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REVIEW ARTICLE

NF-κB and AP-1 as molecular targets for chemoprevention with EGCG, a review

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Abstract There are multiple lines of compelling evidence, mostly from population-based studies, supporting that green tea consumption is associated with reduced risk of several human malignancies. Epigallocatechin gallate (EGCG) is a major antioxidant present in green tea. Chemopreventive effects of EGCG have been extensively investigated and well documented. These include inhibition of metabolic activation of carcinogens and/or stimulation of their detoxification, scavenging of reactive oxygen species, induction of apoptosis or differentiation of malignant or transformed cells, suppression of tumor promotion, inhibition of angiogenesis or metastasis, etc. EGCG targets many intracellular signaling molecules and events responsible for malignant transformation or abnormal cell proliferation, and NF-κB and AP-1 appear to be two of most potential targets of EGCG in its exerting chemopreventive activities.

Keywords Chemoprevention · EGCG · Green tea · Signal transduction · NF-kappaB · AP-1

Accumulating evidence from epidemiologic, clinical and laboratory studies have revealed an inverse-relationship between increased green tea intake and the relative risk for cancers (Yang 1997; Suganuma et al. 1999; Mukhtar and Ahmad 1999). The chemopreventive effects of green tea have been attributed to polyphenolic ingredients that have potent antioxidant properties (Park and Surh 2004). Among many polyphenolic compounds isolated from green tea, epigallocatechin gallate (EGCG) is recognized as a key active constituent in terms of cancer chemopreventive potential. Recently, much attention has been focussed on the intracellular signaling pathways as potential targets for chemoprevention. This paper focusses on effects of EGCG on signaling mediated by NF-κB and AP-1 that are ubiquitous eukaryotic transcription factors implicated in malignant transformation.

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Molecular aspects of chemoprevention

Rational and successful implementation of chemoprevention strategy relies on the precise understanding of carcinogenesis at cellular and molecular levels. Based on the experimentally-induced carcinogenesis models, the process of tumorigenesis consists of three distinct steps, initiation, promotion, and progression. Unique biological and morphological changes occur in cells during each stage of carcinogenesis. Initiation, an irreversible and relatively short-term event, has been ascribed to mutation in the genomic DNA. Promotion, a reversible and relatively lengthy process, is considered to involve an epigenetic mechanism, which results in the clonal expansion of initiated cells to form an actively proliferating premalignant cell population. Progression, an irreversible process, results from genetic instability which leads to additional mutagenic and epigenetic changes, procucing a new clone of tumor cells with increased proliferative capacity, invasiveness, and metastatic potential. Multistage carcinogenesis may span over 20 years, during which opportunities to suppress, reverse, or even delay the critical events in its early and premalignant stages can be provided. Recent advances in our

understanding of the carcinogenic process at the molecular level have identified numerous cellular molecules and events associated with each stage of carcinogenesis that could be potential targets of structurally diverse chemopreventive agents.

Intracellular signaling molecules as novel targets for chemoprevention

The progress in cellular and molecular biology has enabled us to unravel the intracellular signaling events leading to malignant transformation. The cellular signaling pathways that regulate proliferation, survival and transformation of cells are of particular interest in current cancer biology. Many of the molecular alterations associated with carcinogenesis occur in cell signaling pathways that regulate cell proliferation and differentiation. Components of signaling networks include several kinases, such as the family of proline-directed serine/threonine kinases named mitogen-activated protein kinases (MAPKs), protein kinase C (PKC), phosphoinositide-3-kinase (PI3K), etc., which maintain homeostasis of the cell.

Abnormal or improper activation or silencing of these kinases or its downstream transcription factors can result in uncontrolled cell growth, leading to malignant transformation. Therefore, the intracellular signaling pathways are now recognized as important molecular targets for chemoprevention (Bode and Dong 2004). Some chemopreventive phytochemicals 'switch on' or 'turn off' the specific signaling molecules, depending on the nature of the cascade they target, thereby preventing abnormal cell proliferation and growth (Surh 2003).

