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EDITORIAL

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Two stages of cancer prevention with green tea

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Abstract Cancer chemoprevention is a new and important medical science in its own right. On the occasion of my presentation entitled "Natural agents and cancer chemoprevention" at the 90th AACR Meeting in 1999, I summarized our recent results on cancer prevention with green tea. In this article, the present status of clinical trials supported by the Chemoprevention Branch of the National Cancer Institute in the United States is first described by way of introduction. Although various natural products are now under investigation in phase I clinical trials, green tea has, perhaps, the greatest potential for further development. In order to expand our understanding of the effects of tea polyphenols and green tea, I review their ability to inhibit growth and cause apoptosis of cancer cells, their distribution into target organs and their other cancer-preventing properties. In addition, the paper focuses on the significance of reducing tumor necrosis factor α (TNFα) gene expression in cells and TNFα release from cells as essential activities for cancer prevention. As for the amounts of green tea effective in cancer prevention, I present two results from our Research Institute: a prospective cohort study with over 8000 individuals in Saitama Prefecture revealed that the daily consumption of at least ten Japanese-size cups of green tea resulted in delayed cancer onset, and a follow-up study of breast cancer patients conducted at our Hospital found that stages I and II breast cancer patients consuming over five cups per day experienced a lower recurrence rate and longer disease-free period than those consuming fewer than four cups per day. Thus, I propose here, for the first time, the two-stage approach to analyzing cancer prevention with green tea: cancer prevention before cancer onset and cancer prevention following cancer treatment. As an additional example of cancer prevention with natural agents, kava, a daily beverage in Fiji, is mentioned. All the evidence reminds us of the significance of alternative medicine in practical cancer prevention.

Introduction

On the basis of the modern understanding of cancer development in humans, multistage carcinogenesis (Vogelstein et al. 1988; Weinstein et al. 1997) and the classical concept of carcinogenesis and field cancerization (Slaughter et al. 1953), the significance of cancer prevention as a new medical science in its own right is now well established (Hong and Sporn 1997). The Chemoprevention Branch in the Division of Cancer Prevention and Control at the National Cancer Institute (NCI) took the initiative in developing cancer-preventive agents (Kelloff et al. 1992), and had its first success with tamoxifen, the first proved cancer-preventive agent for breast cancer, in 1998 (Fisher et al. 1998). However, neither the concept nor the practice of cancer prevention has been taken on board by cancer researchers in a practical sense. Although our research group in Japan has studied (−)-epigallocatechin gallate (EGCG), the main constituent of green tea and green tea extract, as a cancer preventive for humans since 1983 (Yoshizawa et al. 1987), it is only recently that green tea has gained significant acceptance as a cancer preventive (Yang and Wang 1993; Weisburger 1999; Conney et al. 1999). At the 90th Annual Meeting of the American Association of Cancer Research, in Philadelphia this year, I was invited to give a talk entitled "Natural agents and cancer chemoprevention" at the "Meet-the-expert" sunrise sessions. Since my presentation was well received by the participants, I will introduce our study of cancer pre-

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vention with green tea to those who work in other research fields by means of this Guest Editorial.

Cancer chemoprevention

In 1976, Michael Sporn, who recently left NCI, defined cancer chemoprevention as the prevention of the occurrence of cancer by administration of one or more compounds (Sporn et al. 1976; Sporn 1993). Gary Kelloff, Chief of the Chemoprevention Branch, recently extended the definition as follows: intervention with specific agents to prevent, inhibit or reverse carcinogenesis before malignancy, a relatively new medical science (Kelloff et al. 1997). Before considering cancer chemoprevention itself, we have to realize that cancer prevention is essentially different from the prevention of infectious diseases, such as measles, smallpox and tuberculosis, since cancer is a disease of the aging process with many stages, while infectious disease is the reaction to a pathogen. My own definition of cancer chemoprevention is the administration of cancer preventives to delay the carcinogenic processes in humans, no matter when the carcinogenesis starts, thereby blocking the appearance of clinical symptoms. If we accept my definition, some measure of cancer prevention can be attained through normal daily beverages, such as green tea (Fujiki et al. 1999a).

Clinical trials

Although cancer chemoprevention is principally distinct from chemotherapy, the Chemoprevention Branch has established the following three clinical trials for development of possible cancer-preventive agents, as these trials are applied to anticancer agents: phase I is concerned primarily with the safety and/or toxicity of a possible chemopreventive agent, phase II is a small-scale efficacy study with the evaluation of the modulation of an intermediate marker as an endpoint, and phase III is a large-scale efficacy study with the evaluation of the reduction of cancer as the usual endpoint (Kelloff et al. 1992). The difficulties with these clinical trials are the length of time and the enormous financial resources required to carry out a full clinical evaluation of a possible chemopreventive agent.

The Chemoprevention Branch is sponsoring various phase I, II and III trials with more than 30 agents, listed in Table 1 (Kelloff et al. 1996). Using their own trials, in April 1998, the Food and Drug Administration in the United States found that tamoxifen can prevent breast cancer in humans, making tamoxifen the first recognized cancer-preventive agent for breast cancer (Fisher et al. 1998). While administration of tamoxifen prevented both invasive and non-invasive breast cancers, it increased the incidence of endometrial cancer, which led Richard Klausner, Director of NCI (commenting in *USA Today*), to state that tamoxifen is an imperfect, but very encouraging first step and that we need to look for other and better drugs, i.e., new agents without adverse effects.

