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## Green Tea and Cancer: A Summary of the Evidence

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Green tea consumption has been associated in population studies reviewed below with a decreased risk of cancer in humans. This evidence is particularly strong for gastric cancer, where studies in China have demonstrated that both gastritis, the inflammatory condition that precedes gastric cancer, and gastric cancer are inversely associated with green tea intake ( Setiawan VW, Zhang ZF, Yu GP, Lu QY, Li YL, Lu ML, Wang MR, Guo CH, Yu SZ, Kurtz RC, Hsieh CC. Protective effect of green tea on the risks of chronic gastritis and stomach cancer. Int J Cancer 2001;92:600-4). Green tea consumption has also been associated with a reduced risk of ovarian cancer ( Zhang M, Binns CW, Lee AH. Tea consumption and ovarian cancer risk: a case-control study in China . Cancer Epidemiol Biomarkers Prev 2002; 11:713-8). Green tea polyphenols (GTPs) mainly consist of catechins (3-flavanols), of which (-)-epigallocatechin gallate is the most abundant and the most extensively studied. However, it is not the only active substance and studies from our own lab have shown anti-cancer activities of the other related compounds in green tea extracts. Green tea has been shown to increase energy expenditure and be an adjunct to obesity treatments, and obesity has been related to cancer risk in recent studies from the American Cancer Society. One study in animals reviewed below suggests that both the green tea catechins and caffeine may reduce skin cancer by affecting the fat pads in the skin. Recent observations have raised the possibility that green tea catechins, in addition to their antioxidative properties, also affect the molecular mechanisms involved in angiogenesis, extracellular matrix degradation, regulation of cell death and multidrug resistance. Green tea catechins affects these mechanism each of which plays a crucial role in the development of cancer in humans. These studies have been carried out in cell culture by our group and others in several different models of cancer in animals discussed below. The extraction of polyphenols from green tea, as well as their bioavailability, are also known and affect blood and tissue levels of the GTPs and consequently their biological activities (Lee MJ, Maliakal P, Chen L, Meng X, Bondoc FY, Prabhu S, Lambert G, Mohr S, Yang CS.Pharmacokinetics of tea catechins after ingestion of green tea and (-)-epigallocatechin-3-gallate by humans: formation of different metabolites and individual variability. Cancer Epidemiol Biomarkers Prev 2002;11:1025-32). In addition, research on dietary GTPs as novel antiangiogenic and antitumor compounds has also been conducted. These various studies are summarized below in some detail. Non-supportive studies are limited to a negative population study and one intervention study in late stage advanced prostate cancer that was not hormone-responsive. This is the most advanced stage of prostate cancer and it is not surprising that green tea was not effective in this setting. In fact, these patients were being treated with hormone antagonists at the same time they were receiving green tea extract. The evidence clearly shows that green tea catechins inhibit tumor growth directly via suppressive actions on tumor cell mechanisms involved in the multistep process of carcinogenesis and reduce tumor growth in animal models of cancer. In addition, no tumor can grow to more that 200 microns in diameter unless it grows its own blood supply. This process, known as tumor angiogenesis, is at the crux of an interaction between an invading tumor and the patient's immune system. Green tea catechins have been clearly shown to inhibit this key process in tumor growth in a number of studies reviewed below including some from our research group at UCLA. By inhibiting tumor angiogenesis, green tea catechins can tip the balance in favor of the patient versus the tumor. Therefore, while a minority of cancer patients die from their initial tumor, it is far more common that an initial tumor is successfully treated and that some years later the tumor recurs.

When this recurrence leads to spread of the tumor, a process known as metastasis, it commonly leads to suffering and death. Green tea catechins, by inhibiting new tumor blood vessel growth, could delay or prevent tumor recurrence and this is the subject of several multimillion dollar trials at various universities sponsored by the National Cancer Institute. These trials involving patients with superficial bladder cancer at UCLA and in skin cancer at the University of Arizona would not have been undertaken without the significant biological and scientific work reviewed here which establish the likelihood that these trials will be successful. Therefore, while there are no completed large clinical trials at this time showing the benefits of green tea in preventing cancer , there is a combination of strong evidence drawn from population studies, animal studies and basic studies in cancer cells which establish the biological and scientific basis for the claim that green tea extract may reduce the risks of some forms of cancer.

1. Population-based Studies which are supportive.

Setiawan VW, Zhang ZF, Yu GP, Lu QY, Li YL, Lu ML, Wang MR, Guo CH, Yu SZ, Kurtz RC, Hsieh CC. Protective effect of green tea on the risks of chronic gastritis and stomach cancer. Int J Cancer 2001 May 15;92(4):600-4

Despite the declining trend, stomach cancer remains the second most common cancer worldwide. This study examined the role of green tea consumption on chronic gastritis and stomach cancer risks. A population-based case-control study was conducted in Yangzhong , China , with 133 stomach cancer cases, 166 chronic gastritis cases, and 433 healthy controls. Epidemiologic data were collected by standard questionnaire and odds ratios (OR) and 95% confidence intervals (CI) were estimated using logistic regression models in SAS. Inverse association was observed between green tea drinking and chronic gastritis and stomach cancer risks. After adjusting for age, gender, education, body mass index, pack-years of smoking and alcohol drinking, ORs of green tea drinking were 0.52 (95% CI: 0.29-0.94) and 0.49 (95% CI: 0.31-0.77) for stomach cancer and chronic gastritis, respectively. In addition, dose-response relationships were observed with years of green tea drinking in both diseases. The results provide further support on the protective effect of green tea against stomach cancer. This is the first time that green tea drinking was found to be protective against chronic gastritis, which may be of importance when designing intervention strategies for stomach cancer and its pre-malignant lesions in the high-risk population.