NF-κB and AP-1 as prime regulators of cell signaling

Numerous intracellular signaling pathways converge with the activation of distinct sets of transcription factors. Of particular interest are NF-κB and AP-1, which are the evolutionarily conserved eukaryotic transcription factors, acting independently or co-ordinately to regulate expression of those genes involved in various physiological processes (Dorai and Aggarwal 2004). A wide range of signals including cytokines, mitogens, phorbol esters, growth factors, environmental and occupational particles, toxic metals, intracellular stresses, bacterial toxins, viral products, and ionizing radiation induce expression of early response genes through activation of NF-κB (Karin 1999) and AP-1 (Shaulian and Karin 2002).

Activation of NF-κB and/or AP-1 contributes to tumorigenesis by transactivating several classes of target genes that have inflammatory, immunoregulatory, anti-apoptotic, and the cell cycle regulatory functions (Figs. 1 and 2). The ability of these transcription factors to suppress apoptosis and to promote cell-cycle progression clearly indicates that they may participate in many aspects of oncogenesis (Shaulian and Karin 2002; Greten and Karin 2004). Indeed, the elevation of NF-κB or AP-1 activity is evident in a number of human malignancies. Although NF-κB was initially considered as a pro-apoptotic factor, accumulating data from subsequent studies have altered this view, addressing an anti-apoptotic role of NF-κB in response to a variety of proapoptotic insults (Chen et al. 2001). NF-κB has been shown to antagonize the function of the tumor suppressor protein p53 (Chen et al. 2001). Activation of both NF-κB and AP-1 promotes cell cycle transition by a direct transcriptional up-regulation of *cyclin D1* (Shaulian and Karin 2002; Chen et al. 2001). Up-regulation of anti-apoptotic genes, such as *cIAP1, cIAP2*, *XIAP*, *Bcl-2* and *Bcl-*X_L, by NF-κB provides tumor cells with survival advantages (Chen et al. 2001) Down-regulation of apoptosis-inducing genes, such as $p21^{WAF1/CIP1}$, *p16*, *p19* and *p53*, as well as up-regulation of the anti-apoptotic gene *Bcl-3* by c-Jun activation indicates the anti-apoptotic role of AP-1 as well (Shaulian and Karin 2002; Shaulian and Karin 2001).

Considering the role of NF-κB and AP-1 transcription factors in carcinogenic processes, especially those related to tumor promotion, cell cycle transition and preventing apoptosis, it is plausible that these transcription factors are the prime molecular targets of chemopreventive phytochemicals. Multiple lines of evidence suggest that EGCG interferes with several signaling pathways involved in the activation of NF-κB and AP-1 (Figs. 1 and 2).

Effects of EGCG on cellular signaling network

As mentioned above, NF-κB and AP-1 that are potential mediators of cellular signal transduction events can be potential molecular targets for chemoprevention with EGCG and possibly other chemopreventive tea polyphenols (Bode and Dong 2003; Surh 1999). The plausible mechanism underlying suppression of anchorage-independent transformation by EGCG was examined in the epidermal growth factor- and 12-*O*-tetradecanoylporbol-13-acetate (TPA)-stimulated mouse epidermal JB6 cell line (Dong 2000; Nomura et al. 2000). EGCG was also found to inhibit UVB-induced AP-1 activation as well as NF-κB-dependent transcriptional activation (Nomura et al. 2001). In another study, EGCG inhibited UVB-mediated NF-κB activation in normal human epidermal keratinocytes (Afaq et al. 2003). Recently, we reported that EGCG could suppress COX-2 expression induced by TPA in mouse skin *in vivo* and also in cultured human breast epithelial cells, possibly by inhibiting the activation of both ERK and NF-κB (Kundu et al. 2003). EGCG treatment inhibited expression of matrix metalloproteinase (MMP)-2