Table 1 List of possible cancer-preventing agents in NCI trials. *4-HPR*, all-*trans*-(4-hydroxyphenyl) retinamide, *DFMO* 2-difluoromethylornithine, *DHEA* dehydroepiandrosteronedione, *EGCG* (−)-epigallocatechin gallate (Kelloff et al. 1996)

Phase	Agents
III	Several retinoids (retinol, retinyl palmitate, all-*trans*-retinoic acid, 13-*cis*-retinoic acid), calcium, β-carotene, vitamin E, tamoxifen, finasteride
II	4-HPR alone and in combination with tamoxifen, DFMO, non-steroidal antiinflammatory drugs (aspirin, piroxicam, sulindac), oltipraz, DHEA
I	*S*-Allyl-L-cysteine, curcumin, genistein, indole-3-carbinol, perillyl alcohol, phenethyl isothiocyanate, tea extract (EGCG), DHEA analog, ibuprofen, 9-*cis*-retinoic acid, sulindac sulfone, ursodiol, vitamin D analogs, *p*-xylylselenocyanate

Natural products

Natural products attract our attention as possible sources of cancer-preventive agents for obvious reasons (Wattenberg 1983, 1993; Troll et al. 1992): we believe them to be non-toxic and they are present in things we eat and drink every day. As listed in Table 1, seven products are under investigation in phase I (Kelloff et al. 1996). *S*-Allyl-L-cysteine is a water-soluble organosulfur compound found in garlic, and garlic oil has shown chemopreventive activity in rodent experiments (Belman 1983). Curcumin is the major yellow pigment in turmeric and curry, obtained from the rhizome of the plant *Curcuma longa* (Steele et al. 1994). Genistein is the isoflavone found in soybeans (Naim et al. 1973), and indole-3-carbinol is a non-nutritive component of cruciferous vegetables (Bradfield and Bjeldanes 1987): Consumption of all these vegetables has been associated with decreased risk for cancer in humans (Young and Wolf 1988). Perillyl alcohol is a cyclic monoterpene occurring in numerous species of plants including mints, lavender, perilla and citrus fruits (Karp et al. 1990; Ren and Gould 1994). Phenethyl isothiocyanate occurs naturally as its thioglucoside conjugate in many cruciferous vegetables including watercress, Chinese cabbage and broccoli (Buttery et al. 1976; Spence and Tucknott 1983). The last one is tea, specifically green tea, having (−)-epigallocatechin gallate (EGCG), isolated from *Camellia sinensis*, as its main constituent (Okuda et al. 1985). Many natural products that are present in edible plants and vegetables have been reported to inhibit carcinogenesis in rodents (Wattenberg et al. 1992; Steele et al. 1996), but this does not necessarily mean that all such products will prove to be cancer preventives for humans. What kinds of agents will prove to be effective cancer preventives? Since green tea is now acknowledged to prevent cancer in Japan (Fujiki et al. 1996) and will possibly soon be recognized as such in other countries, I will review our work with EGCG and green tea, seeking an answer to this question.

EGCG and green tea

In 1987, we first reported, in the new British journal *Phytotherapy Research*, that repeated topical applications of EGCG to mouse skin treated with 7,12-dimethylbenz[*a*]anthracene (DMBA) as an initiator inhibited tumor promotion in a two-stage carcinogenesis experiment (Yoshizawa et al. 1987). This evidence opened up a new area of cancer prevention for humans, and since then many scientists have joined the study of green tea as a cancer preventive (Yang and Wang 1993; Weisburger 1999; Conney et al. 1999). Green tea, oolong tea and black tea are originally derived from the same plant, *Camellia sinensis*, green tea being the non-oxidized/non-fermented product that contains several tea polyphenols, such as EGCG, (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC) and (−)-epicatechin (EC) (Fujiki and Okuda 1992). Oolong tea is the partially oxidized/fermented product, and black tea is the fermented product containing low amounts of tea catechins, along with theaflavins and thearubigins (IARC Working Group 1991). One of the most important advantages of green tea as a cancer preventive is its non-toxicity: the Japanese drink several Japanese-size cups throughout the day and I myself am used to drinking over 1 l every day, so I consume as much as 1 g/day of tea polyphenols, along with caffeine, which is found in all of the teas.

Growth inhibition and apoptosis

It is important to know whether each tea polyphenol is equally active. Figure 1 shows that tea polyphenols inhibit growth of human lung cancer cell line, PC-9, dose-dependently, the order of potency being ECG, EGCG and EGC. EC was not effective (Okabe et al. 1997). These results suggested that EGCG is the most important of the tea polyphenols, because of its high content (five-fold that of ECG) and its activity. Although EC is the apparently inactive tea polyphenol, my colleague, Masami Suganuma, recently discovered that EC showed synergistic effects with EGCG on induction of apoptosis in PC-9 cells (Suganuma et al. 1999a). That is, various concentrations of EC with 75 μM EGCG enhanced DNA fragmentation induced by EGCG alone, dose-dependently and synergistically. Thus, we concluded that unfractionated green tea has stronger effects than any single tea polyphenol.

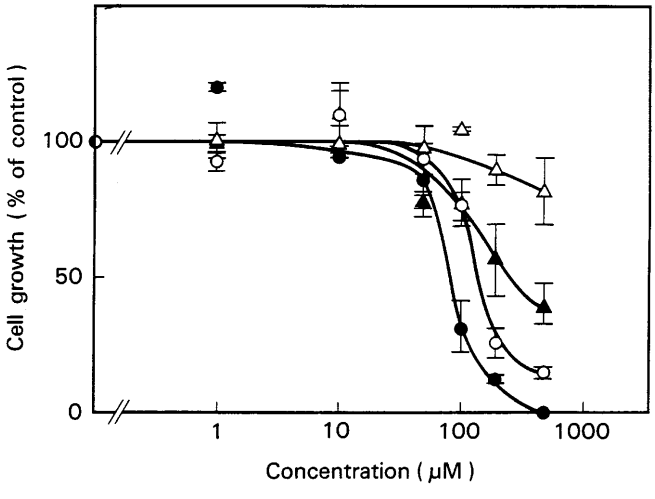

Fig. 1 Growth inhibition of PC-9 cells by tea polyphenols. PC-9 cells (2×10^5/ml) were treated with various concentrations of (−)-epicatechin gallate (●), (−)-epigallocatechin gallate (○), (−)-epigallocatechin (▲) or (−)-epicatechin (△) for 3 days. The number of viable cells was counted by means of the dye-exclusion method