Zhang M, Binns CW, Lee AH. Tea consumption and ovarian cancer risk: a case-control study in China . Cancer Epidemiol Biomarkers Prev 2002 Aug;11(8):713-8

To investigate whether tea consumption has an etiological association with ovarian cancer, a case-control study was conducted in China during 1999-2000. The cases were 254 patients with histologically confirmed epithelial ovarian cancer. The 652 controls comprised 340 hospital visitors, 261 non-neoplasm hospital outpatients, and 51 women recruited from the community. Information on the frequency, type, and duration of tea consumption was collected by personal interview using a validated questionnaire. The risk of ovarian cancer for tea consumption was assessed using adjusted odds ratios based on multivariate logistic regression analysis, accounting for confounding demographic, lifestyle, and familial factors including hormonal status and family ovarian cancer. The ovarian cancer risk declined with increasing frequency and duration of overall tea consumption. The adjusted odds ratio was 0.39 for those drinking tea daily and 0.23 for those drinking tea for >30 years, compared with nontea drinkers. The dose response relationships were significant, and the inverse association with ovarian cancer was observed for green tea consumption. This study demonstrated that increasing frequency and duration of tea drinking, especially green tea, can reduce the risk of ovarian cancer.

2. Basic Studies Supporting the Anti-Cancer Effects of Green Tea

Lee MJ, Maliakal P, Chen L, Meng X, Bondoc FY, Prabhu S, Lambert G, Mohr S, Yang CS.Pharmacokinetics of tea catechins after ingestion of green tea and (-)-epigallocatechin-3-gallate by humans: formation of different metabolites and individual variability. Cancer Epidemiol Biomarkers Prev 2002 Oct;11(10 Pt 1):1025-32

Green tea and tea polyphenols have been studied extensively as cancer chemopreventive agents in recent years. The bioavailability and metabolic fate of tea polyphenols in humans, however, are not clearly understood. In this report, the pharmacokinetic parameters of (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC), and (-)-epicatechin (EC) were analyzed after administration of a single oral dose of green tea or decaffeinated green tea (20 mg tea solids/kg) or EGCG (2 mg/kg) to eight subjects. The plasma and urine levels of total EGCG, EGC, and EC (free plus conjugated forms) were quantified by HPLC coupled to an electrochemical detector. The plasma concentration time curves of the catechins were fitted in a one-compartment model. The maximum plasma concentrations of EGCG, EGC, and EC in the three repeated experiments with green tea were 77.9 +/- 22.2, 223.4 +/- 35.2, and 124.03 +/- 7.86 ng/ml, respectively, and the corresponding AUC values were 508.2 +/- 227, 945.4 +/- 438.4, and 529.5 +/- 244.4 ng x h x ml(-1), respectively. The time needed to reach the peak concentrations was in the range of 1.3-1.6 h. The elimination half-lives were 3.4 +/- 0.3, 1.7 +/- 0.4, and 2.0 +/- 0.4 h, respectively. Considerable interindividual differences and variations between repeated experiments in the pharmacokinetic parameters were noted. Significant differences in these pharmacokinetic parameters were not observed when EGCG was given in decaffeinated green tea or in pure form. In the plasma, EGCG was mostly present in the free form, whereas EGC and EC were mostly in the conjugated form. Over 90% of the total urinary EGC and EC, almost all in the conjugated forms, were excreted between 0 and 8 h. Substantial amounts of 4'-O-methyl EGC, at levels higher than EGC, were detected in the urine and plasma. The plasma level of 4'-O-methyl EGC peaked at 1.7 +/- 0.5 h with a half life of 4.4 +/- 1.1 h. Two ring-fission metabolites, (-)-5-(3',4',5'-trihydroxyphenyl)-gamma-valerolactone (M4) and (-)-5-(3',4'-dihydroxyphenyl)-valerolactone (M6), appeared in significant amounts after 3 h and peaked at 8-15 h in the urine as well as in the plasma. These results are useful for designing the dose and dose frequency in intervention studies with tea and for development of biomarkers of tea consumption. In addition they demonstrate the bioavailability of a green tea extract supplement or green tea in terms of supplying catechins in the blood stream. This study makes the laboratory studies below relevant since the catechins can be elevated by taking green tea or a green tea extract supplement.

