Mechanistic Findings of Green Tea as Cancer Preventive for Humans (44370)

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Abstract. **Based on our initial work with green tea, in which repeated topical applications of (−)-epigallocatechin gallate (EGCG), the main green tea polyphenol, inhibited tumor promotion in a two-stage carcinogenesis experiment on mouse skin (Phytother Res 1, 44–47, 1987), numerous scientists have since provided so much additional evidence of the benefits of drinking green tea that it is now an acknowledged cancer preventive in Japan, and will possibly soon be recognized as such in other countries. Our work has so far produced several important results with EGCG and green tea: a wide range of target organs in animal experiments for cancer prevention, wide bioavailability of ^{3}H-EGCG in various organs of mice, delayed cancer onset of patients with a history of consuming over 10 cups of green tea per day, and absence of any severe adverse effects among volunteers who took 15 green tea tablets per day (2.25 g green tea extracts, 337.5 mg EGCG, and 135 mg caffeine) for 6 months. This paper introduces three new findings: 1) EGCG interacted with the phospholipid bilayer membrane resulting in confirmation of the sealing effect of EGCG; 2) EGCG inhibited TNF-α gene expression in the cells and TNF-α release from the cells; 3) high consumption of green tea was closely associated with decreased numbers of axillary lymph node metastases among premenopausal Stage I and II breast cancer patients, and with increased expression of progesterone and estrogen receptors among postmenopausal ones. These results provide new insights into our understanding of the mechanisms of action of tea polyphenols and green tea extract as a cancer preventive.**

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More than 10 years ago, identification of the main green tea polyphenol, (−)-epigallocatechin gallate (EGCG), opened up a new area of cancer prevention for humans, based on various studies of the inhibitory effects of EGCG and green tea in rodent carcinogenesis experiments (1), along with the results of prospective cohort studies of humans (2). The important features of EGCG and green tea as cancer preventives for humans are as follows: they are nontoxic, for rodents and humans (1); they have a wide range of target organs, which correlates well with wide distribution of ^{3}H-EGCG (3); they have inhibitory effects on growth of cancer cells associated with G_2/M arrest in PC-9 cell line (4); and they have demonstrated inhibitory effects on lung metastasis of B16 melanoma cells associated with reduction of various cytokine levels (5). This paper deals with three new findings related to mechanisms of action of green tea polyphenols.

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Results and Discussion

Sealing Effect of EGCG. We previously reported that repeated topical applications of EGCG to mouse skin inhibited tumor promotion of two different tumor promoters, teleocidin, one of the 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-type tumor promoters, and okadaic acid (1). Since TPA is an activator of protein kinase C (PKC) (6), and okadaic acid is an inhibitor of protein phosphatases 1 and 2 A (7), EGCG inhibits two different mechanisms of tumor promotion. A membrane fraction of mouse skin that was

treated with a single topical application of EGCG showed immediate reduction of specific binding of both TPA and okadaic acid to their receptors (8), a phenomenon we called the sealing effect of EGCG. Since EGCG is also thought to inhibit similar interaction between those of hormones, growth factors and cytokines and their receptors (Fig. 1).

One of our authors (K. S.) attempted to clarify the sealing effect of EGCG (9). Liposomes were first prepared, and various concentrations of EGCG were dispersed to them, after which the effects on PKC activation and protein phosphatase activity were studied. Figure 2 shows inhibition of PKC activation by EGCG, as one of the results. PKC was fully activated by 2 μ*M* TPA without EGCG; with EGCG, it was completely inhibited. Increasing the amount of TPA recovered this inhibition of PKC activation by 50%, but it was never recovered completely because EGCG and TPA bind competitively to PKC in the lipid bilayer membrane. It is assumed that EGCG works its sealing effect of PKC by inhibiting interaction of various ligands with proteins.

In addition, results of the following experiments give support to the sealing effect of EGCG. EGCG is distributed onto the surface region of phospholipid bilayer membrance, and it forms aggregates where aromatic groups of EGCG molecules stack regularly. Thus, it is well established that the sealing effect of tea polyphenols is the key to their inhibitory action (8, 9).