Organ distribution

Based on various studies of the inhibitory effects of EGCG and green tea on rodent carcinogenesis and in cell culture systems, Table 2 summarizes the important features of EGCG and green tea as cancer preventives (Fujiki et al. 1998). Drinking EGCG and green tea inhibited cancers in various organs of rodents, such as those of the digestive tract, including the esophagus, stomach, duodenum and colon, and the liver, lung, pancreas, breast, bladder, prostate and skin (Yang and Wang 1993; NCI, DCPC, Chemoprevention Branch and Agent Development Committee 1996; Fujiki et al. 1999b; Weisburger 1999). These systemic effects of EGCG and green tea suggested that EGCG and tea polyphenols are easily distributed from the digestive tract to various organs. An EGCG solution containing 3.7 MBq [^{3}H]EGCG with a specific activity of 48.1 GBq/mmol was given directly to mice by gastric tube (Suganuma et al. 1998). The radioactivity in the blood of female mice was 24.0×10^3 dpm/ml 1 h after administration, and reached 235.0×10^3 dpm/ml 6 h after. About 2% of the total administered radioactivity had been incorporated into the total blood of the mouse 24 h after incubation. In addition, in feces of female mice, 0.9%, 23.4% and 37.7% of the total administered radioactivity was excreted at different intervals (within 3 h, 6 h and 24 h); in urine, the amounts were 1.7%, 1.4% and 6.6% respectively. As for the digestive tract, the average amounts of total administered radioactivity in the stomach, small intestine and colon, including the dietary content, were 30.7%, 40.6% and 3.9% respec-

Table 2 Important features of EGCG and green tea as cancer preventives (Fujiki et al. 1998)

1. Non-toxic, for rodents and humans
2. Wide-range of target organs, such as digestive tract including esophagus, stomach, duodenum and colon, liver, lung, breast, pancreas, bladder, prostate and skin
3. Inhibitory effect on growth of cancer cells, associated with G2/M arrest in PC-9 cell line
4. Inhibitory effect on lung metastasis of B16 melanoma cells, associated with reduction of various cytokine levels

tively, 1 h after administration, values that had decreased after 24 h. Specifically, the radioactivity per 100 mg tissue in female mice was found to be 328.0×10^3 dpm, 213.7×10^3 dpm and 269.0×10^3 dpm for the stomach, small intestine and colon respectively, after 24 h. The radioactivity per 100 mg tissue of various organs 24 h after administration is shown in Table 3 (Suganuma et al. 1998). All the results showed that radioactivity was present in the organs where EGCG and green tea had previously been shown to inhibit carcinogenesis.

Cancer-preventive activities

Numerous scientists have asked the pertinent question: what are the mechanisms of action of tea polyphenols? Table 4 summarizes the results of many reports on the preventive activities of EGCG, green tea and black tea (NCI, DCPC, Chemoprevention Branch and Agent Development Committee 1996; Fujiki et al. 1997; Jankun et al. 1997; Naasani et al. 1998; Cao and Cao 1999). Initially we found that EGCG inhibited tumor promotion, and proposed as a possible mechanism the sealing effect of EGCG (Fujiki et al. 1992), in which topical application of EGCG to mouse skin inhibited interaction of tumor promoters, hormones, and various growth factors with their receptors. Moreover, many scientists have subsequently reported that EGCG and tea polyphenols have antimutagenic (Okuda et al. 1984) and antimicrobial activities (Hamilton-Miller 1995), and that tea polyphenols inhibit various activities of enzymes and also gene expression of inflammatory cytokines related to carcinogenesis, thereby enhancing the activity of some biochemical reaction supporting anticarcinogenesis (Yang and Wang 1993; NCI, DCPC, Chemoprevention Branch and Agent Development Committee 1996; Fujiki et al. 1997; Conney et al. 1999). Among these various preventive activities, various journals have recently reported new findings related to mechanisms of action of EGCG: inhibition of urokinase (Jankun et al. 1997), which is involved in metastasis, inhibition of telomerase activity associated with entering crisis and senescence (Naasani et al. 1998), inhibition of p38-mitogen-activated protein kinase activation (Chen et al. 1999) and inhibition of angiogenesis (Cao and Cao 1999). In short, tea polyphenols have many functions and are thus completely different from an enzyme inhibitior, which has a specific function. If this is indeed so, what is the essential activity for cancer prevention? More and more, we feel it is the inhibition of the expression and release of tumor necrosis factor α (TNFα).

Table 3 Distribution of radioactivity in various organs after a single administration of [^{3}H]EGCG. (Suganuma et al. 1998)

Organ	$10^3 \times$ Radioactivity (dpm/100 mg tissue)
Blood (ml^{-1})	288.0 ± 2.0
Brain	22.5 ± 10.4
Lung	22.0 ± 5.8
Heart	24.4 ± 8.1
Liver	27.3 ± 3.7
Kidney	24.7 ± 6.5
Spleen	20.0 ± 7.1
Pancreas	21.4 ± 12.3
Uterus and ovary	21.8 ± 8.0
Mammary gland	18.4 ± 4.3
Bladder	6.6 ± 3.6
Bone	18.5 ± 6.5
Skin	19.3 ± 0.4

