

Oral Cancer Prevention Advances with a Translational Trial of Green Tea

Perspective on Tsao et al., p. 931

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Abstract

This perspective on Tsao et al. (beginning on p. 931 in this issue of the journal) discusses green tea extract, which was shown for the first time to have dose-dependent effects in a clinical chemopreventive setting (oral premalignant lesions). This translational trial provides important data on angiogenesis and other biomarkers on which to base future clinical research, which should include trials of green tea extract or polyphenols combined with other natural or synthetic compounds to enhance chemopreventive effects.

Tea and other natural dietary agents have drawn substantial attention from both researchers and the general public because of their ready availability, low toxicity, and potential ability to suppress carcinogenesis and reduce the risk of cancer. Tea is one of the most widely consumed beverages in the world. It contains polyphenols with antioxidant properties and is associated with cancer-preventive effects in many epidemiologic and pre-clinical studies (1). Different processing techniques yield different types of tea. Although both green and black tea have been studied for their chemopreventive potential, green tea has shown greater promise and efficacy against multiple types of cancer. Of the several polyphenols found in different tea preparations, epigallocatechin 3-gallate (EGCG) is the most abundant (50-80% of the antioxidant polyphenol content of tea) and best studied and possibly has the most potent antioxidant activity (2-4). Other tea polyphenols include (-)-epigallocatechin, (-)-epicatechin gallate, and (-)-epicatechin (3, 4).

EGCG and other polyphenols clearly have both direct and indirect molecular effects on tumor-signaling pathways. EGCG directly binds to a number of proteins including laminin, vimentin, Fas, and insulin-like growth factor-1 receptor and has indirect effects on epidermal growth factor receptors (EGFR), signal transducers and activators of transcription, activator protein-1, and nuclear factor- κ B (NF- κ B; refs. 5-14).

Green tea polyphenols can also induce cell cycle arrest or apoptosis by activating p53 and its multiple target genes (Fig. 1). EGCG induced the expression of p53 and its targets p21 and BAX in prostate cancer cells with wild-type but not inactive p53 (12). EGCG also activated p53 and BAX in breast cancer cells (15). Amin et al. reported that EGCG induces apoptosis by activating p73-dependent expression of a subset

of p53 target genes including *p21*, *cyclin G₁*, *MDM 2*, *WIG1*, and *PIG11* (13).

EGCG also acts as a potent inhibitor of NF- κ B pathways (14). It may block one or more steps in the NF- κ B signaling pathway, such as the effects of the most upstream growth factor receptors that activate the NF- κ B signaling cascade, translocation of NF- κ B to the nucleus, DNA binding of the dimers, or interaction with the transcriptional machinery. NF- κ B target genes that are negatively regulated by EGCG include Bcl-2, Bcl-x(L), cyclin D1, matrix metalloproteinase (MMP), and vascular endothelial growth factor (VEGF). VEGF is a critical contributor to angiogenesis, which is a very promising target for chemoprevention (16).

Green tea polyphenols inhibit the angiogenesis of breast cancer cells by inhibiting the expression of VEGF and MMP-9 through suppressing signal transducers and activators of transcription-3 activation (17, 18). EGCG also inhibited cell viability, capillary tube formation, and migration of human umbilical vein endothelial cells and inhibited angiogenic and metastasis markers (VEGF, CD31, MMP-2, MMP-7, MMP-9, and MMP-12) in a xenograft model of pancreatic cancer (19).

It is well established that EGCG treatment inhibits the phosphorylation of EGFR and its downstream targets AKT and ERK and potentiates the effects of the EGFR tyrosine kinase inhibitor erlotinib in head and neck cancer (20). EGCG induced the internalization and ubiquitin-mediated degradation of EGFR, ultimately undermining EGFR signaling. It has also been reported that EGCG inhibited the activation of EGFR and the insulin-like growth factor-1 receptor in human colon cancer cells (21).

