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REVIEW - CANCER RESEARCH

Synergistic enhancement of anticancer effects on numerous human cancer cell lines treated with the combination of EGCG, other green tea catechins, and anticancer compounds

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Abstract
Purpose In 2008, we reported that 10 Japanese-size cups of green tea daily, supplemented with tablets of green tea extract (GTE), reduced the recurrence of colorectal adenoma by 51.6 % in patients after polypectomy. Based on these results, we paid special attention to Japanese cancer patients, who consume green tea every day and are administered anticancer drugs. This encouraged us to study whether the combination of green tea catechins and anticancer drugs has the potential to enhance the efficacy of the drugs.
Results and discussion The combination of GTE and NSAIDs synergistically inhibited tumor development in rodents through the activation of the GADD153–DR5–TRAIL apoptotic pathway. Since then, this study was further extended by various investigators to the combinations of EGCG and other green tea catechins with anticancer compounds, the latter of which include NSAIDs, phytochemicals, and anticancer drugs. In order to demonstrate whether diversity of the combinations would generally induce synergistic anticancer effects on numerous human cancer cell lines, we studied the results of 42 in vitro combination experiments and the synergistic inhibition of tumor volume of 13 combination experiments using xenograft mouse models, which were previously reported by other investigators. The various combinations of EGCG and anticancer compounds induced similar synergistic anticancer effects for both in vitro and in vivo experiments, and showed an average reduction in tumor volume by 70.3 %. Considering the evidence showing that treatment with EGCG inhibited self-renewal of cancer stem cells, the combination shows a great advantage.
Conclusion Green tea is a cancer preventive for humans, showing a new trend of green tea catechins as synergists with anticancer compounds.

Keywords Apoptosis · Cancer stem cells · EGCG · GADD153 · NSAID

Abbreviations

EGCG	(−)-Epigallocatechin gallate
ER	Estrogen receptor
GADD153	*Growth arrest and DNA damage-inducible gene 153*
GTE	Green tea extract
HDAC	Histone deacetylase
NSAIDs	Nonsteroidal anti-inflammatory drugs
TRAIL	TNF-related apoptosis-inducing ligand

Introduction

The study of cancer chemoprevention has confirmed the significant role of green tea as a cancer preventive. The prospective cohort study of Nakachi's group at Saitama Cancer Center Research Institute for the first time revealed the delay of cancer onset in individuals aged over 40 who regularly consumed at least 10 cups of green tea per day (Nakachi et al. 2000). Furthermore, a phase II prevention trial of colorectal adenoma recurrence for patients after polypectomy, conducted by Moriwaki's group at Gifu

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University in collaboration with our group, found 51.6 % prevention of recurrence for those consuming 10 cups of green tea supplemented with tablets of green tea extract (GTE) (Shimizu et al. 2008). Based on this evidence, we decided to study this question: when anticancer drugs are administered to cancer patients, would the drugs be more effective if supplemented with green tea catechins? The usual composition of green tea catechins is 10–15 % (−)-epigallocatechin gallate (EGCG), 6–10 % (−)-epigallocatechin (EGC), 2–3 % (−)-epicatechin gallate (ECG), and 2 % (−)-epicatechin (EC) (Suganuma et al. 1999). The induction of in vitro synergistic anticancer effects on human lung cancer cell line PC-9 was first found by treatment with the combinations of EGCG and sulindac, and EGCG and tamoxifen (Suganuma et al. 1999); subsequently, tumor development in multiple intestinal neoplasia (min) mice was synergistically inhibited by the combination of green tea extract and sulindac (Suganuma et al. 2001). The combination of EGCG and sulindac also induced both apoptosis and up-regulated expression of *growth arrest and DNA damage-inducible 153* (*GADD153*, also known as *CHOP*) and $p21^{WAF1}$ genes in PC-9 cells (Suganuma et al. 2001; Fujiki et al. 2002). Following our reports of these results, numerous investigators joined the study of synergistic anticancer effects on numerous human cancer cell lines treated with various combinations of EGCG and anticancer compounds, including nonsteroidal anti-inflammatory drugs (NSAIDs), phytochemicals, and anticancer drugs (Masuda et al. 2001). Some of the significant results were introduced in our previous review article entitled "Green tea: An effective synergist with anticancer drugs for tertiary cancer prevention" (Fujiki and Suganuma 2012).