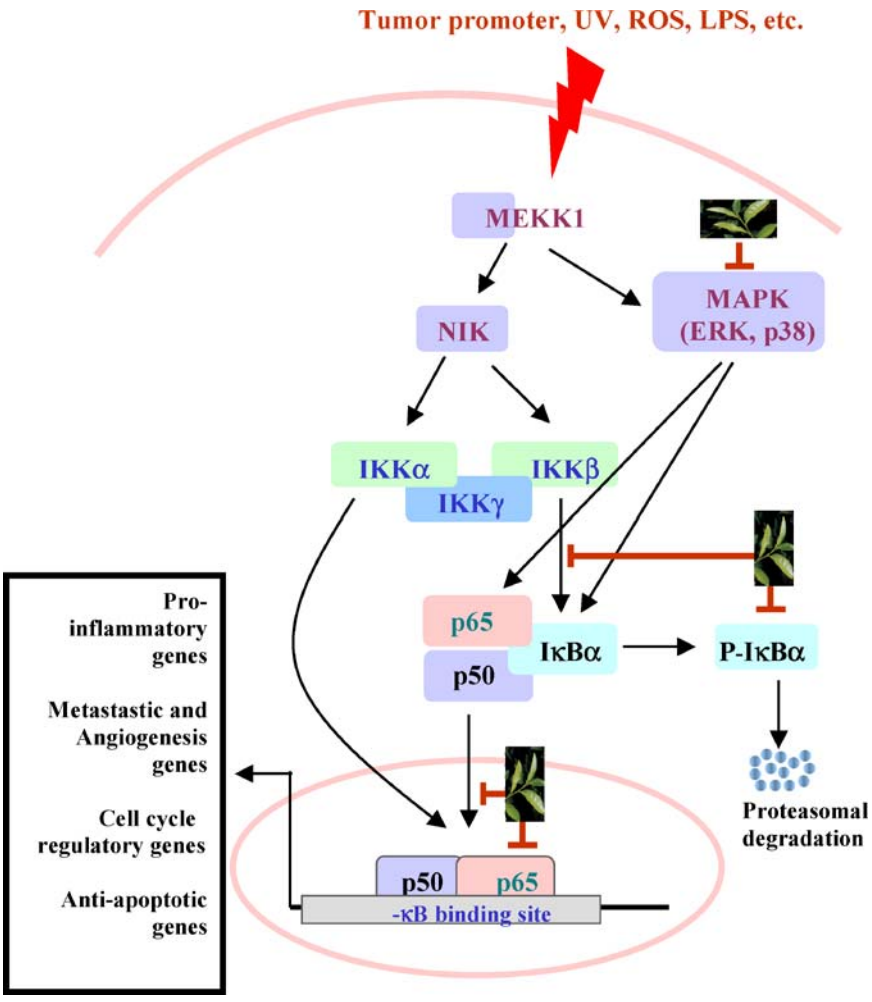

Fig. 1 Intracellular signaling pathways involving activation of NF-κB and its modulation by EGCG

and -9 via inactivation of MAPKs and NF-κB in human prostate carcinoma DU-145 cells (Vayalil and Katiyar 2004). Treatment of human epidermoid A431 cells with EGCG resulted in dose-dependent inhibition of NF-κB/p65, induction of DNA breaks, cleavage of poly(ADP-ribose)polymerase and morphological changes characteristic of apoptosis (Gupta et al. 2004). Furthermore, pretreatment of cells with the caspase inhibitor Z-VAD-FMK led to increased nuclear translocation, DNA binding and transcriptional activity, thereby protecting the cells from EGCG-induced apoptosis. These findings suggest that EGCG-mediated activation of caspases is critical, at least in part, for inhibition of NF-κB and subsequent apoptosis (Gupta et al. 2004). EGCG strongly inhibited the constitutive activation of NF-κB in human head and neck squamous cell carcinoma (HNSCC) and breast carcinoma (MDA-MB-231) cell lines (Masuda et al. 2002).

In addition, the alterations in NF-κB activity by EGCG in fetal rat intestinal IEC-6 cells were accompanied by blockade of inhibitory kappa B (IκB) kinase complex (IKK). The antioxidative capacity of EGCG did not appear to be responsible for this process, but the gallate group was rather essential (Yang et al. 2001). The involvement of the gallic moiety in inhibition of transformed cell growth and activation of AP-1 or MAPKs was also demonstrated in cultured 30.7b Ras 12 and BES21 cells (Yang et al. 2000). The effect of EGCG on AP-1 transcriptional activation was explored in HCL14 (Barthelman et al. 1998) or HaCaT (Chen and Bowden 2000) keratinocytes. EGCG significantly reduced the accumulation of the c-Fos component of AP-1 in both studies. Similar AP-1 inhibition was observed in the epidermis of transgenic mice containing an AP-1 luciferase reporter gene (Barthelman et al. 1998). More recently, EGCG has been reported to inhibit TPA-induced MMP-9 expression by blocking activation of AP-1 and upstream kinases, such as ERK and JNK, in human gastric AGS cells (Kim et al. 2004).