As reported in this issue of the journal (22), Tsao et al. have conducted an important phase II randomized, placebo-controlled trial of green tea extract (GTE) in patients with high-risk oral premalignant lesions. This work joins the seminal contributions that decades of retinoid research have made to the chemoprevention of oral, head and neck, and other cancers. Indeed, chemoprevention entered mainstream cancer research in the 1970s through the pioneering study of retinoids by Sporn et al. (23). Hong et al. conducted the first randomized clinical retinoid trial, which showed that high-dose 13-*cis*-retinoic acid (13-*cRA*) significantly reduced the size of oral premalignant lesions and reversed dysplasia (24). The

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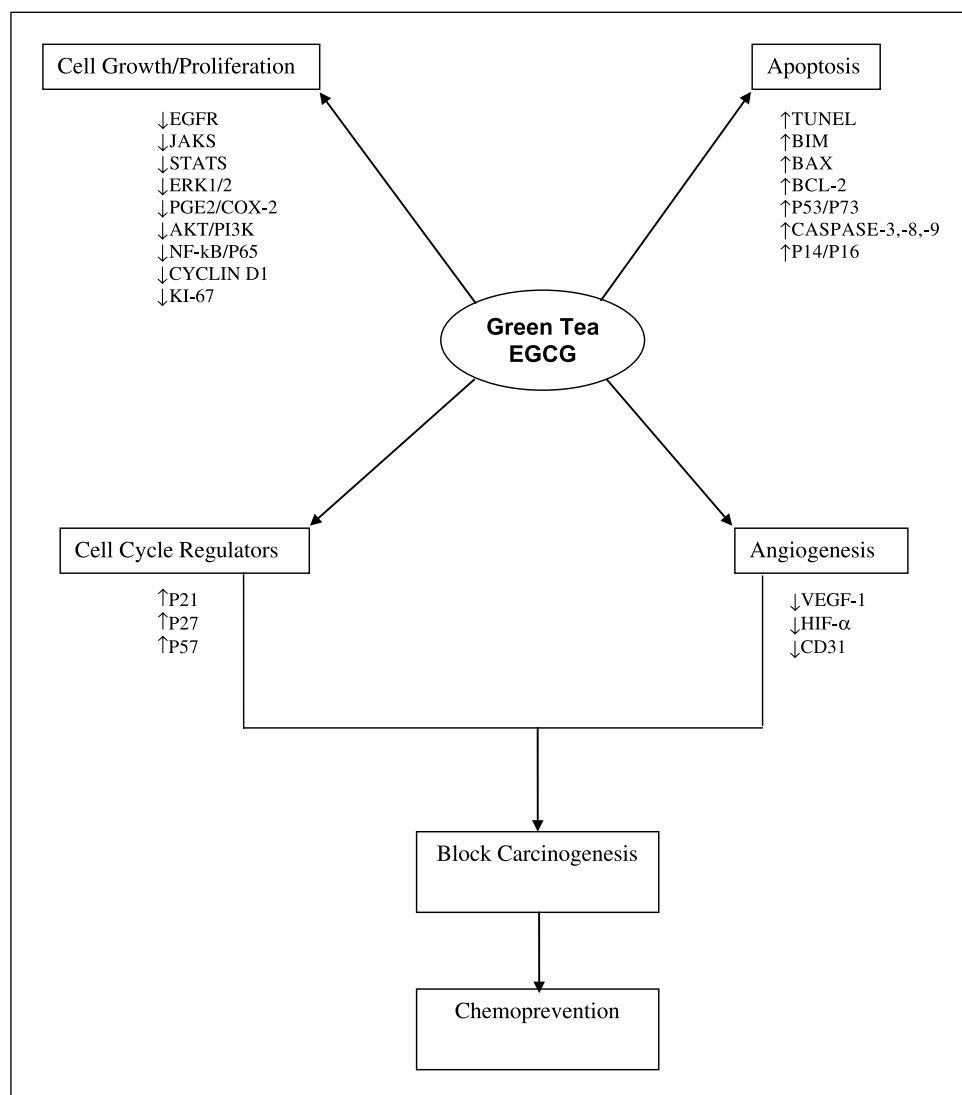


Fig. 1. Molecular mechanisms of EGCG activity.