Wang YC, Bachrach U.The specific anti-cancer activity of green tea (-)-epigallocatechin-3-gallate (EGCG). Amino Acids 2002;22(2):131-43

The effect of the green tea polyphenol-(-)epigallocatechin-3-gallate (EGCG) was tested in cultures of normal and transformed NIH-pATM ras fibroblasts. In this system transformation can be induced at will by the addition of dexamethasone, which induces the expression of H-ras by activating the mammary tumor virus long terminal repeat (MMTV-LTR) promoter. This facilitates a reliable comparison of the susceptibility of normal and transformed cells to EGCG. It has been shown that EGCG inhibited the growth of transformed but not of the normal fibroblasts. In an attempt to elucidate the mode of the preferential inhibitory activity of EGCG, its effect on growth promoting factors has been examined. The level of ornithine decarboxylase (ODC, EC 4.1.1.17), which is a signal for cellular proliferation, was reduced by EGCG in the transformed but not in the normal cells. EGCG also showed strong inhibition of tyrosine kinase and mitogen-activated protein kinase (MAPK) activities, without affecting the kinases in the

normal cells. Similarly, EGCG also preferentially decreased the levels of the oncogenes Ras and Jun in transformed cell. EGCG preferentially induced apoptosis in the transformed fibroblasts. In vitro chemosensitivity tests demonstrated that EGCG inhibited the proliferation of leukemic cells. These findings establish the EGCG, a major green tea catechin, can inhibit many of the key steps in the multistage process of carcinogenesis within tumor cells. These studies establish the basic biologic and scientific rationale for the actions of green tea in inhibiting tumor growth at a cellular level.
Lu YP, Lou YR, Lin Y, Shih WJ, Huang MT, Yang CS, Conney AH.
Inhibitory effects of orally administered green tea, black tea, and caffeine on skin carcinogenesis in mice previously treated with ultraviolet B light (high-risk mice): relationship to decreased tissue fat. Cancer Res 2001 Jul 1;61(13):5002-9

Treatment of SKH-1 hairless mice with ultraviolet B light (UVB; 30 mJ/cm(2)) twice a week for 22 weeks resulted in tumor-free animals with a high risk of developing malignant and nonmalignant skin tumors during the next several months in the absence of additional UVB treatment (high-risk mice). Oral administration of green tea or black tea (6 mg tea solids/ml) to UVB-pretreated high-risk SKH-1 mice for 23 weeks after stopping UVB treatment decreased the number of tumors/mouse, decreased the size of the parametrial fat pads, and decreased the thickness of the dermal fat layer away from tumors and directly under tumors. Administration of the decaffeinated teas had little or no effect on these parameters, and adding caffeine (equivalent to the amount in the regular teas) to the decaffeinated teas restored their inhibitory effects. Administration of caffeine alone also decreased the number of tumors/mouse, the size of the parametrial fat pads, and the thickness of the dermal fat layer away from tumors and under tumors. Using data from individual mice and linear regression and correlation analysis, we found a highly significant positive correlation between the thickness of the dermal fat layer away from tumors and the number of tumors/mouse ($r = 0.34$; $P = 0.0001$), but the correlation between average tumor size/mouse and the thickness of the dermal fat layer away from tumors was weak ($r = 0.16$; $P = 0.034$). The results suggested that p.o. administered tea or caffeine may have decreased tumor multiplicity in part by decreasing fat levels in the dermis. Additional analysis revealed that oral administration of caffeinated beverages (green tea, black tea, decaffeinated green tea plus caffeine, decaffeinated black tea plus caffeine, or caffeine alone) decreased the thickness of the dermal fat layer under large tumors to a much greater extent than under small tumors. This is the first demonstration of a close association between inhibition of carcinogenesis and the lowering of tissue fat levels by a chemopreventive agent.

Afaq F, Adhami VM, Ahmad N, Mukhtar H. Inhibition of ultraviolet B-mediated activation of nuclear factor kappaB in normal human epidermal keratinocytes by green tea Constituent (-)-epigallocatechin-3-gallate. Oncogene 2003 Feb 20;22(7):1035-44
Epigallocatechin-3-gallate (EGCG), the major constituent of green tea, possesses significant anti-inflammatory and cancer chemopreventive properties. Studies have shown the photochemopreventive effects of green tea and EGCG in cell culture, animal models, and human skin. The molecular mechanism(s) of photochemopreventive effects of EGCG are incompletely understood. We recently showed that EGCG treatment of the normal human epidermal keratinocytes (NHEK) inhibits ultraviolet (UV)B-mediated activation of the mitogen-activated protein kinase (MAPK) pathway. In this study, we evaluated the effect of EGCG on UVB-mediated modulation of the nuclear factor kappa B (NF-kappaB) pathway, which is known to play a critical role in a variety of physiological functions and is involved in inflammation and development of cancer. Immunoblot analysis demonstrated that the treatment of NHEK with EGCG (10-40 microM) for 24 h resulted in a significant inhibition of UVB (40

mJ/cm(2))-mediated degradation and phosphorylation of IkappaBalpha and activation of IKKalpha, in a dose-dependent manner. UVB-mediated degradation and phosphorylation of IkappaBalpha and activation of IKKalpha was also observed in a time-dependent protocol (15 and 30 min, 1, 2, 3, 6, 12 h post-UVB exposure). Employing immunoblot analysis, enzyme-linked immunosorbent assay, and gel shift assay, we demonstrate that EGCG treatment of the cells resulted in a significant dose- and time-dependent inhibition of UVB-mediated activation and nuclear translocation of a NF-kappaB/p65. Our data suggest that EGCG protects against the adverse effects of UV radiation via modulations in NF-kappaB pathway, and provide a molecular basis for the photochemopreventive effect of EGCG.