In order to demonstrate whether various combinations would induce synergistic anticancer effects on numerous human cancer cell lines, we studied the results of 42 in vitro combination experiments and the inhibition of tumor volume in 13 in vivo experiments with in vivo xenograft mouse models implanted with human cancer cell lines, which were conducted by other investigators. The results showed that EGCG and green tea extract strongly enhanced the efficacy of all anticancer compounds in vitro and in vivo. Notably, the combinations of EGCG and paclitaxel, and EGCG and docetaxel, completely eliminated the tumor development of human prostate cancer cell line PC-3ML (Stearns and Wang 2011), and EGCG reduced drug resistance in cancer cells (Milligan et al. 2009; Liang et al. 2010). In addition, treatment with EGCG has been reported to inhibit the self-renewal of cancer stem cells (Tang et al. 2012). The medical concept of Japanese pathology Professor Tomizo Yoshida—"The final goal of cancer therapy was the survival of cancer patient with coexistence of cancer cells"—is realized in the therapy of a combination of green tea catechins and anticancer compounds. We present here the progress of green tea from a cancer preventive to a synergist with anticancer compounds for personalized cancer therapy.

Green tea as a cancer preventive

Green tea is a beverage for human health, looking at Japanese tradition for some 800 years. When our research in cancer chemoprevention began in Japan in 1983, we studied green tea, having in mind a possible cancer-preventive agent. We found that three of the four catechins—EGCG, EGC, and ECG—have cancer-preventive activity, and that the combination of the active catechins with inactive EC has synergistic effects on the induction of apoptosis and inhibition of cell growth for PC-9 cells. The combination synergistically inhibits the release of TNF-α an endogenous tumor promoter, from BALB/c-3T3 cells induced by okadaic acid (Suganuma et al. 1999). Our results indicate that whole green tea is a more effective and practical cancer preventive than any of the green tea catechins alone.

Here are the results of three cancer prevention studies of green tea for humans

1. The prospective cohort study with 8,552 individuals aged over 40 in Saitama Prefecture found that cancer onset for female patients who were consuming at least 10 Japanese-size cups (120 ml/cup) of green tea per day was 7.3 years later than that for female patients who consumed less than three cups per day (Nakachi et al. 2000). This study also revealed that green tea most significantly prevented lung cancer—a relative risk of 0.33 with at least 10 cups of green tea per day—and that high consumption also prevented cancers of the colorectum, liver, and stomach, in that order (Nakachi et al. 2000). The preventive effects on a wide range of target organs were supported by studying the incorporation of ^{3}H-EGCG in the organs (Suganuma et al. 1998).
2. A clinical phase II prevention trial of colorectal adenoma recurrence in patients showing no polyps after polypectomy revealed that the recurrence rate of the control group, which maintained their usual daily consumption of green tea, was 31 % (determined by endpoint colonoscopy 12 months later), and that of the GTE group, which drank at least 10 cups of green tea, consisting of daily consumption of green tea supplemented with GTE, was 15 % (Shimizu et al. 2008). Tablets of green tea extract (GTE) are produced by the Green Tea Laboratory of Saitama Prefectural Agriculture and Forestry Research Center, and they confirmed the absence of pesticides (Fujiki et al. 2001). Although this prevention trial did not use a placebo, since green

tea is a daily beverage for most Japanese, 51.6 % prevention was successfully achieved with patients in the recurrence of metachronous colorectal adenomas.

3. Among a total of 472 breast cancer patients (stages I and II) at Saitama Cancer Center Hospital, the group consuming over five cups of green tea per day (average eight cups) showed a lower recurrence rate, 16.7 %, and a longer disease-free period, 3.6 years, than the other group that consumed less than four cups per day (average three cups), 24.3 % and 2.8 years. The results strongly indicate that green tea is effective against cancer recurrence, leading to more hopeful prognoses for breast cancer patients, after removal of the primary cancer (Nakachi et al. 1998). Since there are many healthy cancer patients following treatment in Japan, we strongly recommend a cancer treatment strategy with green tea.

