*., 1982). This cell culture system model is well developed for studying tumor promotion and anti-tumor promotion *in vitro*.

The use of specific transcription factor-luciferase reporter transfected cells and transgenic mouse models represent another important paradigm for studying transcription activation. The cells and mice carry a transcription factor

sequence linked to a luciferase reporter gene, which allows for visualization of the particular transcription factor-linked luciferase activity by luminometer. This model is used extensively to monitor the activity of specific transcription factors *in vivo*.

Carcinogenesis, Signal Transduction, and Transcription Factors

The process by which information from an extracellular signal is transmitted from the plasma membrane into the cell and along an intracellular chain of signaling molecules to stimulate a cellular response is known as "signal transduction." A cell may respond to a stimulus by activating gene transcription through proteins known as transcription factors. A transcription factor is comprised of one or more proteins that bind to specific DNA sequences in a gene. Gene transcription is the most common result of the protein binding to the DNA and is referred to as transcriptional activation.

When cells are exposed to mitogenic or stress stimuli, a complex response occurs that involves discrete phosphorylation cascades. The result is an activation of members of MAPK family. Three classes of MAPKs are known and include the c-Jun N-terminal kinases/stress-activated protein kinases (JNKs/ SAPKs), p38 kinases and the extracellular signal-regulated protein kinases (ERKs) (Boulton *et al*., 1991; Davis, 1994; Kallunki *et al*., 1994; Kyriakis *et al*., 1994). JNKs/SAPKs and p38 kinases are activated potently by various forms of stress, including ultraviolet (UV) irradiation (Kallunki *et al.*, 1994). In contrast, ERKs have been shown to be strongly activated and play a critical role in transmitting signals initiated by tumor promoters such as TPA and growth factors including EGF and platelet-derived growth factor (PDGF) (Cowley *et al.*, 1994; Minden *et al*., 1994). However, the activation of these pathways is not mutually exclusive. For example, heat shock and UV irradiation partially activate the ERKs cascade and EGF partially activates the JNKs pathway (Davis, 1994; Minden, Lin *et al*., 1994). Evidence strongly indicates that activation of MAPKs by tumor promoting agents plays a functional role in tumor promoter-induced malignant transformation (Dong *et al*., 1997a; Dong *et al*., 1997b; Huang *et al.*, 1997a; Huang *et al*., 1998; Watts *et al*., 1998). MAP kinases are activated by translocation (Kharbanda *et al*., 2000) to the nucleus, where they influence target transcription factors. AP-1 and NF-κB are transcription factors that are known to be extremely important in tumor promoter-induced transformation and tumor promotion. Both transcription factors are influenced differentially by the MAP kinase pathways.

Tea, MAP Kinase and AP-1 Signaling Pathways

Many of the molecular alterations that are associated with carcinogenesis occur in cell-signaling pathways responsible for regulating cellular proliferation or apoptosis. Tea polyphenols have been shown to interact with MAP kinase pathways and mediate signaling by influencing activation and phosphorylation of these molecules. Evidence indicates that tea inhibits tumor promoter- or growth factor-induced cell transformation and AP-1 activation. AP-1 is a well-characterized transcription factor composed of homodimers and/or heterodimers of the *jun* and *fos* gene families (Cohen and Curran, 1988; Halazonetis *et al.*, 1988; Hirai *et al.*, 1989; Angel and Karin, 1991) and it regulates the transcription of various genes associated with cellular inflammation, proliferation and apoptosis (Angel and Karin, 1991). In cell culture and animal models, AP-1 was shown to be involved in tumor progression and metastasis (Crawford and Matrisian, 1996) and to play a key role in preneoplastic-to-neoplastic transformation (Bernstein and Colburn, 1989; Barthelman *et al.*, 1998). By comparing P^+ and P^- derivatives of the mouse epidermal JB6 cell line, we found that the transcriptional factor AP-1 plays a critical role in tumor promotion (Dong *et al.*, 1994; Dong *et al*., 1995). Significantly, when tumor promoter-induced AP-1 activity was blocked, neoplastic transformation was inhibited (Dong *et al.*, 1994). AP-1 thus appears to be a key target for chemopreventive agents such as tea (McCarty, 1998).