follow-up phase III trial of high-dose 13-cRA induction followed by either low-dose 13-cRA or β -carotene maintenance suggested that low-dose 13-cRA was superior to β -carotene in maintaining the remission of premalignant lesions (25). Another phase III trial of high-dose 13-cRA showed a significant reduction in the incidence of second primary tumors after the 1-year treatment period and lasting for a total of 3 years (26). A number of single-agent, low-dose 13-cRA trials followed, with negative results (27–29). The combination of high-dose 13-cRA, α -IFN, and α -tocopherol seemed to be very effective in delaying head and neck-associated second primary tumors and recurrence (30), and a subsequent phase III trial of this combination was initiated but stopped because of slow patient accrual. High-dose 13-cRA was active against oral premalignant lesions but too toxic for long-term administration. Programmatic effort to extend the early high-dose promise and reduce toxicity with a lower dose retinoid or retinoid combinations ultimately did not reduce the incidences of oral and other head and neck cancers (29).

Following in these footsteps, Tsao et al. randomly assigned patients with high-risk oral premalignant lesions to receive placebo or GTE at 500, 750, or 1,000 mg/m² thrice a day for

12 weeks, evaluating biomarkers in baseline and 12-week biopsies (22). The clinical response was greater in the combined higher dose (750 and 1,000 mg/m²) GTE arms (58.5%) than in the 500 mg/m² (36.4%) or placebo (18.2%; $P = 0.03$) arms, suggesting a dose-response effect of GTE in oral premalignant lesions. GTE treatment was well tolerated, although higher doses increased insomnia and nervousness (albeit without grade 4 toxicities). This study also provided some very important biomarker data, including data on VEGF that are consistent with previously reported effects of GTE (18, 31). Baseline stromal VEGF levels correlated with clinical ($P = 0.04$) but not histologic response. Baseline levels of other biomarkers (epithelial VEGF, p53, Ki-67, cyclin D1, and p16 promoter methylation), however, were not associated with outcome. Furthermore, stromal VEGF and cyclin D1 expression were downregulated in the lesions of clinically responsive patients and upregulated in nonresponsive patients at 12 weeks (versus baseline levels), consistent with the results of preclinical green tea studies (discussed earlier). A similar cyclin D1 effect was seen in a clinical trial of a high-dose 13-cRA-based combination in patients with advanced oral premalignant lesions (32). Tsao et al. conclude that GTE may suppress oral

pre-malignant lesions, in part through blocking angiogenic stimuli. This randomized phase II study not only contributes very important findings to the field but also provides significant support and guidance for the incorporation of biomarkers in clinical trials; it provides an important basis for future phase III studies.

It is important to emphasize that conducting biomarker evaluations within a clinical trial is very difficult and challenging—requiring, for example, tremendous effort in performing multiple biopsies and biomarker analyses in the Tsao et al. study. Undertaking clinical chemoprevention trials that incorporate biomarkers, however, is the only way to ascertain whether or not a chemopreventive agent is working at the molecular level and to understand mechanisms of response and resistance (33). Such biomarker studies will be critical in clinical trials of natural compounds because these agents are relatively nontoxic and thus may not be well-suited to the traditional phase I therapy end points of maximum tolerated dose and dose-limiting toxicity. Determining biological activity by molecular marker modulation will be an excellent guide

to the best methods and chemoprevention settings for these agents. Therefore, Tsao et al. are to be congratulated for their enormous effort in incorporating biomarkers into their clinical trial of GTE in patients with oral pre-malignant lesions, and for their contribution to the development of chemoprevention strategies using natural compounds with the potential to reduce cancer risk. Combined-agent approaches are of emerging importance to cancer chemoprevention (34), and it will be particularly important to examine tea product-based combinations (e.g., EGCG) with the potential to maximize chemopreventive efficacy (versus single agents), in part, by suppressing the emergence of resistant clones, a typical obstacle to long-term chemoprevention. Indeed, our recent preclinical study of EGCG combined with an EGFR inhibitor showed promising effects, including NF- κ B suppression, in head and neck carcinogenesis (35).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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