Synergistic anticancer effects with the combinations of EGCG and sulindac, or EGCG and celecoxib, and their molecular mechanisms

We previously reported that the combination of green tea extract and sulindac synergistically inhibited intestinal tumors in min mice: the average number of tumors per mouse treated with the vehicle, green tea extract, sulindac, or the combination was 72.3, 56.7, 49.0, and 32.0, respectively, at 16 weeks of age (Suganuma et al. 2001), and that the combination of green tea extract and celecoxib synergistically inhibited lung tumors of A/J mice: the average number of tumors per mouse treated with the vehicle, green tea extract, celecoxib, or the combination at 16 weeks was 3.2, 2.2, 1.5, and 1.1, respectively (Suganuma et al. 2011).

To find out the molecular mechanisms of the synergistic anticancer effects, we first looked at the induction of apoptosis in PC-9 cells. The percentages of apoptotic cells after 40 h incubation with the vehicle, EGCG, sulindac, or the combination were 5.0, 6.1, 4.1, and 42.9, respectively: The combination resulted in an 8.6-fold enhancement of apoptosis. Similarly, treatment of PC-9 cells with the vehicle, EGCG, celecoxib, or the combination for 40 h showed percentages of apoptotic cells to be 5.0, 6.1, 3.5, and 56.3, respectively: The combination enhanced apoptosis 11.3-fold (Table 1) (Suganuma et al. 2006).

We previously studied the levels of gene expression in PC-9 cells using CLONTECH'S Atlas™ cDNA expression array, which includes 588 cancer-related human genes (Okabe et al. 2001). The combination of EGCG and sulindac dramatically increased up-regulated expression of *growth arrest and DNA damage-inducible 153* (*GADD153*, also known as *CHOP*) and $p21^{WAF1}$ genes: increases were 12-fold and threefold, whereas those genes were not affected by treatment with either EGCG or sulindac alone (Table 2) (Fujiki et al. 2002). GADD153 protein is a transcription factor, one of the CCAAT/enhancer-binding proteins (C/EBPs), and is induced by cellular stress and protein misfolding in the endoplasmic reticulum (Maytin et al. 2001). GADD153 acts as an up-regulator of death receptor (DR) 5 induction, through direct binding to the site on the *DR5* promoter (Yoshida et al. 2005). DR5 protein is an apoptosis-inducing membrane receptor for TNF-related apoptosis-inducing ligand (TRAIL): it is a cell surface protein consisting of 281 amino acids with 30 kDa (Shiraishi et al. 2005), which suggests that the combination induces strong apoptosis via the GADD153 and DR4/5 pathway (Fig. 1). The combination of EGCG and celecoxib activates extracellular signal-regulated kinase (ERK) 1/2 and p38 mitogen-activated protein kinase (MAPK), whereas neither EGCG nor celecoxib alone induced phosphorylation of these kinases in PC-9, indicating that the ERK signaling

Table 1 Up-regulated induction of apoptosis associated with the expression of *GADD153* gene in human lung cancer cell line PC-9

Treatments	Induction of apoptosis[a] (% of apoptotic cells)		Expression of *GADD153* gene[b] (fold expression)	
	Without	With EGCG[c]	Without	With EGCG[c]
Control	5.0 ± 1.6	6.1 ± 1.2	1.0	3.0 ± 0.2
Sulindac[c]	4.1 ± 1.6	42.9 ± 3.6	1.1 ± 0.2	11.2 ± 0.8
Celecoxib[c]	3.5 ± 0.5	56.3 ± 4.2	1.3 ± 0.1	16.8 ± 0.6
Aspirin[c]	6.9 ± 2.0	13.2 ± 3.0	1.3 ± 0.5	3.3 ± 1.0