EGCG has been shown to effectively inhibit cell transformation in A172 and *sis*-transfected NIH 3T3 cells (Ahn *et al*., 1999). In addition, we have previously shown that both EGCG and theaflavins inhibit EGF- or TPA-induced cell transformation in a dose-dependent manner (Dong *et al*., 1997b). At a dose range similar to that which inhibited cell transformation, EGCG and theaflavins also repressed AP-1-dependent transcriptional activity and AP-1 DNA binding activity induced by TPA. Furthermore, these tea compounds inhibited TPA- or EGF-induced c-Jun phosphorylation and JNKs activation but not ERKs phosphorylation. Based on these results and what is known about tumor-promoted activation of AP-1, EGCG and theaflavins appear to exert their chemopreventive effects primarily through the inhibition of AP-1 transactivation and subsequent AP-1 DNA binding activity (Dong *et al.*, 1997b).

The *Ras* pathway is also important in the activation of AP-1. *Ras* gene mutation, which perpetually turns on the growth signal transduction pathway, occurs frequently in many cancer types. JB6 cells, transfected with a mutant H-*ras* gene to mimic carcinogenesis *in vitro*, express high levels of AP-1 activity, which was determined to be a major growth stimulant (Chung *et al.*, 1999). The effect of green and black tea polyphenols on growth of *ras*-transfected cells and AP-1 activity was compared in these cells and almost all of the tea polyphenols showed strong inhibition of cell growth and AP-1 activity (Chung *et al*., 1999). Both EGCG and theaflavin-3,3'-digallate inhibited ERKs phosphorylation, and theaflavin-3,3'-digallate also inhibited p38 kinase phosphorylation. Both ERKs and p38 kinase phosphorylation are implicated in AP-1

activation. The phosphorylation and protein levels of c-Jun and Fra-1, important protein components of AP-1, were also decreased by EGCG and theaflavin-3,3'-digallate (Chung *et al*., 1999). Further studies (Chung *et al*., 2001a) indicated that EGCG and theaflavin-3,3'-digallate decreased phosphorylation of ERK1/2, MEK1/2 and Elk1. Theaflavin-3,3digallate but not EGCG effectively decreased total Raf-1 protein levels. EGCG treatment resulted in a decreased Raf-1 association with MEK1 possibly by binding to proline-rich sequence on MEK1 (see final section of this review). Thus tea polyphenols were suggested to exert their inhibitory effect by interfering with the binding of the protein substrate to the kinase (Chung, Park *et al*., 2001a). Because the *ras* genes are activated in many animal carcinogenesis models and in human cancers, the inhibition of the phosphorylation of c-Jun and ERKs may be important for the repression of cancer formation and growth and thus may be significant chemopreventive targets for tea.

Green tea polyphenols have been reported to induce transcription of ARE (antioxidant-responsive element)-dependent reporter genes and also to strongly activate ERK2 and JNK1 (Yu *et al.*, 1997; Kong *et al*., 2001; Owuor and Kong, 2002). This activation was shown to be associated with increased mRNA levels of the immediate-early response genes, *c-jun* and *c-fos*. The biological actions of five green tea catechins were compared (Chen *et al.*, 2000a) and results indicated that EGCG and ECG induced ARE-mediated luciferase activity. Only EGCG activated all 3 MAP kinases, ERKs, JNKs and p38 kinase. However, EGC activated ERKs and p38 kinase, and EGCG activated caspase-3 and induced apoptosis in HeLa cells. These authors showed that EGCG-induced caspase-3 activation occurred much later than activation of MAP kinases. They concluded that low concentrations of EGCG lead to ARE-mediated gene expression through MAP kinases but that higher concentrations of EGCG result in a sustained activation of MAP kinases, especially JNKs, that ultimately leads to apoptosis (Chen *et al*., 2000a).

Tea Inhibits UV-induced AP-1 Activation

UV-induced signal transduction pathways play a critical role in tumor promotion. Black tea has been reported to inhibit proliferation and enhance apoptosis of skin tumors in mice (Lu *et al*., 1997). Using the JB6 mouse epidermal cell line, we compared the effects of theaflavins and EGCG on UVB irradiation-induced AP-1-dependent transcriptional activation (Nomura *et al.*, 2000b). Theaflavins and EGCG inhibited UVB-induced AP-1 activation in a concentration-dependent manner and the inhibitory effects of theaflavins were stronger than those of EGCG. We also found that theaflavins significantly inhibited UVB-induced activation of ERKs and JNKs (Nomura *et al.*, 2000b). Because the transcription factor AP-1 is important in the process of tumor promotion, the inhibitory effect of these polyphenols on AP-1 activation may further support the anti-tumor promotion action of these tea constituents. These results confirm the findings of others who observed that black tea polyphenols were more effective than EGCG in inhibiting TPA-induced protein kinase C translocation and activation and AP-1 binding activities in NIH3T3 cells (Chen *et al.*, 1999b).