Zhang H, Spitz MR, Tomlinson GE, Schabath MB , Minna JD, Wu X.
Modification of lung cancer susceptibility by green tea extract as measured by the comet assay. Cancer Detect Prev 2002;26(6):411-8

Green tea is known to possess various beneficial properties that may affect carcinogen metabolism, free radical scavenging, or formation of DNA adducts. Therefore, it is plausible that green tea extract may modify BPDE-induced DNA damage. In this report, the comet assay was used to (1) evaluate BPDE-induced DNA damage as a potential marker of cancer susceptibility and (2) assess the ability of green tea to modify BPDE-induced DNA damage. DNA damage in individual comet cells was quantified by (1) visually measuring the proportion of cells exhibiting migration versus those without and (2) the length of damaged DNA migration (comet tail). We detected a dose-response between BDPE concentration and mean comet tail length in EBV-immortalized lymphoblastiod (lymphoid) cell lines. As the concentration of BPDE increased from 0.5 to 3 microM, the length of the mean comet tail length increased proportionally in the 3590P (derived from a healthy subject) and 3640P (derived from a patient with head and neck cancer) cell lines. In separate experiments using lymphoid cells from 21 lung cancer cases and 12 healthy subjects, the mean comet tail length was significantly higher in the lung cancer cases (80.19 +/- 15.55) versus the healthy subjects (59.94 +/- 14.23) ($P < 0.01$). Similar findings were observed when analyzing the mean percentage of comet induced cells (84.57 +/- 8.85 and 69.04 +/- 12.50, respectively) ($P < 0.01$). When green tea extract was added in conjunction with BPDE, there was a notable reduction of the mean comet tail length (13.29 +/- 0.97) as compared to BPDE treatment alone (80.19 +/- 15.55) ($P < 0.01$) in lung cancer cases. There were no statistical differences between the baseline (no treatments) (12.74 +/- 0.63) and the green tea extract treatment (13.06 +/- 0.97) ($P = 0.21$). These data suggest the modification of lung cancer susceptibility by the green tea extract. Similar results were observed for the percentage of induced comet cells and the statistical trends were similar for the 12 healthy subjects. This preliminary study demonstrated that the detection of BPDE-induced DNA damage via the comet assay may be a useful biologic marker of lung cancer susceptibility. The differential effects in BPDE-induced DNA damage between lung cancer cases and healthy subjects suggests predisposed cancer susceptibility to lung cancer risk. This reports also demonstrated the chemopreventive effects of green tea extract on BPDE-induced DNA damage. These observations provide further support for the application of the comet assay in molecular epidemiologic studies.
Li N, Chen X, Liao J, Yang G, Wang S, Josephson Y, Han C, Chen J, Huang MT, Yang CS.Inhibition of 7,12-dimethylbenz[a]anthracene (DMBA)-induced oral carcinogenesis in hamsters by tea and curcumin. Carcinogenesis 2002 Aug;23(8):1307-13

In this study, the effects of tea and curcumin on 7,12-dimethylbenz[a]anthracene (DMBA)-induced oral carcinogenesis in hamsters were examined. DMBA solution (0.5% in mineral oil,

0.1 ml) was applied topically to the left cheek pouch of male Syrian golden hamsters 3 times/week for 6 weeks. Two days after the last treatment of DMBA, the animals received green tea (6 mg tea solids/ml) as drinking fluid, or 10 mmol curcumin applied topically 3 times/week, or the combination of green tea and curcumin treatment, or no treatment for 18 weeks. The combination of tea and curcumin significantly decreased the oral visible tumor incidence from 92.3% (24/26) to 69.2% (18/26) and the squamous cell carcinoma (SCC) incidence from 76.9% (20/26) to 42.3% (11/26). The combination of tea and curcumin also decreased the number of visible tumors and the tumor volume by 52.4 and 69.8%, as well as the numbers of SCC, dysplasic lesions and papillomas by 62.0, 37.5 and 48.7%, respectively. Green tea or curcumin treatment decreased the number of visible tumors by 35.1 or 39.6%, the tumor volume by 41.6 or 61.3% and the number of SCC by 53.3 or 51.3%, respectively. Green tea also decreased the number of dysplasic lesions. Curcumin also significantly decreased the SCC incidence. Tea and curcumin, singly or in combination, decreased the proliferation index in hyperplasia, dysplasia and papillomas. Only the combination treatment decreased the proliferation index in SCC. Tea alone and in combination with curcumin significantly increased the apoptotic index in dysplasia and SCC. Curcumin, alone and in combination with tea, significantly inhibited the angiogenesis in papilloma and SCC. The results suggested that green tea and curcumin had inhibitory effects against oral carcinogenesis at the post-initiation stage and such inhibition may be related to the suppression of cell proliferation, induction of apoptosis and inhibition of angiogenesis.