[a] Percentage of apoptotic cells was determined by flow cytometer

[b] Expression of *GADD153* gene is expressed as fold expression compared with non-treated PC-9 cells (control)

[c] Cells treated with 50 μM sulindac, 10 μM celecoxib, 100 μM aspirin, with or without 100 μM EGCG 40 h for apoptosis, and 24 h for gene expression (Suganuma et al. 2006)

Table 2 Effects of the combination of EGCG and sulindac on gene expression in human lung cancer cell line PC-9

Genes	Relative expression level compared with control (fold)		
	EGCG	Sulindac	EGCG + sulindac
UP-regulated			
GADD153	0.87	0.78	11.61
$p21^{WAF1}$	1.02	1.43	2.97
Down-regulated			
T-plasminogen activator	1.87	0.64	0.05
TIMP3	0.77	0.74	0.29
IL-1β	0.78	0.95	0.30
Integrin β4	0.87	0.89	0.34

(Fujiki et al. 2002)

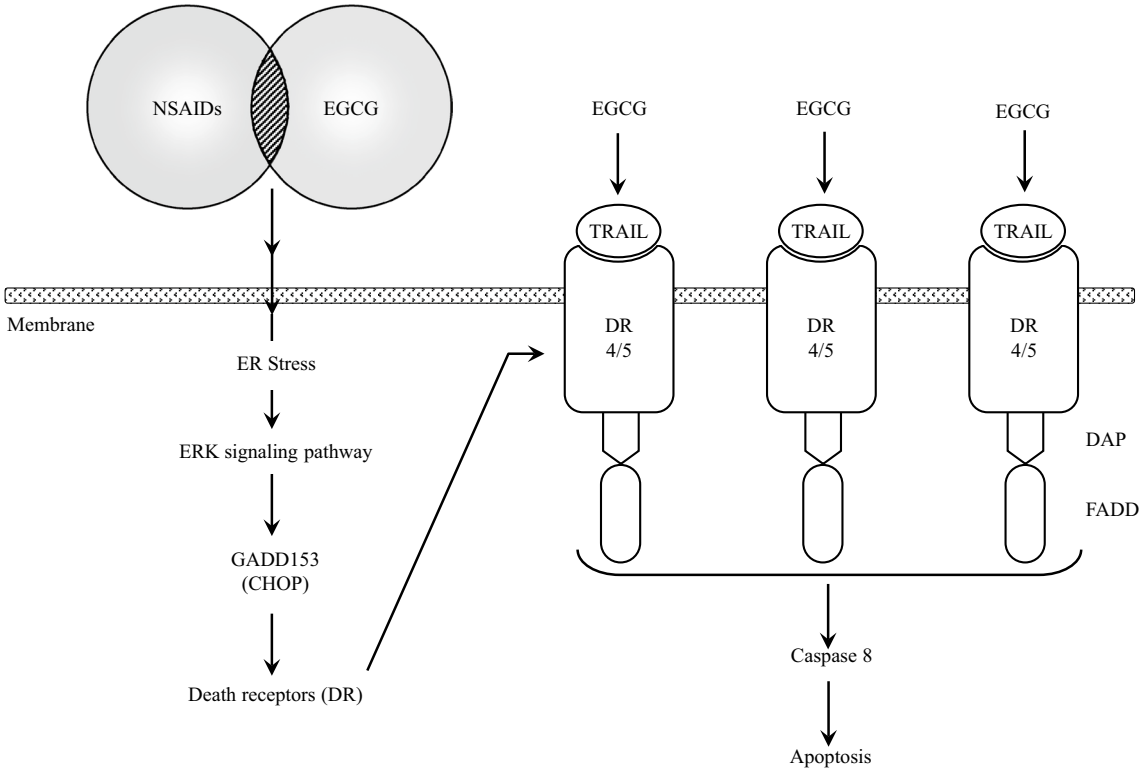

Fig. 1 Schematic illustration of apoptotic pathway with the combination of EGCG and NSAIDs in relation to GADD153, death receptors (DR) 4/5, and TRAIL

pathway is required for *GADD153* gene expression (Fig. 1) (Suganuma et al. 2006).

