

# Primary Cancer Prevention by Green Tea, and Tertiary Cancer Prevention by the Combination of Green Tea Catechins and Anticancer Compounds

REVIEW

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Green tea is a daily beverage, a non-oxidized non-fermented product containing at least four green tea catechins. Considering our first results when repeated applications of (-)-epigallocatechin gallate (EGCG) prevented tumor promotion in mouse skin, we have continued to look at green tea as a possible cancer preventive agent. 1) The 10-year prospective cohort study by Drs. K. Nakachi and K. Imai revealed that drinking 10 Japanese-size cups (120 mL/cup) of green tea per day delayed cancer onset in humans by 7.3 years among females and by 3.2 years among males. The delay of cancer onset is of course significant evidence of primary cancer prevention in humans. 2) In collaboration with Dr. H. Moriwaki's group we successfully presented a prototype of tertiary cancer prevention showing that 10 Japanese-size cups of green tea daily, supplemented with tablets of green tea extract (G.T.E), reduced recurrence of colorectal adenomas in polypectomy patients by 51.6% (from 31% to 15%). 3) In 1999, we first reported that the combination of green tea catechins and non-steroidal anti-inflammatory drugs showed synergistic anticancer effects in both in vitro and in vivo experiments, along with elucidation of the mechanism. 4) Further studies by other investigators have revealed that various combinations of EGCG or green tea extract and anticancer compounds inhibit tumor volume in xenograft mouse models implanted with various human cancer cell lines. Green tea is a cancer preventive, and green tea catechins act as synergists with anticancer compounds.

**(J Cancer Prev 2015;20:1-4)****Key Words:** EGCG, GADD153, Cancer onset, Recurrence of colon polyps

## INTRODUCTION

Numerous phytochemicals have been reported to have cancer preventive activity.<sup>1</sup> Since the study on cancer prevention began in Japan in 1983, we have studied green tea as a cancer preventive at the National Cancer Center Research Institute in Tokyo and Saitama Cancer Center Research Institute. Green tea contains four main tea catechins: 10% to 15% (-)-epigallocatechin gallate (EGCG), 6% to 10% (-)-epigallocatechin (EGC), 2% to 3% (-)-epicatechin gallate (ECG), and 2% (-)-epicatechin (EC).<sup>2</sup> EGCG, EGC and ECG have cancer preventive activity, while EC is usually inactive. In 1987, we first reported that repeated applications of EGCG prevented tumor promotion of both 12-*O*-tetradecanoylphorbol-13-acetate and okadaic acid in mouse skin initiated with 7,12-

dimethylbenz[a]anthracene: EGCG inhibited tumor promotion through both protein kinase C inactivation, and inhibition of protein phosphatases 1 and 2A.<sup>3,4</sup>

Numerous investigators since then have reported that EGCG and green tea catechins can prevent carcinogenesis in rodents in a wide-range of target organs;<sup>5</sup> the systemic effect of EGCG was proved by incorporation of <sup>3</sup>H-EGCG into various organs in mice.<sup>6</sup> Moreover, frequent drinking of green tea each day plays a significant role in cancer prevention, based on the result that duplicate administrations of <sup>3</sup>H-EGCG at 6 hours intervals enhanced incorporation of <sup>3</sup>H-EGCG 4 to 9 fold in most organs compared with a single administration.<sup>6</sup>

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## DELAY OF CANCER ONSET WITH 10 CUPS OF GREEN TEA PER DAY

In 1986, Drs. Nakachi and Imai at Saitama Cancer Center Research Institute surveyed 8,552 individuals aged over 40 on their living habits, including their daily consumption of green tea. During the 10 years after 1986, a total of 419 cancer patients, 244 males and 175 females, were found. These 419 cancer patients were divided into three groups, based on daily consumption of green tea: under 3 cups, 4 to 9 cups and over 10 cups. Next, the average age at cancer onset was obtained from National Health Insurance receipts.<sup>7</sup>

Cancer onset in female patients who had consumed over 10 cups of green tea per day was 7.3 years later than that of patients who had consumed less than three cups per day, and in male patients who had consumed over 10 cups of green tea per day it was 3.2 years later than that of patients who had consumed less than three cups per day.<sup>7</sup> The difference between females and males may be partly due to higher tobacco consumption by males. This prospective cohort study resulted in a hugely significant finding: drinking 10 cups of green tea per day results in delay of cancer onset among the general population.

## PREVENTION OF COLORECTAL ADENOMA RECURRENCE WITH 10 CUPS OF GREEN TEA PER DAY

As the multistage carcinogenesis of Vogelstein et al.<sup>8</sup> indicated, colon polyps are the early stage of colon cancer. In collaboration with Dr. Moriwaki's group at Gifu University, we conducted a double-blind randomized clinical Phase II prevention trial to study recurrence of colorectal adenoma. Patients who had no polyps 12 months after the 1st colonoscopy were divided into two groups: Control group maintained daily consumption of green tea without placebo, and the tablets of green tea extract (G.T.E) group took daily 10 cups of green tea supplemented with G.T.E for 12 months. G.T.E was produced by the Green Tea Laboratory of Saitama Prefecture. The results were exciting: The recurrence rate of the control group was 31%, and that of the G.T.E group was 15%—as determined by end-point colonoscopy 12 months later<sup>9</sup>—so drinking 10 Japanese-size cups of green tea supplemented with G.T.E reduced recurrence of colorectal adenomas by 51.6%, even when a placebo was not used in prevention trial.<sup>9</sup> Shin<sup>10</sup> at Seoul National University presented similar results at the International Conference on the 19th Annual Meeting of Korean Society of Cancer Prevention: The results confirmed prevention

of colorectal adenoma recurrence.