Orner GA, Dashwood WM, Blum CA, Diaz GD, Li Q, Al-Fageeh M, Tebbutt N, Heath JK, Ernst M, Dashwood RH. Response of Apc(min) and A33 (delta N beta-cat) mutant mice to treatment with tea, sulindac, and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP). Mutat Res 2002 Sep 30;506-507:121-7

This study compared the inhibitory effects of white and green teas with sulindac, a nonsteroidal anti-inflammatory agent, in two different mouse models of intestinal tumorigenesis. In the Apc(min) mouse, white and green teas given at human-relevant concentrations (1.5% w/v, 2-min brew), and sulindac (80 ppm in the drinking water), each suppressed polyp formation by approximately 50%, and the combination of white tea plus sulindac was more effective than either treatment alone (P=0.05). Mice expressing an N-terminally truncated, oncogenic version of beta-catenin (A 33(delta N beta-cat) mutant mice) developed colonic aberrant crypt foci (ACF) spontaneously, but PhIP treatment increased the incidence and number of ACF per colon. In the normal-looking intestinal mucosa of Apc(min) and A 33(delta N beta-cat) mice, white tea plus sulindac treatment markedly attenuated the expression of beta-catenin protein, and this was recapitulated in vitro in cells transiently transfected with beta-catenin plus Tcf-4 and treated with tea or the major tea polyphenol epigallocatechin-3-gallate (EGCG). Expression of a beta-catenin/Tcf reporter was inhibited by EGCG in the transfected cells, and the beta-catenin/Tcf target genes cyclin D1 and c-jun were downregulated in vivo by tea plus sulindac treatment. Collectively, the data support a chemopreventive role for tea and sulindac against intermediate and late stages of colon cancer, via effects on the beta-catenin/Tcf signaling pathway.

Zhou JR, Yu L, Zhong Y, Blackburn GL Soy phytochemicals and tea bioactive components synergistically inhibit androgen-sensitive human prostate tumors in mice.J Nutr 2003

Feb;133(2):516-21

This study identified synergistic effects between soy and tea components on prostate tumor progression in a mouse model of orthotopic androgen-sensitive human prostate cancer. Soy phytochemical concentrate (SPC), black tea and green tea were compared with respect to tumorigenicity rate, primary tumor growth, tumor proliferation index and microvessel density, serum androgen level and metastases to lymph nodes. SPC, black tea and green tea significantly reduced tumorigenicity. SPC and black tea also significantly reduced final tumor weights. Green tea did not reduce final tumor weight, although it tended to elevate (P = 0.14) the serum dihydrotestosterone (DHT) concentration. The combination of SPC and black tea synergistically inhibited prostate tumorigenicity, final tumor weight and metastases to lymph nodes in vivo. The combination of SPC and green tea synergistically inhibited final tumor weight and metastasis and significantly reduced serum concentrations of both testosterone and DHT in vivo. Inhibition of tumor progression was associated with reduced tumor cell proliferation and tumor angiogenesis. This study demonstrated activity of green tea in an animal model where human prostate tumor tissue was implanted in the mouse prostate. The study suggests green tea may be useful in prevention of prostate tumor progression. However, similar effects of black tea and soy protein as well as the interactions require further research.

Gupta S, Hussain T, Mukhtar H. Molecular pathway for (-)-epigallocatechin-3-gallate-induced cell cycle arrest and apoptosis of human prostate carcinoma cells. Arch Biochem Biophys 2003 Feb 1;410(1):177-85

Epigallocatechin-3-gallate (EGCG), the major polyphenolic constituent present in green tea, is a promising chemopreventive agent. We recently showed that green tea polyphenols exert remarkable preventive effects against prostate cancer in a mouse model and many of these effects are mediated by the ability of polyphenols to induce apoptosis in cancer cells [Proc. Natl. Acad. Sci. USA 98 (2001) 10350]. Earlier, we showed that EGCG causes a G0/G1 phase cell cycle arrest and apoptosis of both androgen-sensitive LNCaP and androgen-insensitive DU145 human prostate carcinoma cells, irrespective of p53 status [Toxicol. Appl. Pharmacol. 164 (2000) 82]. Here, we provide molecular understanding of this effect. We tested a hypothesis that EGCG-mediated cell cycle dysregulation and apoptosis is mediated via modulation of cyclin kinase inhibitor (cki)-cyclin-cyclin-dependent kinase (cdk) machinery. As shown by immunoblot analysis, EGCG treatment of LNCaP and DU145 cells resulted in significant dose- and time-dependent (i) upregulation of the protein expression of WAF1/p21, KIP1/p27, INK4a/p16, and INK4c/p18, (ii) down-modulation of the protein expression of cyclin D1, cyclin E, cdk2, cdk4, and cdk6, but not of cyclin D2, (iii) increase in the binding of cyclin D1 toward WAF1/p21 and KIP1/p27, and (iv) decrease in the binding of cyclin E toward cdk2. Taken together, our results suggest that EGCG causes an induction of G1 phase ckis, which inhibits the cyclin-cdk complexes operative in the G0/G1 phase of the cell cycle, thereby causing an arrest, which may be an irreversible process ultimately leading to apoptotic cell death. This is the first systematic study showing the involvement of each component of cdk inhibitor-cyclin-cdk machinery during cell cycle arrest and apoptosis of human prostate carcinoma cells by EGCG.

Yoo HG, Shin BA, Park JC, Kim HS, Kim WJ, Chay KO, Ahn BW, Park RK, Ellis LM, Jung YD.Induction of apoptosis by the green tea flavonol (-)-epigallocatechin-3-gallate in human endothelial ECV 304 cells. Anticancer Res 2002 Nov-Dec;22(6A):3373-8
We have previously shown that treatment with (-)-epigallocatechin-3-gallate (EGCG) inhibited vascularity and tumor growth in human colon cancer xenografts in nude mice (Jung et al: Br J

Cancer 84, 2001). In this study, we examined whether endothelial cell death by EGCG is mediated by apoptosis and which molecular mechanisms are involved in this process. EGCG was found to suppress cell growth and induce apoptosis largely through mitochondrial depolarization, activation of caspase-3 and cleavage of DNA fragmentation factor-45 in human endothelial ECV 304 cells. The induction of apoptosis by EGCG was confirmed by cleaved and condensed nuclear chromatin and DNA hypoploidy. These results suggest that EGCG may exert at least part of its anticancer effect by inhibiting angiogenesis through inducing endothelial apoptosis.