Table 4 Preventive activities of EGCG, green and black tea. *8-OHdG* 8-hydroxy-2′-deoxyguanosine, *ODC* ornithine decarboxylase, *PKC* protein kinase C, *TNFα* tumor necrosis factor α, *IL-1* interleukin-1, *GSH* glutathione *GSH-Px* GSH peroxidase. *GST* glutathione-S-transferase (NCI, DCPC, Chemoprevention Branch and Agent Development Committee 1996; Fujiki et al. 1997; Jankun et al. 1997; Naasani et al. 1998; Cao and Cao 1999)

Anticarcinogenic	antipromotion, including tumor growth, invasion, metastasis, and cell transformation
Sealing effect	inhibiting interaction of tumor promoters, hormones, and various growth factors with their receptors
Antimutagenic	antiinitiation, including food mutagens, endogenous nitrosation products, carcinogen-DNA adducts, and 8-OHdG
Antimicrobial	
Inhibition	oncogene expression (c-*myc*, c-H-*ras*, c-*raf*), lipid peroxidation, angiogenesis, free radicals, ODC, urokinase, PKC, lipooxygenase, cyclooxygenase, 5α-reductase, nitric oxide synthase, telomerase TNFα gene expression and TNFα release, and IL-1 gene expression
Enhancement	phase II (GST), GSH-Px, catalase, NAD(P)H:quinone reductase, and gap junction

Inhibition of TNFα expression and TNFα release

First, I will briefly describe the results of our study of TNFα in carcinogenesis (Fujiki and Suganuma 1999). In 1988, we added the okadaic acid class to the list of tumor promoters on mouse skin initiated with DMBA (Suganuma et al. 1988); okadaic acid is structurally different from 12-*O*-tetradecanoylphorbal 13-acetate (TPA) and inhibits protein phosphatases-1 and -2A (PP-1 and PP-2A) (Hescheler et al. 1988). In addition, okadaic acid in drinking water induced tumor promotion in rat glandular stomach initiated with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (Suganuma et al. 1992). Repeated i.p. injections of microcystin-LR and nodularin, which are also inhibitors of PP-1 and PP-2A, induced tumor promotion in rat liver initiated with diethylnitrosamine (Nishiwaki-Matsushima 1992; Ohta et al. 1994). Since the tumor promoters of the okadaic acid class, which includes microcystin and nodularin, induced tumor promotion in three different organs initiated with three different carcinogens, we concluded that the okadaic acid pathway, mediated through inhibition of PP-1 and PP-2A, is a general tumor promotion pathway for various organs (Fujiki and Suganuma 1993). Applying the

okadaic acid pathway of tumor promotion to human carcinogenesis, we found a possible link between tumor promoters and TNFα, based on evidence that okadaic acid mimics TNFα/interleukin-1(IL-1), in inducing phosphorylation of the same proteins, and that it also induces similar expression of early-response genes in human fibroblasts, along with activation of NF-κB in Jurkat cells (Guy et al. 1992).

We obtained the following results. (1) A single application of TPA and okadaic acid induced TNFα gene expression in mouse skin (Suganuma et al. 1995), and treatment with TPA and okadaic acid induced TNFα release from various cells including BALB/3T3, HL-60 and KATO-III cells (Fujiki and Suganuma 1999). (2) Since TNFα is a protein of 17 kDa, topical applications of TNFα would not be effective on mouse skin. In a two-stage cell transformation assay, TNFα stimulated transformation of BALB/3T3 cells initiated with 3-methylcholanthrene, and was about 1000 times stronger than TPA at their molar concentrations (Komori et al. 1993). (3) TNFα, IL-1α and IL-1β, the latter two being functionally similar to TNFα, induced clonal growth of Bhas 42 cells, which are v-H-*ras*-transfected BALB/3T3 cells, whereas they were not effective in BALB/3T3 cells (Suganuma et al. 1999b). (4) Quite recently our very important results showed that repeated topical applications of okadaic acid and TPA to DMBA-initiated skin of TNFα-deficient mice induced tumor promotion much less weakly than in normal CD-1 mice initiated with DMBA (Suganuma et al. 1999b). Thus, we are increasingly certain that TNFα is an endogenous tumor promoter and a central mediator of cancer development.

The next step was to determine whether EGCG and other tea polyphenols inhibit TNFα gene expression in the cells and its release from the cells. We found that ECG, EGCG and EGC dose-dependently inhibited TNFα release from a human stomach cancer cell line, KATO-III cells treated with okadaic acid, whereas EC, an inactive tea polyphenol, did not (Okabe et al. 1999). Since various structurally different cancer inhibitors, such as 9-*cis*-retinoic acid, sulindac, the active form of vitamin D_3, and tamoxifen, also inhibited TNFα release (Suganuma et al. 1996), we began looking at the reduction of levels of TNFα and similar cytokines in the tumor development process as the common, key criterion for cancer inhibitors and cancer-preventive agents (Fujiki et al. 1999a).

Prospective cohort study

We concentrated on what we see as one of the most important questions: can green tea affect the development of cancer in humans, and, if so, how many cups per day should one drink to get the benefits? Kei Nakachi and Kazue Imai at our Research Institute conducted a prospective cohort study with 8552 individuals of Yoshimi town in Saitama Prefecture, beginning in 1986 (Imai and Nakachi 1995). These individuals, all over 40 years of age, answered 90 questions on their living habits, including their daily consumption of green tea. During the period of the study, 153 men and 109 women died of cancer, and these deaths were analyzed by two parameters: age at death and daily consumption of green tea. The results were strongly suggestive: male cancer patients who consumed over ten cups per day died 3.6 years later, among males, and female patients died 7.8 years later, than cancer patients drinking fewer than three cups per day. Most Japanese use a medium-size (180 ml) cup for drinking green tea, and will ingest 0.8–1.3 g green tea extract, including 340–540 mg EGCG, in ten cups per day (Nakachi et al. 1997a).