Using a B6D2 transgenic mouse expressing the AP-1 luciferase reporter gene, Barthelman *et al.* (Barthelman, Bair *et al.*, 1998) studied the role of AP-1 activity in tumor promotion and progression. They found that in mouse skin epidermis, UVB irradiation induced a nearly 40-fold increase in luciferase activity, as compared with acetone-treated controls. They then demonstrated that EGCG blocked the signal transduction pathway whereby UVB induces AP-1 activation and suggested that by inhibiting AP-1 activity in UVB-irradiated mouse skin, EGCG may be preventing nonmelanoma skin cancer at the level of tumor promotion (Barthelman *et al.*, 1998). Chen *et al* (Chen *et al*., 1999a) have provided additional evidence supporting the finding that in human keratinocytes EGCG blocks UVB-induced expression of c-Fos which, through inhibition of p38 kinase activation is associated with UVB-induced AP-1 DNA binding and transactivation (Chen *et al.*, 1998). Thus because AP-1 is important for tumor promotion and c-Fos is a potentially important protein component of AP-1, the inhibitory effects of EGCG on c-Fos expression may offer a further explanation for the anti-tumor-promoting effects of EGCG. However, at least one study (Lu *et al*., 2000b) has shown that oral consumption of green tea also enhances UV-induced increases in p53 and p21 proteins and apoptosis suggesting that green tea affects other pathways in addition to AP-1.

In contrast to the studies outlined above, the effects of EGCG on AP-1 activity, MAP kinase signaling, and expression of the AP-1-regulated differentiation marker, human *involucrin (hINV)*, in normal human epidermal keratinocytes were reported in a recent and interesting study (Balasubramanian *et al*., 2002). EGCG increased *hINV* promoter activity and endogenous hINV protein levels in a manner similar to TPA (Balasubramanian *et al*., 2002). AP-1 DNA binding was enhanced and the AP-1 complex contained primarily Fra-1 and JunD. Interestingly we have found that inhibition of UVB-induced AP-1 activation was also associated with an upregulation of JunD and Fra-2, and a suppression of c-Jun and c-Fos expression and composition in bound AP-1 in response to UVB stimulation (Liu *et al*., 2001). Balasubramanian *et al* (Balasubramanian *et al*., 2002) further showed that Ras, MEKK1, MEK3 and p38 kinase were involved in the cells' response to EGCG and additional studies suggested that p38 kinase was primarily responsible for the mediation of the response. The changes were also associated with characteristics of keratinocyte differentiation suggesting the occurrence of a markedly different response to EGCG under normal conditions compared to stress conditions (Balasubramanian *et al*., 2002).

Tea Inhibits Arsenic-induced Apoptosis and AP-1 Activation

Other potentially beneficial effects of green tea include arsenic detoxification. Green tea has been used for many years as a traditional Chinese remedy for detoxification of arsenite-caused toxicity. We showed that EGCG and theaflavins effectively blocked arsenite-induced apoptosis of JB6 cells and inhibited arsenite-induced AP-1 transcriptional activation and AP-1 DNA binding activity (Chen *et al*., 2000b). EGCG and theaflavins potently inhibited arsenite-induced ERKs, but not p38 kinase activity, suggesting that ERKs and JNKs may be involved in mediating arsenite-induced apoptosis, and the inhibition of arsenite-induced apoptosis by EGCG and theaflavins may be due to a decreased phosphorylation of ERKs and JNKs. Furthermore, these results provide a possible mechanism for the detoxification effect of tea on arsenite-induced toxicity (Chen *et al*., 2000b).