A relationship between induction of apoptosis and expression of *GADD153* gene in PC-9 cells treated with sulindac or celecoxib, with or without EGCG, was clear (Table 1), indicating that GADD153 protein plays an important role in induction of apoptosis in cancer cells treated with the combination. However, the combination of EGCG and aspirin did not have any effect, suggesting different mechanisms of action (Table 1). In addition, the combination of EGCG and TRAIL significantly diminished the proliferation of human pancreatic cancer cell line MIA PaCa-2, and increased both apoptosis and cleavage of procaspase-2 (Basu and Haldar 2009). All the results suggest that the combinations of EGCG and NSAIDs activate the GADD153–DR5–TRAIL apoptotic pathway (Fig. 1) (Fujiki and Suganuma 2012).

In vitro synergistic anticancer effects of the combinations on human cancer cell lines from different organs

In order to demonstrate the therapeutic benefit of the combination for humans, we studied whether these synergistic anticancer effects could be generally induced in various human cancer cell lines by treatment with a diversity of combinations of EGCG and anticancer compounds, such as NSAIDs, phytochemicals, and anticancer drugs. For this study, we chronologically collected the results of 42 in vitro experiments on human cancer cell lines treated with the combinations, which had been conducted by numerous investigators. To look at the anticancer effects more systematically, the results were classified into nine groups of cancer cell lines depending on tissue, they are as follows: (1) head, neck, and lung cancer; (2) breast cancer; (3) prostate cancer; (4) liver cancer; (5) colon cancer; (6) ovarian cancer; (7) malignant neuroblastoma; (8) leukemia; and (9) cancers from other organs. The diversity of the combinations and their specific anticancer effects is emphasized in Tables 3, 4, 5, 6, 7, 8, 9, 10, and 11.

Human head, neck, and lung cancer cell lines

Table 3 summarizes the results of nine in vitro experiments on 14 human head, neck, and lung cancer cell lines treated with the combinations of EGCG and anticancer compounds including sulindac, tamoxifen, celecoxib, luteolin, and curcumin, along with 5-fluorouracil (5-FU) and erlotinib. It is important to note that the combinations of EGCG and NSAIDs, phytochemicals, or anticancer drugs all induced

Table 3 Combination of green tea catechins and anticancer compounds enhances anticancer effects in human head, neck, and lung cancer cell lines

Catechins +	Anticancer compounds	Cell lines	Anticancer effects	References
EGCG	Sulindac	PC-9	Induction of apoptosis, enhanced expression of *GADD153* gene	Suganuma et al. (1999)
EGCG	Tamoxifen	PC-9	Induction of apoptosis, enhanced expression of *GADD153* gene	Suganuma et al. (1999)
EGCG	5-Fluorouracil	YCU-N861, YCU-H891	Synergistic inhibition of cell proliferation and colony formation	Masuda et al. (2001)
EGCG	Celecoxib	PC-9, A549, ChaGo K-1	Induction of apoptosis, enhanced expression of *GADD153* gene	Suganuma et al. (2006)
EGCG	Erlotinib	Tu177, Tu212, 886LN, SQCCY1, 38	Inhibition of pEGFR, pAKT, activation of caspase-9 and caspase-3	Zhang et al. (2008)
EGCG	Erlotinib	H2122, H358, H460	Inhibition of cell proliferation, increase in response to erlotinib	Milligan et al. (2009)
EGCG	Luteolin	H292, A549, H460, Tu212,	Induction of caspase-8 and caspase-3 cleavage, increase in apoptosis	Amin et al. (2010)
EC	Curcumin	PC-9, A549	Induction of apoptosis, expression of *GADD153* and *GADD45* genes	Saha et al. (2010)
EGCG	Curcumin	A549, NCI-H460	Enhancement of cell cycle arrest at G_1 and S/G_2 phases	Zhou et al. (2013)

Table 4 Combination of green tea catechins and anticancer compounds enhances anticancer effects in human breast cancer cell lines