Vergote D, Cren-Olive C, Chopin V, Toillon RA, Rolando C, Hondermarck H, Le Bourhis X.
(-)-Epigallocatechin (EGC) of green tea induces apoptosis of human breast cancer cells but not of their normal counterparts. Breast Cancer Res Treat 2002 Dec;76(3):195-201

(-)-Epigallocatechin (EGC), one of green tea polyphenols, has been shown to inhibit growth of cancer cells. However its mechanism of action is poorly known. We show here that EGC strongly inhibited the growth of breast cancer cell lines (MCF-7 and MDA-MB-231) but not that of normal breast epithelial cells. The inhibition of breast cancer cell growth was due to an induction of apoptosis, without any change in cell cycle progression. MCF-7 cells are known to express a wild-type p53 whereas MDA-MB-231 cells express a mutated p53. The fact that EGC induced apoptosis in both these cell lines suggests that the EGC-triggered apoptosis is independent of p53 status. Moreover, neutralizing antibodies against the death receptor Fas and inhibitors of caspases, such as caspase-8 and -10, efficiently inhibited the EGC-triggered apoptosis. In addition, immunoblotting revealed that EGC treatment was correlated with a decrease in Bcl-2 and an increase in Bax level. These results suggest that EGC-triggered apoptosis in breast cancer cells requires Fas signaling.

Sartippour MR, Heber D, Ma J, Lu Q, Go VL, Nguyen M.
Green tea and its catechins inhibit breast cancer xenografts.
Nutr Cancer 2001;40(2):149-56

It is widely accepted that the main active component of green tea is epigallocatechin-3-gallate (EGCG). In this study, we examined the effect of green tea extracts on breast cancer growth and endothelial cells in in vitro assays and in animal models. Furthermore, we compared the potency of the different catechin components of green tea extract (GTE), including EGCG. Our data showed that mixed GTE and its individual catechin components were effective in inhibiting breast cancer and endothelial cell proliferation. In mouse experiments, GTE suppressed xenograft size and decreased the tumor vessel density.
**These results demonstrated the value of all catechins and argue for the use of a mixed GTE as a botanical dietary supplement, rather than purified EGCG, in future clinical trials.**
Sartippour MR, Heber D, Zhang L, Beatty P, Elashoff D, Elashoff R, Go VL, Brooks MN.
Inhibition of fibroblast growth factors by green tea. Int J Oncol 2002 Sep;21(3):487-91
In a previous study, the effect of green tea on breast cancer growth and endothelial cells both in in vitro assays and in animal models was studied. The data show that both mixed green tea extract (GTE) as well as its individual catechin components are effective in inhibiting breast cancer and endothelial cell proliferation in vitro, and that GTE suppresses breast cancer xenograft size and decreases the tumor blood vessel density in vivo. In the present study, further results demonstrate that 40 microg/ml GTE or EGCG can decrease the levels of the angiogenic factor bFGF (basic fibroblast growth factor) levels in the cells. This phenomenon is observed in both human umbilical vein endothelial cells (HUVECs) and in human breast cancer

cells MDA-MB231. This effect is dose dependent. Furthermore, GTE and EGCG decrease the transcript levels of bFGF and aFGF (acidic fibroblast growth factor) in HUVECs and MDA-MB231 cells. Our findings suggest that the inhibition of the angiogenic fibroblast growth factors could account for one of the mechanisms of green tea's actions. Since cancer is angiogenesis dependent, this may partially explain the antineoplastic effects associated with green tea consumption.

Sartippour MR, Shao ZM, Heber D, Beatty P, Zhang L, Liu C, Ellis L, Liu W, Go VL, Brooks MN. Green tea inhibits vascular endothelial growth factor (VEGF) induction in human breast cancer cells. J Nutr 2002 Aug;132(8):2307-11
A previous study showed that green tea extract (GTE) as well as its individual catechin components inhibited MDA-MB231 breast cancer cell and human umbilical vein endothelial cell (HUVEC) proliferation. Further, GTE suppressed breast cancer xenograft size and decreased the tumor vessel density in vivo. In the current study, the effect of GTE on the major angiogenic factor vascular endothelial growth factor (VEGF) was examined in an in vitro experiment. GTE or EGCG (40 mg/L) significantly decreased the levels of the VEGF peptide secreted into conditioned media. This occurred in both HUVEC and human breast cancer cells and the effect was dose dependent. Furthermore, GTE and EGCG decreased the RNA levels of VEGF in MDA-MB231 cells. This inhibition occurred at the transcriptional regulation level and was accompanied by a significant decrease in VEGF promoter activity. The experiments also showed that GTE decreased c-fos and c-jun RNA transcripts, suggesting that activator protein (AP)-1-responsive regions present in the human VEGF promoter may be involved in the inhibitory effect of GTE. Furthermore, GTE suppressed the expression of protein kinase C, another VEGF transcription modulator, in breast cancer cells. Inhibition of VEGF transcription appeared to be one of the molecular mechanism(s) involved in the antiangiogenic effects of green tea, which may contribute to its potential use for breast cancer treatment and/or prevention.