Tea Components Inhibit Growth Factor-induced Signal Transduction

EGCG was shown to selectively inhibit the tyrosine phosphorylation of the PDGF-R and its downstream ERKs in cultured vascular smooth muscle cells (VSMCs) from rat aorta (Ahn *et al.*, 1999). But EGCG had no effect on EGF-induced MAP kinase activation. Additionally, PDGF-induced, but not EGF-induced mRNA expression of *c-fos* and *egr-1* was completely inhibited in EGCG-treated VSMCs (Ahn *et al*., 1999). In contrast, others have shown that theaflavin-3,3-digallate was more effective than EGCG in inhibiting EGF- or PDGF-induced phosphorylation of the EGF receptor (A431 cells) and PDGF receptor (NIH3T3 cells), respectively (Liang *et al*., 1999). In addition, EGCG and theaflavins strongly inhibited the proliferation of both A431 and NIH3T3 cells (Liang *et al.*, 1999). Another group showed that EGCG inhibits S phase entry in EGF-stimulated MCF10A breast epithelial cells when cells were treated in G0 or mid G1, but not when treated after G1 (Liberto and Cobrinik, 2000). Other effects included an induction of p21(CIP1/WAF1/SDI1), an inhibition of cyclin D1-associated pRB kinase activity, and an impairment of pRB phosphorylation (Liberto and Cobrinik, 2000).

The molecular effects of EGCG on the EGFR signaling pathway in human head and neck squamous cell carcinoma (HNSCC) cell lines were assessed (Masuda *et al.*, 2001). EGCG treatment resulted in cell cycle arrest and apoptosis. Decreases were observed in the cyclin D1 protein and in the hyperphosphorylated form of pRB and increases were seen in $p21^{Cip1}$ and $p27^{Kip1}$ proteins. Other changes induced by EGCG included decreases in the Bcl-2 and Bcl-X(L) proteins, an increase in the Bax protein, and activation of caspase 9, suggesting a mitochondrial pathway leading to apoptosis. Phosphorylation of the EGFR, signal transducer and activator of transcription3 (STAT3) and ERKs was also inhibited by EGCG (Masuda *et al.*, 2001).

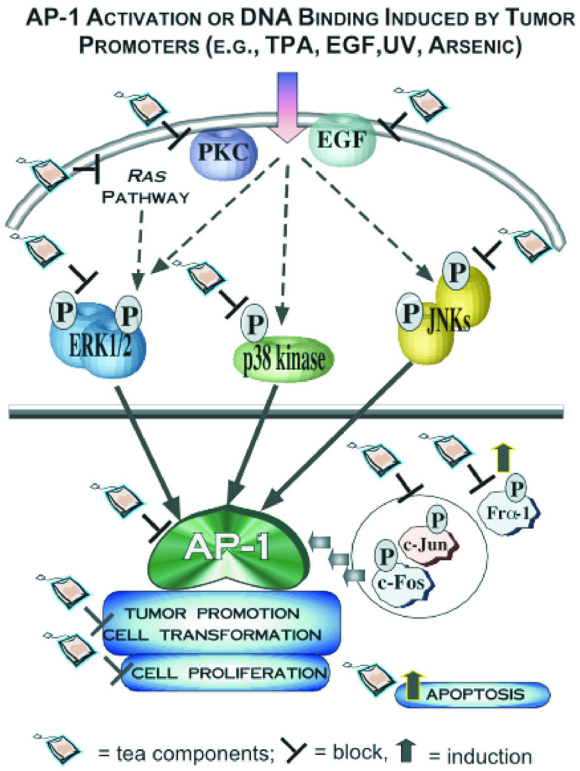

Fig. 1. Effect of tea components on AP-1activation induced by tumor promoters, including TPA, EGF, and UV irradiation.

Generally, the tea polyphenols seem to have an inhibitory effect on MAP kinase signaling but at least one group showed that in the macrophage cell line, Raw 264.7, COX-2 expression and activity and prostaglandin production are increased by EGCG treatment and are associated with the activation of both the ERKs and protein-tyrosine phosphatase signaling pathways (Park *et al*., 2001). EGCG appeared to stimulate the COX-2 promoter region suggesting a transcriptional effect as well as post-transcriptional effects (Park *et al*., 2001).

Still overall, studies strongly suggest that EGCG and theaflavins have a marked inhibitory effect on signal transduction pathways leading to AP-1 activation (see Fig. 1). An accretion of evidence indicates that inhibition of AP-1 signaling is important for the repression of cancer formation and growth and thus may be a significant chemopreventive target for tea.