Concluding remarks

Evidence for cancer chemopreventive activities of EGCG and related green tea polyphenols has been accumulating, but the intracellular events or molecules that these

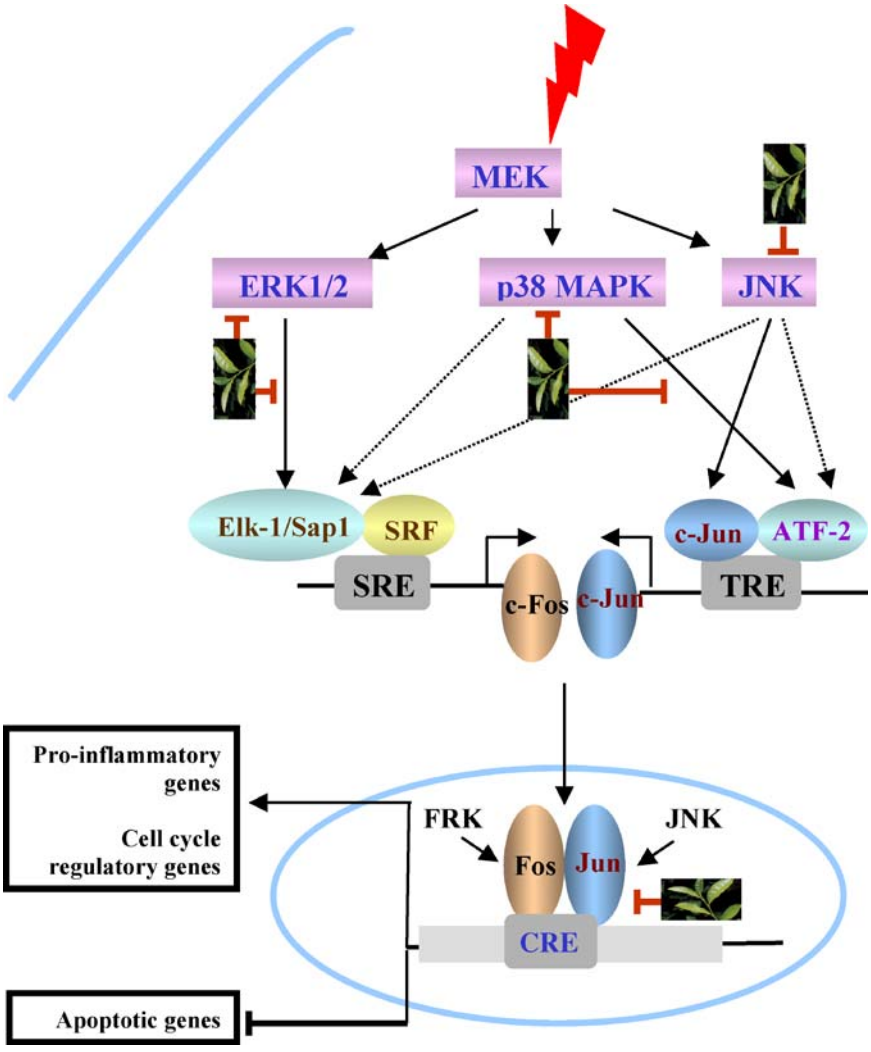

Fig. 2 AP-1 as an alternative molecular target of EGCG

phytochemicals target still need further investigation. Since NF-κB and AP-1 mediate pleiotropic effects of both external and internal stimuli in the cellular signaling cascades, they are potential targets of EGCG. Recent advances in DNA microarray technologies and mammalian genome sequencing have enabled us to quantitatively assess the expression profiles of a distict set of genes under specified conditions. Attempts have been made to identify the genes affected by EGCG using a cDNA expression array that allows simultaneous identification of a milieu of genes regulated (Fujiki et al. 2001; Wang and Mukhtar 2002; Weinreb et al. 2003). Such approaches will facilitate identification of genes whose expression is modulated by EGCG under the control of NF-κB and AP-1 transcription factors. More work is warranted to clarify the gene expression profile in conjunction with the intracellular signaling modulated by EGCG.

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