Catechins +	Anticancer compounds	Cell lines	Anticancer effects	References
EGCG	4-OHT	MDA-MB-231	Synergistic cytotoxicity in ERα+ and ERα− breast cancer cells	Chisholm et al. (2004)
EGC	4-OHT	HS578T	Less cytotoxicity than EGC alone or 4-OHT alone	Chisholm et al. (2004)
EGCG	4-OHT	MDA-MB-231	Induction of condensed chromatin and twofold greater apoptosis	Stuart et al. (2007)
EGCG	Tamoxifen	MDA-MB-231	Suppression of EGFR, pEGFR, mTOR, and CYP1B1	Scandlyn et al. (2008)
EGCG	Curcumin	MDA-MB-231	Synergistic cytotoxicity to the cells, G_2/M-phase cell cycle arrest	Somers-Edgar et al. (2008)
EGCG	Raloxifene	MDA-MB-231	Decrease in cell number, reduced phosphorylation of EGFR and AKT	Stuart and Rosengren (2008)
EGCG	Resveratrol	MCF-7	Suppression of cell viability and colony formation	Hsieh and Wu (2008)
EGCG	γ-Tocotrienol	MCF-7	Suppression of cell viability, increase in catalase activity	Hsieh and Wu (2008)
EGCG	Tricostatin A	MDA-MB-231	Re-activation of ERα expression, sensitization of ERα-dependent cellular response to activator 17β-estradiol	Li et al. (2010)
EGCG	Tamoxifen	MCF-7	Anticarcinogenic effects	Sakata et al. (2011)

synergistic anticancer effects. Specifically, EGCG showed a dose-dependent inhibition of cell growth in three non-small cell lung carcinoma cell lines regardless of sensitivity to erlotinib (Milligan et al. 2009). Although (−)-epicatechin (EC) is an inert catechin, the combination of EC and curcumin increased apoptosis and expression of *GADD153* and *GADD45* genes in PC-9 and A549 cells, suggesting that expensive EGCG can be replaced by less expensive EC (Saha et al. 2010). The combinations of EGCG and erlotinib for Tu212 and H460 cells, EGCG and luteolin for Tu212 and A549 cells, and EGCG and curcumin for A549 cells showed synergistic inhibition of tumor volume in xenograft mouse models (Zhang et al. 2008; Milligan et al. 2009; Amin et al. 2010; Zhou et al. 2013) (Table 12).

Human breast cancer cell lines

Table 4 summarizes the results of 10 in vitro experiments on three human breast cancer cell lines, including estrogen receptor-negative (ERα−) and -positive (ERα+), treated with combinations of EGCG and anticancer compounds,

Table 5 Combination of EGCG and anticancer compounds enhances anticancer effects in human prostate cancer cell lines

EGCG +	Anticancer compounds	Cell lines	Anticancer effects	References
EGCG	Resveratrol	ALVA-41, PC-3	Down-regulation of casein kinase 2 and protein expression	Ahmad et al. (2007)
EGCG	NS398	LNCaP, CWR22Rv1, PC-3,	Inhibition of cell growth, induction of apoptosis, expression of Bax, pro-caspase-6 and pro-caspase-9	Ahmad et al. (2007)
EGCG	Quercetin	CWR22Rv1	Synergistic expression of androgen receptor, p53, and NQO1	Hsieh and Wu (2009)
EGCG	Genistein	CWR22Rv1	Synergistic expression of androgen receptor, p53, and NQO1	Hsieh and Wu (2009)
EGCG	Sulforaphane	PC-3 AP-1	Diminished induction of AP-1 activity, down-regulation of Nrf2-dependent genes	Nair et al. (2010)
EGCG	Doxorubicin	PC-3ML, IBC-10a, PCa-20a	Synergistic effects in blocking tumor cell growth and colony-forming ability	Stearns et al. (2010)
EGCG	Quercetin	Cancer stem cells of PC-3 and LNCaP	Inhibition of self-renewal capacity, migration, and invasion	Tang et al. (2010)
EGCG	Paclitaxel	PC-3ML	Reduction in growth rate, increase in the expression of apoptotic genes, such as p53, p73, p21, and caspase-3	Stearns and Wang (2011)
EGCG	Docetaxel	PC-3ML	Reduction in growth rate, increase in the expression of apoptotic genes, such as p53, p73, p21, and caspase-3	Stearns and Wang (2011)
EGCG	Bortezomib	RPMI8226 MM	No abrogated antitumor activity	Bannerman et al. (2011)