Tea and NF-κB Activation

In many cell lines, tumor promoters also induce activation of NF-κB, which is a rapidly-induced stress-responsive transcription factor that functions to intensify the transcription

of a variety of genes including cytokines, growth factors and acute response proteins (Baeuerle and Henkel, 1994; Baeuerle and Baltimore, 1996; Baldwin, 1996; Karin, 1998; Karin and Ben-Neriah, 2000; Karin and Delhase, 2000). NF-κB activation has also been linked to MAP kinase signaling pathways, especially p38 kinase (Schulze-Osthoff *et al*., 1997). The mechanism for NF-κB activation is well understood. In its inactive form, NF-κB is found in the cytosol bound to an inhibitory protein called inhibitory kappa B (IκB). When stimulated, IκB is phosphorylated, ubiquinated, released from NF-κB and subsequently degraded. Following separation from IκB, NF-κB is translocated into the nucleus where it activates gene transcription by binding to its distinct DNA sequence found in specific genes. NF-κB activation is associated with initiation or acceleration of tumorigenesis (Gilmore, 1997) and in JB6 cells, inhibition of NF-κB also blocked tumor promoter-induced cell transformation (Li *et al*., 1997). Even though less is known about the effects of tea polyphenols on NF-κB activation, like AP-1, NF-κB may also be a potential chemopreventive or therapeutic target for tea components.

EGCG treatment has been shown previously (Ahmad *et al.*, 1997) to induce apoptosis in cancer cells but not in normal cells. These studies have been supported by new reports from others (Lu *et al*., 2000a). Ahmad *et al*. (2000b) recently reported that EGCG treatment inhibited cell growth, induced G0/G1-phase cell cycle arrest, and caused apoptosis in human epidermoid carcinoma (A431) cells but not in normal human epidermal keratinocytes (NHEK). The mechanism was suggested to be related to a differential inhibition by EGCG of TNF-α- or LPS-mediated NF-κB activation in cancer compared to normal cells, with the cancer cells generally being more sensitive to EGCG (Ahmad *et al*., 2000b).

EGCG and Theaflavins Block Phosphorylation and/or Degradation of IκB

We have shown that in JB6 cells, EGCG and theaflavins inhibit TPA-induced NF-κB activity and NF-κB sequence specific DNA binding in a concentration-dependent manner (Nomura *et al.*, 2000a). The mechanism for the inhibition appeared to occur through a blockade of the TPA-induced phosphorylation of IκBα at Ser32 in the same concentration range that prevented DNA binding. Others showed in Jurkat T cells and extracts that EGCG inhibits the chymotrypsin-like activity of the 20S proteasome, which targets a variety of proteins including p21, p53, Bax, $p27^{Kip1}$ and IκBα, for degradation (Nam *et al*., 2001). In this study, the net result of the inhibition was cell growth arrest and an accumulation of proteasome target proteins, $p27^{Kip1}$ and IκBα (Nam *et al*., 2001).

Theaflavin-3,3'-digallate also was shown to effectively block NF-κB activation, phosphorylation of cytosolic IκB and lipopolysaccharide-induced nuclear accumulation of NF-κB p65 and p50 subunits (Lin *et al*., 1999). These results are supported by others who observed that theaflavin-3,3'-digallate inhibited IKK1 and IKK2 activity in activated macrophages more strongly than did the other tea polyphenols (Pan *et al*., 2000). In that study, the inhibition of IKKs by theaflavin-3,3-digallate prevented the phosphorylation and degradation of IκB and inhibited NF-κB activity. These results suggest that tea polyphenols such as theaflavin-3,3-digallate may exert chemopreventive actions by suppressing the activation of NF-κB through inhibition of IKK activity and subsequent IκB phosphorylation and degradation.

Tea Components May Exert Their Anti-cancer Effects through Their Antioxidant Properties

Several studies suggest that the effects of EGCG and other tea components on NF-κB may be related to their antioxidant properties. For example, EGCG was shown to exhibit a protective effect against chromium (VI)-induced DNA damage and to inhibit both TPA- and chromium (IV)-induced NF-κB activation (Shi *et al*., 2000). Nitric oxide has also been suggested to play a key role in carcinogenesis. Tea polyphenols were shown to inhibit inducible nitric oxide synthase (iNOS) in activated macrophages and EGCG inhibited nitric oxide generation (Lin and Lin, 1997). EGCG treatment significantly reduced both protein and mRNA levels of iNOS. These results agreed with the finding that EGCG blocked NF-κB activation, which is necessary for iNOS induction. The mechanism appeared to be related to an inhibition of IκB degradation by EGCG agreeing with the studies discussed above. This same group also reported that theaflavin-3,3'-digallate inhibited generation of nitric oxide and iNOS protein in lipopolysaccharide-activated macrophages more effectively than EGCG (Lin *et al*., 1999).