3. Non- Supportive Studies

Tsubono Y, Nishino Y, Komatsu S, Hsieh CC, Kanemura S, Tsuji I, Nakatsuka H, Fukao A, Satoh H, Hisamichi S. Green tea and the risk of gastric cancer in Japan. N Engl J Med 2001 Mar 1;344(9):632-6

In January 1984, a total of 26,311 residents in three municipalities of Miyagi Prefecture , in northern Japan (11,902 men and 14,409 women 40 years of age or older), completed a self-administered questionnaire that included questions about the frequency of consumption of green tea. During 199,748 person-years of follow-up, through December 1992, 419 cases of gastric cancer were identified (in 296 men and 123 women). Cox regression was used to estimate the relative risk of gastric cancer according to the consumption of green tea. In this study, green-tea consumption was not associated with the risk of gastric cancer. After adjustment for sex, age, presence or absence of a history of peptic ulcer smoking status, alcohol consumption, other dietary elements, and type of health insurance, the relative risks associated with drinking one or two, three or four, and five or more cups of green tea per day, as compared with less than one cup per day, were 1.1 (95 percent confidence interval, 0.8 to 1.6), 1.0 (95 percent confidence interval, 0.7 to 1.4), and 1.2 (95 percent confidence interval, 0.9 to 1.6), respectively (P for trend=0.13). The results were similar after the 117 cases of gastric cancer that were diagnosed in the first three years of follow-up had been excluded, with respective relative risks of 1.2 (95 percent confidence interval, 0.8 to 1.8) 1.0 (95 percent confidence

interval, 0.7 to 1.5), and 1.4 (95 percent confidence interval, 1.0 to 1.9) (P for trend=0.07). In this population-based prospective cohort study in Japan , there was no association between green-tea consumption and the risk of gastric cancer.

While the nearly 200,000 patient years of follow-up sounds impressive in the above study, when one divides this number by the over 26,000 participants, it amounts to less than 8 years of follow-up on average. As indicated in the attempt of these investigators to adjust for potentially confounding factors, smoking and alcohol intake are major causative factors in Japan of gastric cancer. Green tea catechins have their major effects on gastritis and progression to cancer as shown in the study by Zhang et al. Therefore, it is not surprising that a study with a relatively short period of study of less than 8 years on average would fail to find an association. Furthermore, some individuals were excluded from a subsequent analysis who apparently already had gastric cancer at the time of entry into the study. Excluding these individuals helped the analysis in one way by removing a confounding factor (the presence of a cancer). However, this exclusion markedly reduced the power of the study by reducing the number of cases detected during the follow-up period of less than 8 years from 419 to 302 cases. Therefore, this study may have easily missed an association between green tea consumption and gastric cancer.

Ohno Y, Yoshimura T; Japan Collaborative Cohort Study Group.
A prospective study of stomach cancer death in relation to green tea consumption in Japan . Br J Cancer 2002 Jul 29;87(3):309-13

To evaluate whether green tea consumption provides protection against stomach cancer death, relative risks were calculated using Cox proportional hazards regression analysis in the Japan Collaborative Study for Evaluation of Cancer Risk, sponsored by the Ministry of Health and Welfare (JACC Study). The study was based on 30 370 men and 42 481 women aged 40-79. After adjustment for age, smoking status, history of peptic ulcer, family history of stomach cancer along with certain dietary items, the risks associated with drinking one or two, three or four, five to nine, and 10 or more cups of green tea per day, relative to those of drinking less than one cup per day, were 1.6 (95% CI: 0.9-2.9), 1.1 (95% CI: 0.6-1.9), 1.0 (95% CI: 0.5-2.0), and 1.0 (95% CI: 0.5-2.0), respectively, in men (P for trend=0.669), and 1.1 (95% CI: 0.5-2.5), 1.0 (95% CI: 0.5-2.5), 0.8 (95% CI: 0.4-1.6), and 0.8 (95% CI: 0.3-2.1), respectively, in women (P for trend=0.488). No inverse association between green tea consumption and the risk of stomach cancer death was found in this study.

This study is was designed as a case-control study. These types of population studies by comparing characteristics of those diagnosed with cancer to controls studied at the same regardless of the numbers of participants can miss associations which are detected in long-term follow-up of the same populations over decades. The latter studies are called cohort studies and represent a higher standard of proof in population-based cancer epidemiology. Factors at the time of diagnosis including poor memory of dietary intake, metabolic effects of the tumor on metabolism, and other changes secondary to the presence of the tumor can confound studies designed as case-control comparisons. While positive findings in such a study would be supportive, the finding of no association is not proof of the absence of any relationship and does not contradict the supportive studies from China reviewed above which have much more biological information relevant to the prevention of gastric cancer including information on the protective role of green tea in chronic gastritis which is a pre-malignant lesion in a high-risk population.