## ANTICANCER ACTIVITY WITH THE COMBINATION OF EGCG AND NSAIDS

In 1999, we found that the combination of EGCG and sulindac, or EGCG and celecoxib, synergistically enhanced apoptosis in human lung cancer cell line PC-9 cells 11-fold or 15-fold, whereas EGCG, sulindac or celecoxib alone did not induce any apoptosis.<sup>11,12</sup>

The treatments of multiple intestinal neoplasia (Min) mice with a combination of green tea extract and sulindac synergistically reduced the number of tumors per mouse from 72.3 to 32.0, a decrease of 55.7%, whereas green tea extract alone or sulindac alone inhibited tumor development to a much lesser degree.<sup>13</sup>

The study of molecular mechanisms involved in synergistic enhancement of anticancer activity revealed that the combination of EGCG and sulindac induced a dramatic up-regulation of two genes, growth arrest and DNA damage-inducible gene 153 (*GADD 153*), and *p21*, about 12 fold and 3 fold in PC-9 cells. Whereas treatment with either EGCG or sulindac alone had little effect on gene expression.<sup>14</sup>

## SYNERGISTIC ANTICANCER EFFECTS WITH THE COMBINATION IN IN VITRO EXPERIMENTS

Numerous investigators have reported that the combination of EGCG (green tea catechins) with 36 anticancer compounds (Table 1) enhanced in vitro synergistic anticancer effects in 55 human cancer cell lines (Table 2); EGCG and green tea catechins generally increase the anticancer effects of numerous anticancer compounds in various human cancer cell lines, derived from various cancer tissues.<sup>15</sup>

## ANTICANCER ACTIVITY IN XENOGRFT MOUSE MODELS IMPLANTED USING HUMAN CANCER CELL LINES WITH THE COMBINATION

The inhibition of tumor volume in 13 xenograft mouse models implanted using human cancer cell lines has been reported by other investigators. Average inhibition of tumor volume by the combination of EGCG, or green tea extract, and 13 tested anticancer compounds was 70.3%, while those by anticancer compounds alone, EGCG alone or vehicle for control were 33.7%,

**Table 1.** List of anticancer compounds that have shown synergistic anticancer effects with EGCG, or other green tea catechins

Cancer tissue	Effective anticancer compounds used in experiment
Head, neck and lung	Celecoxib, curcumin, erlotinib, 5-fluorouracil, luteolin, sulindac, tamoxifen
Breast	Curcumin, 4-hydroxytamoxifen, raloxifene, resveratrol, tamoxifen, $\gamma$ -tocotrienol, trichostatin A
Prostate	Bortezomib, docetaxel, doxorubicin, genistein, NS398, paclitaxel, quercetin, resveratrol, sulforaphane
Liver	Doxorubicin, 5-fluorouracil
Colon	Sodium butyrate, sulforaphane
Ovaries	Cisplatin, sulforaphane, <i>trans</i> -palladiums
Malignant neuroblastoma	Retinoids (ATRA, 13-cis-RA, 4-HPR), SU5416
Leukemia	Benzyl isothiocyanate, celastrol, curcumin, cytosine arabinoside, H <sub>2</sub> O <sub>2</sub>
Pancreas	Celecoxib, thymoquinone, TRAIL
Cervix	Retinoic acid
Melanoma	Vorinostat
Skin	3-Deazaneplanocin
Stomach	Docetaxel

EGCG: (-)-epigallocatechin gallate.

**Table 2.** List of human cancer cell lines that have shown the synergistic anticancer effects with the combination

Human cancer tissues	Human cancer cell lines used in experiment
Head, neck and lung	A549, ChaGo K-1, H292, H358, H460, H2122, NCI-H460, PC-9, SQCCY1, Tu177, Tu212, YCU-N861, YCU-H891, 38, 886LN
Breast	MDA-MB-231, HS578T, MCF-7
Prostate	ALVA-41, CWR22Rv1, IBC-10a, LNCaP, PC-3, PC-3 AP-1, PC-3ML, PCa-20a, cancer stem cells of PC-3, RPM18226 MM
Liver	BEL-7404/DOX, Hep3B
Colon	HCT-116, HT-29, RKO
Ovaries	A2780, A2780 (cisR), SKOV-ip1 (paclitaxel-sensitive), SKOVTR-ip2 (paclitaxel-resistant)
Malignant neuroblastoma	SH-SY5Y, SK-N-BE2
Leukemia	B-cell chronic leukemia, HL-60, Jurkat T leukemia, K-562, myelogenous leukemia
Pancreas	Colo357, PANC-1, MIA PaCa-2
Cervix	HeLa, TMCC-1
Melanoma	A-375, G-361, Hs-294T
Skin	A431, SCC-13
Stomach	BGC-823

26.5%, or 0%, respectively.<sup>15</sup> As one specific example, the combinations of EGCG and paclitaxel, and EGCG and docetaxel, completely eliminated tumor development of human prostate cancer cell line PC-3ML in xenograft mouse models.<sup>16</sup> The amount of EGCG necessary for complete elimination of tumor in mice usually corresponds to 6 to 9 Japanese-size-cups of green tea (1.37-2.05 g EGCG) for humans.<sup>15</sup> Since mice treated with the combination did not show any toxic effects, the achievement of improved quality of life is anticipated.

## CONCLUSION

It is essential to discuss the effects of EGCG on various cancer stem cells. The combination of EGCG and quercetin synergistically inhibited stem cell characteristics of human prostate

cancer cells,<sup>17</sup> and EGCG alone also inhibited viability of human pancreatic cancer stem cells in primary and secondary spheroids in a dose-dependent manner (0-60  $\mu$ M).<sup>18</sup> Similar results are increasingly reported by other investigators, indicating that EGCG and other green tea catechins target cancer stem cells in numerous human cancer tissues.

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## CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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