Table 6 Combination of green tea catechins and anticancer drugs enhances anticancer effects in human liver cancer cell lines

Catechins +	Anticancer drugs	Cell lines	Anticancer effects	References
EGCG	Doxorubicin	BEL-7404/DOX	Enhanced sensitivity to doxorubicin, accumulation of DOX in cells	Liang et al. (2010)
ECG	Doxorubicin	BEL-7404/DOX	Enhanced sensitivity to doxorubicin, accumulation of DOX in cells	Liang et al. (2010)
EGCG	5-Fluorouracil	Hep3B	Increase in antitumor effects, abrogation of COX-2 overexpression and PGE_2 secretion	Yang et al. (2012)

Table 7 Combination of EGCG and anticancer compounds enhances anticancer effects in human colon cancer cell lines

EGCG +	Anticancer compounds	Cell lines	Anticancer effects	References
EGCG	Sulforaphane	HT-29	Decrease in cell viability, enhanced transcriptional activation of AP-1 reporter, attenuation of cellular senescence	Nair et al. (2008)
EGCG	Sodium butyrate	RKO, HCT-116, HT-29	Induction of apoptosis and cell cycle arrest, decrease in the expression of HDAC1, DNMT1, and HDAC activity	Saldanha et al. (2014)

Table 8 Combination of EGCG and anticancer drugs enhances anticancer effects in human ovarian cancer cell lines

EGCG +	Anticancer drugs	Cell lines	Anticancer effects	References
EGCG	Cisplatin	A2780, A2780 (*cis*R)	Inhibition of cell viability, cellular accumulation of platinum and platinum–DNA binding in cells	Mazumder et al. (2012)
EGCG	*trans*-palladiums			
EGCG	Sulforaphane	SKOV-ip1(paclitaxel-sensitive), SKOVTR-ip2 (paclitaxel-resistant)	Inhibition of cell viability, arrest in G_2/M and S phases, increase in apoptosis, reduction in hTERT expression	Chen et al. (2013)

Table 9 Combination of EGCG and anticancer compounds enhances anticancer effects in human malignant neuroblastoma cell lines

EGCG +	Anticancer compounds	Neuroblastoma	Anticancer effects	References
EGCG	Retinoids (ATRA, 13-*cis* RA, 4-HPR)	SH-SY5Y	Increase in apoptosis, down-regulation of telomerase activity, induction of neuronal differentiation, overexpression of NFP	Das et al. (2009)
EGCG	SU5416	SH-SY5Y, SK-N-BE2	Inhibition of cell survival, induction of apoptosis, suppression of VEGFR-2 gene expression, and cell migration	Mohan et al. (2011)

Table 10 Combination of EGCG and anticancer compounds on human leukemia cell lines

EGCG +	Anticancer compounds	Leukemia	References
EGCG	Cytosine arabinoside	HL-60	Xu and Zhen (2003)
EGCG	Benzyl isothiocyanate	Jurkat T leukemia	Wu et al. (2008)
EGCG	H_2O_2	Jurkat T leukemia	Wu et al. (2008)
EGCG	Curcumin	B-cell chronic leukemia	Ghosh et al. (2009)
EGCG	Celastrol (proteasome inhibitor)	Myelogenous leukemia, K-562, Jurkat T leukemia	Davenport et al. (2010)

Table 11 Combination of EGCG and anticancer compounds on human cancer cell lines of various organs