Jatoi A, Ellison N, Burch PA, Sloan JA, Dakhil SR, Novotny P, Tan W, Fitch TR, Rowland KM, Young CY, Flynn PJ. A phase II trial of green tea in the treatment of patients with

androgen independent metastatic prostate carcinoma. Cancer 2003 Mar 15;97(6):1442-6

Recent laboratory and epidemiologic studies have suggested that green tea has antitumor effects in patients with prostate carcinoma. This Phase II trial explored green tea's antineoplastic effects in patients with androgen independent prostate carcinoma. This study, which was conducted by the North Central Cancer Treatment Group, evaluated 42 patients who were asymptomatic and had manifested, progressive prostate specific antigen (PSA) elevation with hormone therapy. Continued use of luteinizing hormone-releasing hormone agonist was permitted; however, patients were ineligible if they had received other treatments for their disease in the preceding 4 weeks or if they had received a long-acting antiandrogen therapy in the preceding 6 weeks. Patients were instructed to take 6 grams of green tea per day orally in 6 divided doses. Each dose contained 100 calories and 46 mg of caffeine. Patients were monitored monthly for response and toxicity. Tumor response, defined as a decline >/= 50% in the baseline PSA value, occurred in a single patient, or 2% of the cohort (95% confidence interval, 1-14%). This one response was not sustained beyond 2 months. At the end of the first month, the median change in the PSA value from baseline for the cohort increased by 43%. Green tea toxicity, usually Grade 1 or 2, occurred in 69% of patients and included nausea, emesis, insomnia, fatigue, diarrhea, abdominal pain, and confusion. However, six episodes of Grade 3 toxicity and one episode of Grade 4 toxicity also occurred, with the latter manifesting as severe confusion.

This study concluded that green tea has limited anticancer activity, as defined by a decline in PSA levels, among patients with androgen independent prostate carcinoma. This is the most advanced stage of prostate cancer and it is not surprising that green tea was not effective in this setting. In fact, these patients were being treated with hormone antagonists, which are the last option prior to chemotherapy in prostate cancer treatment and follows primary treatment and recurrence of the tumor as judged by a rising PSA level in most applications of this agent. ( Heber, D., The Leuprolide Study Group (1 of 22 authors). Leuprolide Versus Diethylstilbestrol for Metastatic Prostate Cancer. New Eng. J. Med., 311:1281-1286, 1984). at the same time they were receiving green tea extract. The hormone antagonists are so effective that when failure occurs it is not surprising that green tea extract was not effective in stopping the advancement of tumor cells that had developed resistance both to androgens(male hormones which stimulate the growth of early prostate cancer) and to this antagonist treatment. The evidence from a number of studies reviewed above clearly shows that green tea catechins inhibit tumor growth directly via suppressive actions on tumor cell mechanisms involved in the multistep process of carcinogenesis and reduce tumor growth in animal models of cancer. In addition, no tumor can grow to more that 200 microns in diameter unless it grows its own blood supply. This process, known as tumor angiogenesis, is at the crux of an interaction between an invading tumor and the patient’s immune system. Green tea catechins have been clearly shown to inhibit this key process in tumor growth in a number of studies reviewed below including some from our research group at UCLA. By inhibiting tumor angiogenesis, green tea catechins can tip the balance in favor of the patient versus the tumor. Therefore, while a minority of cancer patients die from their initial tumor, it is far more common that an initial tumor is successfully treated and that some years later the tumor recurs. When this recurrence leads to spread of the tumor, a process known as metastasis, it commonly leads to suffering and death. This is not the time when you would use a preventive agent such as green tea. This study is an example of an unfortunate trend of studies being designed to disprove the utility of dietary supplements by placing them in situations where they cannot be effective and publishing the results. A celebrated example of this was the well-publicized trial of St.John’s wort in major depression published in the prestigious Journal of the American Medical Association.

( *Hypericum Depression Trial Study Group* .Effect of Hypericum perforatum ( St John's wort) in major depressive disorder: a randomized controlled trial. JAMA 2002 Apr 10;287(14):1807-14). St. John's wort is intended for the treatment of mood disorders and mild depression, and was never intended to treat major depression which is resistant to the most active antidepressant drugs. In fact, sertraline (Serzone) was included as a positive control. Neither the results from sertraline nor St.John's wort were different from placebo. However, the conclusion drawn both in the abstract and the publicity which followed was that St. John's wort was ineffective for depression. The above trial green tea in advanced prostate cancer represents a parallel example of this type of negative interpretation of a study. In this instance, there was progression of advanced stage prostate cancer in the presence of green tea extract used in addition to a very potent hormone blockade. If green tea is to be effective in prostate cancer, it would need to be used to reduce the risk of prostate cancer in healthy men or to reduce the recurrence risk of prostate cancer immediately after initial treatment with surgery or radiation. Testing green tea in advanced prostate cancer has no bearing on its application in prostate cancer prevention.

Conclusion

In sum, based on all of the publicly available scientific evidence, I conclude that significant and credible scientific evidence supports the conclusion that:

- Some scientific evidence suggests that consumption of green tea may reduce the risk of gastric cancer.
- Some scientific evidence suggests that consumption of green tea extract may reduce the risk of gastric cancer.
- Some scientific evidence suggests that consumption of green tea may reduce the risk of certain forms of cancer.
- Some scientific evidence suggests that consumption of green tea extract may reduce the risk of certain form of cancer.

Respectfully submitted,

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David Heber, M.D., Ph.D.


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