Inhibition of TNF-α Release. Our recent study of tumor promotion revealed that a tumor promoter induces TNF-α gene expression in its target organs, and an inhibitor of tumor promotion inhibits both TNF-α gene expression and TNF-α release, resulting in reduction of the amount of endogenous tumor promoter in cancer cells and their surrounding tissue. (−)-Epicatechin gallate (ECG), EGCG, and (−)-epigallocatechin (EGC) dose-dependently inhibited TNF-α release from the human stomach cancer cell line, KATO III cells treated with okadaic acid, whereas EC, an inactive compound, did not (Fig. 3). The results suggest that three tea polyphenols containing a galloyl moiety inhibit TNF-α gene expression in the cells.

The tumor promoter okadaic acid induces expression of the TNF-α gene as well as early response genes, such as the *jun* and *fos* gene families. Table I shows results of our study of whether inhibitors of tumor promotion and cancer preventive agents inhibit expression of the TNF-α gene as well as early response genes. It is extremely important to note that all of these agents, EGCG, sarcophytol A, canventol, and tamoxifen, commonly inhibited TNF-α gene expression, but not early response gene expression (10).

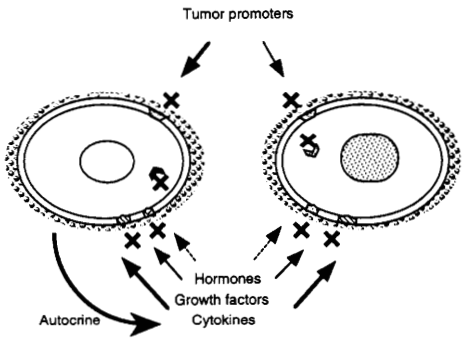

Figure 1. A schematic illustration of the sealing effect of EGCG, mediated partly through a sealed cell membrane by tea polyphenols.

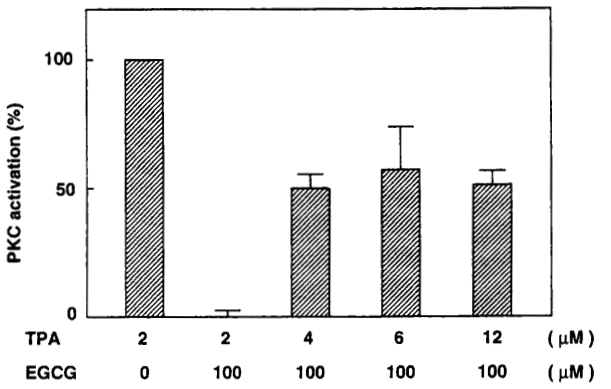

Figure 2. Inhibition by EGCG of protein kinase C activation induced by TPA.

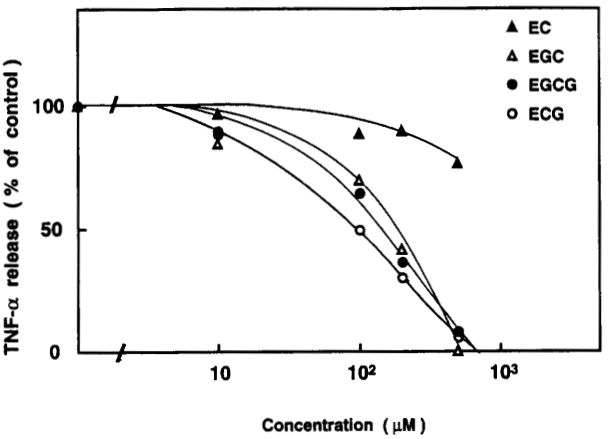

Figure 3. Inhibition by tea polyphenols of TNF-α release from KATO III cells treated with 50 n*M* okadaic acid.

Table I. Effects of Cancer Preventive Agents on Expression of TNF-α Gene and Early Response Genes[a]

	TNF-α	c-*jun*	*jun* B	*jun* D	c-*fos*	*fos* B
Sarcophytol A (20 μ*M*)	5	160	230	80	4360	570
Canventol (100 μ*M*)	15	140	230	80	540	190
EGCG (100 μ*M*)	57	30	40	120	640	130
Tamoxifen (20 μ*M*)	4	n.d.[b]	200	100	n.d.	370

[a] Expression of mRNA induced by 0.2 μ*M* okadaic acid was expressed as 100%.
[b] n.d., not determined.