Table 5 Average age at cancer onset and green tea consumption from prospective cohort study (Imai et al. 1997)

	Age (years) at cancer onset after daily green tea consumption of:		
Gender	≤3 cups	4–9 cups	≥10 cups
Male (220)	65.3 ± 1.5 (54)	67.6 ± 1.0 (102)	68.3 ± 1.2 (64)
Female (164)	65.7 ± 1.7 (49)	66.8 ± 1.2 (94)	74.4 ± 2.5 (21)

During the 10 years of the study, Imai and Nakachi found a total of 384 cancer patients, 220 male and 164 female, among participants in the cohort study. These cancer patients, male and female, were divided into three groups by daily green tea consumption of under three cups to over ten cups. Next, the average age of cancer onset was calculated in each group, and it was found that cancer onset of the patients who had consumed over ten cups of green tea per day, was 8.7 years later among women, and 3.0 years later among men, than those of patients who had consumed under three cups per day (Table 5) (Imai et al. 1997). The difference between women and men is partly due to the higher tobacco consumption of the latter. This was the first evidence that drinking green tea delays cancer onset, probably because of the inhibition of growth of dormant initiated cells (Imai et al. 1997).

With the 419 cancer patients from the 11 years of the same study, our colleagues extended their research by determining the relative risk of major cancers based on consumption of green tea. They found that women who consumed over ten cups of green tea per day showed a lower relative risk of lung cancer, colon cancer and liver cancer. Although the relative risk of stomach cancer was not significantly lower (Nakachi et al. 1997b), we felt greatly encouraged by the results from this prospective cohort study. In brief, many cancers can be prevented, or at least their onset can be delayed.

Cancer prevention following cancer treatment

Nakachi and his associates found decreased recurrence of human breast cancer with increased consumption of

green tea, on the basis of results obtained from 472 cancer patients at our hospital (Nakachi et al. 1998). Consumption of green tea by the patients was roughly divided into two classes, fewer than four cups per day and over five cups per day. In stages I and II cancer patients, the group consuming over five cups per day showed a lower recurrence rate, 16.7%, and a longer disease-free period, 3.6 years, than those consuming fewer than four cups per day (Table 6) (Nakachi et al. 1998). However, stage III cancer patients did not show any significant difference and we assume that breast cancer of stage III involves more accumulated genetic changes in the cells than that of stages I and II. This suggests that green tea is more effective in the early stage of second tumor development, even after the removal of the primary cancer.

For stages I and II breast cancer patients, two clinical parameters showed 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 axillarly lymph nodes, and with increased expression of both types of receptor. Thus, we think that drinking green tea prior to the onset of clinical cancer will lead to more hopeful prognoses for breast cancer patients (Nakachi et al. 1998).

Synergistic effects of EGCG with sulindac

Masami Suganuma at our Research Institute demonstrated that EGCG with other cancer-preventive agents, sulindac and tamoxifen, synergistically and additively enhanced apoptosis of PC-9 cells (Suganuma et al. 1999a), quantitatively measuring apoptosis by DNA fragmentation. Specifically, sulindac at a concentration of 10 μM induced almost no apoptosis of PC-9 cells, while 10 μM sulindac with 75 μM EGCG induced apoptosis eight times as strongly as sulindac alone. Sulindac sulfide, at a concentration of 1 μM, with 75 μM EGCG resulted in a more than 20-fold enhancement. We think, then, that a combination of sulindac and green tea will reduce the adverse effects of sulindac in humans while retaining its preventive effects, thus making it possible to administer green tea to a high-risk population (Suganuma et al. 1999a). The effects of EGCG with tamoxifen were found to be additive.

Two stages of cancer prevention

On the basis of all of the above results, I will now summarize my view of the place of green tea in cancer prevention. Taken as a daily beverage, it can delay the onset of many types of cancer; taken in conjunction with sulindac or tamoxifen (Suganuma et al. 1999a), it can lead to more hopeful prognoses for cancer patients following cancer treatment, including prevention of recurrence, second primary tumor and metastasis. In both stages, I believe that drinking green tea is the most practical method of cancer prevention for clinically healthy persons (Fig. 2).

We then have to look at two groups: those who are used to drinking green tea every day, and those who are not. For the former group, I recommend ten Japanese-size cups per day, especially in Japan, where development of cancer-preventive drugs is lagging. Most Japanese use a medium-size (180 ml) cup for drinking green tea, and will injest 0.8–1.3 g green tea extract including 340–540 mg EGCG with ten medium size cups per day. For the latter group, we have now prepared green tea tablets in collaboration with Saitama Prefecture Tea Experiment Station. We hope that the tablets will be used as a dietary supplement, to allow a healthy population and cancer patients to receive the benefits of ten cups of green tea without changing their life style. Each tablet is 500 mg and contains 96% green tea extract. In the U.S. also, under Waun Ki Hong's leadership, two phase I clinical trials with green tea capsules, manufactured by ITO EN Ltd. in Japan, have been conducted (Newman et al. 1999), and a phase II trial is anticipated.