Overall these results suggest that inhibition of NF-κB activation is also important in accounting for the anti-tumor promotion effects of EGCG and theaflavins (see Fig. 2). Thus NF-κB and its components are prime targets for chemopreventive compounds including tea polyphenols.

Tea and PI-3K Activation

Within the last year or so, significant additional signaling molecules have been implicated in the mechanism(s) responsible for the anti-tumor effects of the tea polyphenols. Phosphatidylinositol-3 kinase (PI-3K) is an important factor in carcinogenesis and an inhibitory effect of EGCG and theaflavins on activation of PI-3K and its downstream effects may further explain the anti-tumor promotion action of these tea constituents (Fig. 3). EGCG was recently shown to inhibit mouse mammary tumor virus (MMTV)-Her-2/neu NF639 cell growth in culture and colony formation in soft agar (Pianetti *et al*., 2002). The inhibition was associated with

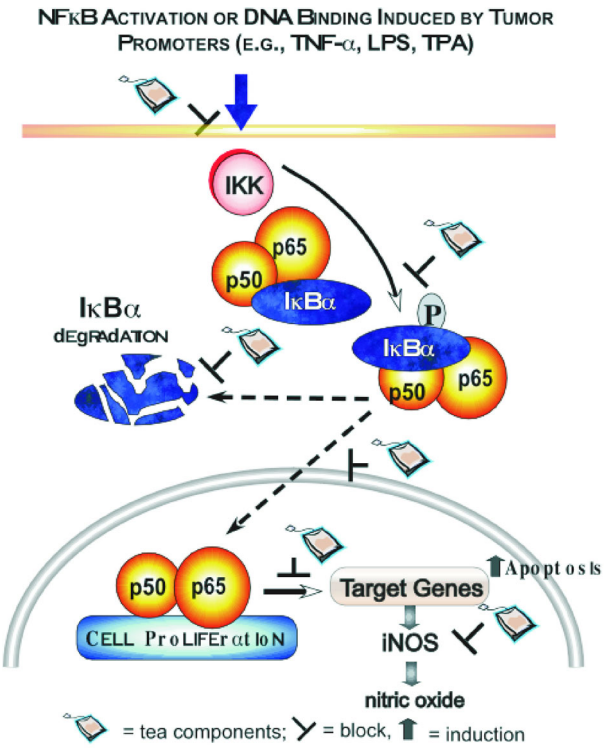

Fig. 2. Effect of tea components on NF-κB activation induced by tumor promoters, including TNF-α, LPS, and TPA.

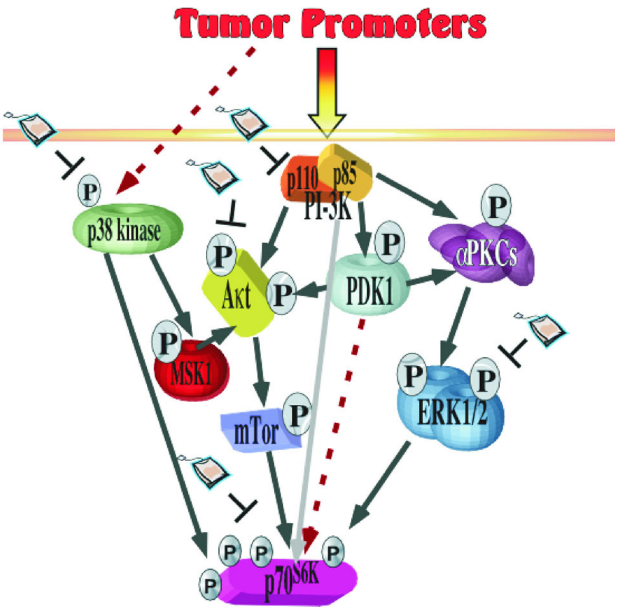

Fig. 3. Effect of tea components on PI-3K pathways.

decreases in PI-3K/Akt signaling, which apparently mediated decreases in NFκB DNA binding and activation. In addition, the high level basal phosphorylation and constitutive activation of the Her-2/neu receptor also appeared to be suppressed (Pianetti *et al.*, 2002). We have shown that EGCG or theaflavins inhibit UVB-induced PI-3K activation in mouse epidermal JB6 Cl41 cells (Nomura *et al.*, 2001). Furthermore, UVB-induced phosphorylation and activation of PI-3K downstream effectors, Akt and ribosomal p70 S6 kinase ($p70^{S6K}$), was also suppressed by the polyphenols. Results using various specific inhibitors indicated that UVB-activation of Akt and $p70^{S6K}$ was mediated through PI-3K, ERKs and p38 kinase but not JNKs. Interestingly, the addition of EGCG or theaflavins directly blocked UVB-induced $p70^{S6K}$ activation but only weakly inhibited UVB-induced Akt activation (Nomura, Kaji *et al.*, 2001). Obviously the anti-cancer effects of tea polyphenols are generated from a complex response of a multitude of signaling pathways and molecules.