Table II. Recurrence Rate of Breast Cancer in Relation to Daily Consumption of Green Tea

	Daily consumption of green tea (cups) (n = 472 patients)	
	≦4	≧5
Stages I and II (390 patients)		
Recurrence rate (%)	24.3	16.7
Disease-free years	2.8	3.6
Stage III (82 patients)		
Recurrence rate (%)	48.8	58.5
Disease-free years	1.9	1.9

Epidemiological Results with Drinking Green Tea. Our colleagues (K. I., K. N.) conducted a prospective cohort study with 8552 individuals in Yoshimi town in Saitama Prefecture beginning in 1986 (11). Their 10-year follow-up study identified 419 cancer patients (175 females and 244 males), and they reported the first evidence that cancer onset of patients who had consumed over 10 cups of green tea per day was 7.3 years later among females, and 3.2 years later among males, compared with patients who had consumed under 3 cups per day (2). The difference between females and males is partly due to higher tobacco consumption by males. This was the first association found between delayed cancer onset and high consumption of green tea. In addition, a lower relative risk was observed for lung cancer, colon cancer, and liver cancer (2).

Next our colleagues (KS, KI, KN) examined the association between consumption of green tea prior to clinical cancer onset and clinical parameters of 472 breast cancer patients (12). Breast cancer patients were histologically classified into Stages I, II, and III. Consumption of green tea by the patients was roughly divided into two classes, less than four cups per day and more than five cups per day. Among Stage I and II cancer patients, the group consuming over 5 cups per day showed a lower recurrence rate (16.7%) and a longer disease-free period (3.6 years) than those consuming less than four cups per day (Table II) (12). Stage III cancer patients did not show any significant differences (Table II). It is assumed that Stage III breast cancer contains more accumulated genetic changes in the cells than Stages I and II, suggesting that green tea is more effective in the early stages of cancer development.

For Stage I and II breast cancer patients, two clinical parameters show a significant association with daily consumption of green tea: the mean number of metastasized axillary lymph nodes in premenopausal patients, and the mean expression of progesterone and estrogen receptors in postmenopausal patients. Specifically, increased consumption of green tea was closely associated with a decreased number of metastasized axillary lymph nodes, and with increased expression of PgR and ER (Table III) (12). Since these clinical parameters are the most reliable predictors of recurrence, we think green tea in drinking water prior to clinical cancer onset will lead to more hopeful prognoses for breast cancer patients.

Additional Evidence. Our colleague, Dr. Yoshibumi Matsushima, recently discovered an unusual mouse that shows the aged appearance associated with reduction of hair growth. The cholesterol level of this mouse was found to be 10 times higher than that of normal mice, and the cause of the disease was the lack of the apolipoprotein E (apo E) gene. Thus, we named the mouse the spontaneously hyperlipidemic (SHL) mouse (13).

The cholesterol level of this mutant mouse slowly decreased after birth but remained constantly high. This brought to mind the results of a prospective cohort study with 1330 men in Saitama Prefecture, a study in which a significant reduction of serum total cholesterol and triglyceride concentration was found in the group that consumed over 10 cups of green tea per day (11). In a similar manner, giving a 0.1% green tea extract solution to male SHL mice resulted in reduction of their serum cholesterol levels (Matsushima, personal communication).

The evidence that EGCG and green tea are natural and readily available inhibitors of TNF-α gene expression makes it possible for us to extend the idea to various human diseases. Since TNF-α is involved in various other pathological states, such as rheumatoid arthritis, Crohn's disease, multiple sclerosis, graft versus host disease, HIV replication, malaria, sepsis, and cachexia associated with cancer (14), green tea could be beneficial not only for cancer prevention but also in the therapy and prevention of other diseases.

Table III. Association Between Consumption of Green Tea and Number of Metastasized Axillary Lymph Nodes and Expression of PgR and ER

	Daily consumption of green tea (cups)			
	≦4	5~7	≧8	
Premenopausal breast cancer (236 patients)				
Mean number of metastasized axillary lymph nodes	2.24	1.17	0.95	(P = 0.02)
Postmenopausal breast cancer (133 patients)				
Mean expression of				
PgR (fmol/mg protein)	32.2	91.3	98.4	(P = 0.06)
ER (fmol/mg protein)	112.8	112.9	180.4	

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