Kava

Herbal remedies are nothing new, but every so often we stumble on evidence that some of them can save people's

Table 6 Recurrence rate of breast cancer in relation to daily consumption of green tea (472 patients) (Nakachi et al. 1998)

	Daily green tea consumption	
Parameter	≤4 cups	≥5 cups
Stages I and II (390 patients)		
Recurrence rate(%)	24.3	16.7
Disease-free period (years)	2.8	3.6
Stage III (82 patients)		
Recurrence rate (%)	48.8	58.5
Disease-free period (years)	1.9	1.9

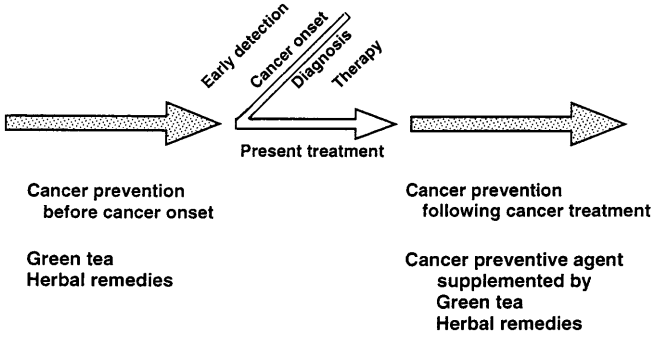

Fig. 2 Two stages of cancer prevention

lives. Green tea is certainly one of these and, while looking for similar preparations in Japan, we found that, in 1985, Brian E. Henderson had reported unusually low rates of many cancers in Fiji, among them, stomach, breast, lung and prostate cancers (Henderson et al. 1985). Naturally we wondered what the people of Fiji might be drinking or eating that was peculiar to Fiji. I was informed that people in Fiji commonly drink kava, or yongona, at evening parties, according to tradition or custom. Kava is a beverage prepared from the powdered roots of a tropical plant, *Piper methysticum* (Sotheeswaran 1987), and it has a slightly numbing effect in the mouth. In collaboration with Yoshinori Asakawa, we isolated five kava lactones from kava extract and subjected them to a TNFα-release assay of BALB/3T3 cells. One of the kava lactones, kava-3, demethoxyyangonin (Achenbach et al. 1972; Sotheeswaran 1987), significantly inhibited TNFα release, with a potency as great as EGCG (unpublished results). We are now studying whether kava lactones can inhibit cancer development, particularly in the lung, in rodent carcinogenesis experiments.

Conclusion

That EGCG, green tea and kava are acknowledged cancer preventives in Japan and Fiji makes it possible for us to extend the idea of the two stages of prevention: cancer prevention before cancer onset can be achieved by drinking green tea and herbal remedies, including kava, and cancer prevention following cancer treatment can be achieved by using conventional cancer-preventive agents such as tamoxifen, sulindac and all-*trans*-(4-hydroxyphenyl) retinamide, supplemented by green tea and probably herbal remedies. There is, indeed, a valid conclusion, and to me it is an obvious one: alternative medicine, including kava and herbal remedies, should be intensively investigated for the development of new cancer preventives for the 21st century.

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References

Achenbach H, Karl W, Regel W (1972) 11-Hydroxy-12-methoxydihydrokawain und 11.12-Dimethoxydihydrokawain, zwei neue Kawa-Lactone aus Rauschpfeffer (*Piper methysticum* Forst.). Chem Ber 105:2182–2187

Belman S (1983) Onion and garlic oils inhibit tumor promotion. Carcinogenesis 4:1063–1065

Bradfield CA, Bjeldanes LF (1987) High-performance liquid chromatographic analysis of anticarcinogenic indoles in *Brassica oleracea*. J Agric Food Chem 35:46–49

Buttery RG, Guadagni DG, Ling LC, Seifert RM, Lipton W (1976) Additional volatile components of cabbage, broccoli, and cauliflower. J Agric Food Chem 24:829–832

Cao Y, Cao R (1999) Angiogenesis inhibited by drinking tea. Nature 398:381

Chen W, Dong Z, Valcic S, Timmermann BN, Bowden GT (1999) Inhibition of ultraviolet B-induced c-*fos* gene expression and p38 mitogen-activated protein kinase activation by (−)-epigallocatechin gallate in a human keratinocyte cell line. Mol Carcinog 24:79–84

Conney AH, Lou YR, Xie J-G, Osawa T, Newmark HL, Liu Y, Chang RL, Huang M-T (1999) Some perspectives on dietary inhibition of carcinogenesis: studies with curcumin and tea. Proc Soc Exp Biol Med (in press)

Fisher B, Costantino JP, Wickerham DL, Redmond CK, Kavanah M, Cronin WM, Vogel V, Robidoux A, Dimitrov N, Atkins J, Daly M, Wieand S, Tan-Chiu E, Ford L, Wolmark N, and other National Surgical Adjuvant Breast and Bowel Project Investigators (1998) Tamoxifen for prevention of breast cancer. Report of the national surgical adjuvant breast and bowel project P-1 study. J Natl Cancer Inst 90:1371–1388

Fujiki H, Okuda T (1992) (−)-Epigallocatechin gallate. Drugs Future 17:462–464

Fujiki H, Suganuma M (1993) Tumor promotion by inhibitors of protein phosphatases 1 and 2A: the okadaic acid class of compounds. Adv Cancer Res 61:143–194

Fujiki H, Suganuma M (1999) Unique features of the okadaic acid activity class of tumor promoters. J Cancer Res Clin Oncol 125:150–155

Fujiki H, Yoshizawa, S, Horiuchi T, Suganuma M, Yatsunami J, Nishiwaki S, Okabe S, Nishiwaki-Matsushima R, Okuda T, Sugimura T (1992) Anticarcinogenic effects of (−)-epigallocatechin gallate. Prev Med 21:503–509

Fujiki H, Suganuma M, Okabe S, Komori A, Sueoka E, Sueoka N, Kozu T, Sakai Y (1996) Japanese green tea as a cancer preventive in humans. Nutr Rev 54:S67–S70

Fujiki H, Komori, A, Suganuma M (1997) Chemoprevention of cancer. In: Bowden GT, Fischer SM (eds) Comprehensive toxicology, vol 12. Pergamon, Cambridge, pp 453–471

Fujiki H, Suganuma M, Okabe S, Sueoka N, Komori A, Sueoka E, Kozu T, Tada Y, Suga K, Imai K, Nakachi K (1998) Cancer inhibition by green tea. Mutat Res 402:307–310