Future Studies and Conclusions

Others and we have demonstrated that EGCG and theaflavins inhibit tumor promoter-induced activation of protein kinases, which are upstream of AP-1, NF-κB and other transcription factors. Although much is known, the exact mechanisms for the inhibition are not clear. We do know that tea polyphenols have a high affinity for binding to proline-rich proteins and many protein kinases in tumor promoter-induced signal transduction pathways contain proline-rich regions. We hypothesize that the chemopreventive effect of the tea polyphenol, EGCG, and its derivatives is a consequence of their ability to bind to protein kinases in signal transduction pathways through proline-rich regions (Fig. 4). This hypothesis is based on (a) data showing that tea polyphenols exert their effects through direct interactions with proline-rich regions of the protein (Hagerman and Butler, 1981; McManus *et al.*, 1985; Mehansho *et al.*, 1987a; Mehansho *et al.*, 1987b; Luck *et al.*, 1994; Murray and Williamson, 1994; Murray *et al.*, 1994); (b) the observation that many protein kinases in signal transduction pathways contain proline-rich regions; and (c) results showing that these proline-rich protein kinases play a critical role in tumor promoter-induced cell carcinogenesis transformation (Fig. 4). Support for the hypothesis comes from data showing that in JB6 cells, EGCG and theaflavins inhibit TPA-, arsenite- or UVB-induced phosphorylation or activation of proline-rich proteins, PI-3K, $p70^{S6K}$, and JNKs (Nomura *et al.*, 2000b; Nomura *et al.*, 2001), but not p38 kinases, which do not contain a proline-rich region. The dose ranges for inhibition of the activities of these kinases are similar to those for inhibition of malignant cell transformation (Huang *et al.*, 1997b).

Research findings suggest that components of either green tea or black tea effectively inhibit tumor promoter- or growth factor-induced cell transformation and AP-1 and NF-κB activation mediated through proline-rich kinase signaling cascades. Taken together, these results strongly suggest that tea polyphenols may be highly effective as chemopreventive agents that act by targeting specific tumor promoter-induced protein kinases and/or transcription factors. Obviously, the

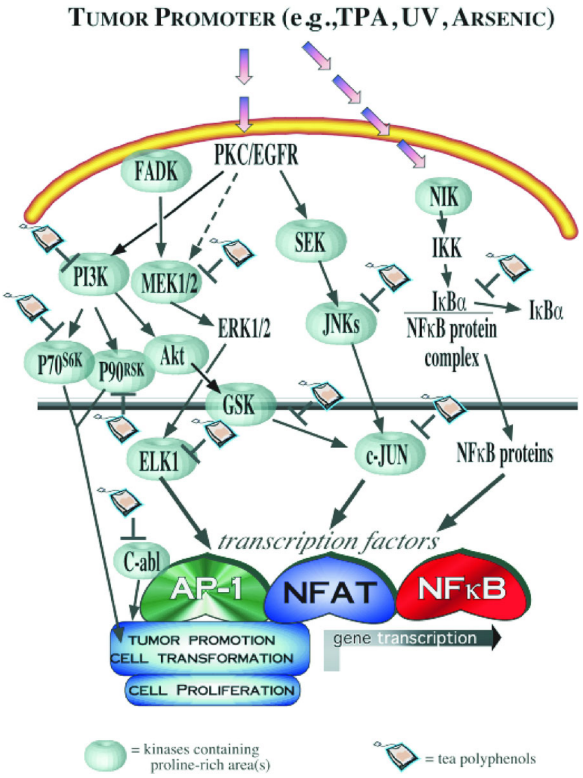

Fig. 4. Tea polyphenols target proline-rich kinases.

elucidation of the molecular mechanisms/targets associated with the anti-tumor effects exhibited by tea polyphenols will contribute greatly to the development and design of chemopreventive agents and more effective cancer chemoprevention trials, respectively.

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