Fujiki H, Suganuma M, Okabe S, Sueoka E, Suga K, Imai K, Nakachi K (1999a) A new concept of tumor promotion by tumor necrosis factor-α, and cancer preventive agents (−)-epigallocatechin gallate and green tea. Cancer Detect Prev (in press)

Fujiki H, Suganuma M, Okabe S, Sueoka E, Suga K, Imai K, Nakachi K, Kimura S (1999b) Mechanistic findings of green tea as cancer preventive for humans. Proc Soc Exp Biol Med 220:225–228

Guy GR, Cao X, Chua SP, Tan YH (1992) Okadaic acid mimics multiple changes in early protein phosphorylation and gene expression induced by tumor necrosis factor or interleukin-1. J Biol Chem 267:1846–1852

Hamilton-Miller JMT (1995) Antimicrobial properties of tea (*Camellia sinensis* L.). Antimicrobial Agents Chemother 39:2375–2377

Henderson BE, Kolonel LN, Dworsky R, Kerford D, Mori E, Singh K, Thevenot H (1985) Cancer incidence in the islands of the Pacific. Natl Cancer Inst Monogr 69:73–81

Hescheler J, Mieskes G, Ruegg JC, Takai A, Trautwein W (1988) Effects of a protein phosphatase inhibitor, okadaic acid, on membrane currents of isolated guinea-pig cardiac myocytes. Pflugers Arch 412:248–252

Hong WK, Sporn MB (1997) Recent advances in chemoprevention of cancer. Science 278:1073–1077

IARC Working Group (1991) Tea. IARC Monogr 51:207–271

Imai K, Nakachi K (1995) Cross sectional study of effects of drinking green tea on cardiovascular and liver diseases. Br Med J 310:693–696

Imai K, Suga K, Nakachi K (1997) Cancer-preventive effects of drinking green tea among a Japanese population. Prev Med 26:769–775

Jankun J, Selman SH, Swiercz R, Skrzypczak-Jankun E (1997) Why drinking green tea could prevent cancer. Nature 387:561

Karp F, Mihaliak CA, Harris JL, Croteau R (1990) Monoterpene biosynthesis: specificity of the hydroxylations of (−)-limonene by enzyme preparations from peppermint (*Mentha piperita*), spearmint (*Mentha spicata*), and perilla (*Perilla frutescens*) leaves. Arch Biochem Biophys 276:219–226

Kelloff GJ, Boone CW, Malone WF, Steele VE (1992) Chemoprevention clinical trials. Mutat Res 267:291–295

Kelloff GJ, Boone CW, Crowell JA, Steele VE, Lubet RA, Doody LA, Malone WF, Hawk ET, Sigman CC (1996) New agents for cancer chemoprevention. J Cell Biochem 26S:1–28

Kelloff GJ, Hawk ET, Karp JE, Crowell JA, Boone CW, Steele VE, Lubet RA, Sigman CC (1997) Progress in clinical chemoprevention. Semin Oncol 24:241–252

Komori A, Yatsunami J, Suganuma M, Okabe S, Abe S, Sakai A, Sasaki K, Fujiki H (1993) Tumor necrosis factor acts as a tumor promoter in BALB/3T3 cell transformation. Cancer Res 53:1982–1985

Naasani I, Seimiya H, Tsuruo T (1998) Telomerase inhibition, telomere shortening, and senescence of cancer cells by tea catechins. Biochem Biophys Res Commun 249:391–396

Naim M, Gestetner B, Kirson I, Birk Y, Bondi A (1973) A new isoflavone from soya beans. Phytochemistry 12:169–170

Nakachi K, Imai K, Suga K (1997a) Epidemiological evidence for prevention of cancer and cardiovascular disease by drinking green tea. In: Ohigashi H, Osawa T, Terao J, Watanabe S, Yoshikawa T (eds) Food factors for cancer prevention. Springer, Tokyo, pp 105–108

Nakachi K, Imai K, Suga K (1997b) Cancer-preventive effects of drinking green tea in a Japanese population. Proc Am Assoc Cancer Res 38:261

Nakachi K, Suemasu K, Suga K, Takeo T, Imai K, Higashi Y (1998) Influence of drinking green tea on breast cancer malignancy among Japanese patients. Jpn J Cancer Res 89:254–261

NCI, DCPC, Chemoprevention Branch and Agent Development Committee (1996) Clinical development plan: tea extracts green tea polyphenols epigallocatechin gallate. J Cell Biochem 26S:236–257

Newman RA, Hutto T, Felix E, Lee JS, Pisters KMW, Hong WK (1999) Clinical pharmacology of catechins and caffeine in adult solid tumor patients enrolled in a phase I trial of green tea extract. Proc Am Assoc Cancer Res 40:82

Nishiwaki-Matsushima R, Ohta T, Nishiwaki S, Suganuma M, Kohyama K, Ishikawa T, Carmichael WW, Fujiki H (1992) Liver tumor promotion by the cyanobacterial cyclic peptide toxin microcystin-LR. J Cancer Res Clin Oncol 118:420–424

Ohta T, Sueoka E, Iida N, Komori A, Suganuma M, Nishiwaki R, Tatematsu M, Kim SJ, Carmichael WW, Fujiki H (1994) Nodularin, a potent inhibitor of protein phosphatases 1 and 2A, is a new environmental carcinogen in male F344 rat liver. Cancer Res 54:6402–6406

Okabe S, Suganuma M, Hayashi M, Sueoka E, Komori A, Fujiki H (1997) Mechanisms of growth inhibition of human lung cancer cell line, PC-9, by tea polyphenols. Jpn J Cancer Res 88:639–643

Okabe S, Ochiai Y, Aida M, Park K, Kim SJ, Nomura T, Suganuma M, Fujiki H (1999) Mechanistic aspects of green tea as cancer preventive on human stomach cancer cell lines. Jpn J Cancer Res 90:733–739

Okuda T, Mori K, Hayatsu H (1984) Inhibitory effect of tannins on direct-acting mutagens. Chem Pharm Bull (Tokyo) 32:3755–3758

Okuda T, Mori K, Hatano T (1985) Relationship of the structure of tannins to the binding activities with hemoglobin and methylene blue. Chem Pharm Bull (Tokyo) 33:1424–1433

Ren Z, Gould MN (1994) Inhibition of ubiquinone and cholesterol synthesis by the monoterpene perillyl alcohol. Cancer Lett 76:185–190

Slaughter DP, Southmick HW, Smeikal W (1953) ''Field cancerization'' in oral stratified squamous epithelium. Cancer 6:963–968

Sotheeswaran S (1987) Kawa and the Australian aborigine. Chem Australia, Oct:377–378

Spence RMM, Tucknott OG (1983) An apparatus for the comparative collection of headspace volatiles of watercress. J Sci Food Agric 34:412–416

Sporn MB (1993) Chemoprevention of cancer. Lancet 342:1211–1213

Sporn MB, Dunlop NM, Newton DL, Smith JM (1976) Prevention of chemical carcinogenesis by vitamin A and its synthetic analogs (retinoids). Fed Proc 35:1332–1338

Steele VE, Moon RC, Lubet RA, Grubbs CJ, Reddy BS, Wargovich M, McCormick DL, Pereira MA, Crowell JA, Bagheri D, Sigman CC, Boone CW, Kelloff GJ (1994) Preclinical efficacy evaluation of potential chemopreventive agents in animal carcinogenesis models: methods and results from the NCI Chemoprevention Drug Development Program. J Cell Biochem 20:32–54

Steele VE, Sharma S, Mehta R, Elmore E, Radpath L, Rudd C, Bagheri D, Sigman CC, Kelloff GJ (1996) Use of in vitro assays to predict the efficacy of chemopreventive agents in whole animals. J Cell Biochem 26S:29–53

Suganuma M, Fujiki H, Suguri H, Yoshizawa S, Hirota M, Nakayasu M, Ojika M, Wakamatsu K, Yamada K, Sugimura T (1988) Okadaic acid: an additional non-phorbol-12-tetradecanoate-13-acetate-type tumor promoter. Proc Natl Acad Sci USA 85:1768–1771

Suganuma M, Tatematsu M, Yatsunami J, Yoshizawa S, Okabe S, Uemura D, Fujiki H (1992) An alternative theory of tissue specificity by tumor promotion of okadaic acid in glandular stomach of SD rats. Carcinogenesis 13:1841–1845

Suganuma M, Okabe S, Sueoka E, Nishiwaki R, Komori A, Uda N, Isono K, Fujiki H (1995) Tautomycin: an inhibitor of protein phosphatases 1 and 2A but not a tumor promoter on mouse skin and in rat glandular stomach., J Cancer Res Clin Oncol 121:621–627

Suganuma M, Okabe S, Sueoka E, Iida N, Komori A, Kim S-J, Fujiki H (1996) A new process of cancer prevention mediated through inhibition of tumor necrosis factor α expression. Cancer Res 56:3711–3715

Suganuma M, Okabe S, Oniyama M, Tada Y, Ito H, Fujiki H (1998) Wide distribution of [^{3}H](−)-epigallocatechin gallate, a cancer preventive tea polyphenol, in mouse tissue. Carcinogenesis 19:1771–1776

Suganuma M, Okabe S, Kai Y, Sueoka N, Sueoka E, Fujiki H (1999a) Synergistic effects of (−)-epigallocatechin gallate with (−)-epicatechin, sulindac, or tamoxifen on cancer-preventive activity in human lung cancer cell line PC-9. Cancer Res 59:44–47

Suganuma M, Okabe S, Marino MW, Sakai A, Sueoka E, Fujiki H (1999b) Essential role of tumor necrosis factor-α in tumor promotion as revealed by TNF-α deficient mice. Cancer Res (in press)

Troll W, Lim JS, Belman S (1992) Protease inhibitors suppress carcinogenesis in vivo and in vitro. In: Wattenberg LW, Lipkin M, Boone CW, Kelloff GJ (eds) Cancer Chemoprevention. CRC, Boca Raton Fla pp 503–512

Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smith AMM, Bos JL (1988) Genetic alterations during colorectal-tumor development. N Engl J Med 319:525–532

Wattenberg LW (1983) Inhibition of neoplasia by minor dietary constituents. Cancer Res 43:2448s–2453s

Wattenberg LW (1993) Prevention-therapy-basic science and the resolution of the cancer problem: presidential address. Cancer Res 53:5890–5896

Wattenberg LW, Lipkin M, Boone CW, Kelloff GJ (eds) (1992) Cancer chemoprevention CRC, Boca Raton, Fla

Weinstein IB, Begemann M, Zhou P, Han EK-H, Sgambato A, Doki Y, Arber N, Ciaparrone M, Yamamoto H (1997) Disorders in cell circuitry associated with multistage carcinogenesis: exploitable targets for cancer prevention and therapy. Clin Cancer Res 3:2696–2702

Weisburger J (ed) (1999) Proceedings of the Society For Experimental Biology and Medicine vol 220. Blackwell Science, New York, NY

Yang CS, Wang Z-Y (1993) Tea and cancer. J Natl Cancer Inst 85:1038–1049

Yoshizawa S, Horiuchi T, Fujiki H, Yoshida T, Okuda T, Sugimura T (1987) Antitumor promoting activity of (−)-epigallocatechin gallate, the main constituent of ''tannin'' in green tea. Phytother Res 1:44–47

Young TB, Wolf DA (1988) Case-control study of proximal and distal colon cancer and diet in Wisconsin. Int J Cancer 42:167–175