EGCG +	Anticancer compounds	Human organs	Cell lines	References
EGCG	Thymoquinone (mitochondria-targeted antioxidant)	Pancreas	PANC-1	Tan et al. (2006)
EGCG	Celecoxib	Pancreas	Colo357	Härdtner et al. (2012)
EGCG	TRAIL	Pancreas	MIA PaCa-2	Basu and Haldar (2009)
EGCG	Retinoic acid	Cervix	HeLa, TMCC-1	Yokoyama et al. (2008)
EGCG	Vorinostat (first HDAC inhibitor)	Melanoma	A-375, Hs-294T G-361	Nihal et al. (2010)
EGCG	3-Deazaneplanocin	Skin	SCC-13, A431	Choudhury et al. (2011)
EGCG	Docetaxel	Stomach	BGC-823	Wu et al. (2012)

including 4-hydroxytamoxifen (4-OHT), tamoxifen, curcumin, raloxifene (selective estrogen receptor modulator), resveratrol, γ-tocotrienol, and tricostatin A. Synergistic anticancer effects were found in both ERα− human breast cancer cell lines MDA-MB-231 and HS578T, and ERα+ human breast cancer cell line MCF-7. The combination of EGCG and trichostatin A synergistically induced re-activation of ERα expression in MDA-MB-231 cells (Li et al. 2010), and combinations of EGCG and 4-OHT, and EGC and 4-OHT, had the same effects (Chisholm et al. 2004). The treatment of MDA-MB-231 cells with combinations of EGCG and tamoxifen, and EGCG and curcumin, showed synergistic inhibition of tumor volume in xenograft mouse models (Scandlyn et al. 2008; Somers-Edgar et al. 2008) (Table 12).

Human prostate cancer cell lines

Table 5 summarizes the results of 10 in vitro experiments on nine human prostate cancer cell lines treated with combinations of EGCG and anticancer compounds. Anticancer drugs used were doxorubicin, paclitaxel, docetaxel, and bortezomib, and the other compounds included phytochemicals and NS398 (a specific COX-2 inhibitor). The combination of EGCG and doxorubicin showed synergistic anticancer effects on tumor cell growth and colony-forming ability with PC-3ML, IBC-10a, and PCa-20a, but it did not reduce the growth rates of human WI38 fibroblasts derived from lung tissue (Stearns et al. 2010). The combinations of EGCG and paclitaxel, and EGCG and docetaxel, had synergistic effects on reducing growth rates of PC-3ML to near zero in 8 days, and both combinations increased the expression of apoptotic genes (p53, p73, p21, and caspase-3) (Stearns and Wang 2011). Although the combination of EGCG and bortezomib had no impact on the cytotoxicity of RPMI8226 MM cells (Bannerman et al. 2011), bortezomib exerts anticancer effects by reversibly blocking the activity of 26S proteasome (Adams 2004). Two polyphenols, EGCG and resveratrol, have separately induced apoptosis, associated with a significant down-regulation

Table 12 Enhanced inhibition of tumor volume in xenograft mouse models implanted with human cancer cell lines after treatment with the combinations

Cancer cell lines	Tumor volume (% of control)					
	Drugs	Vehicle	EGCG alone	Drugs alone	Combination	References
Head, neck, and lung cancer cell lines						
Tu212	Erlotinib	100	48.3	41.7	15.8	Zhang et al. (2008)
H460	Erlotinib	100	86.6	75.0	46.6	Milligan et al. (2009)
Tu212	Luteolin	100	100	86.5	15.4	Amin et al. (2010)
A549	Luteolin	100	72.3	67.9	45.5	Amin et al. (2010)
A549	Curcumin	100			41.8	Zhou et al. (2013)
Breast cancer cell lines						
MDA-MB-231(ERα−)	Tamoxifen	100	69.4	100	29.0	Scandlyn et al. (2008)
MDA-MB-231(ERα−)	Curcumin	100	73.2	109	49.1	Somers-Edgar et al. (2008)
Prostate cancer cell lines						
CWR22Rv1	Celecoxib	100	62.6[a]	58.6	26.3	Adhami et al. (2007)
PC-3ML	Paclitaxel	100	40.9[b]	44.3	0	Stearns et al. (2010)
	Docetaxel	100	54.1[b]	42.4	0	Stearns et al. (2010)
CWR22	Bortezomib	100	96.0	46.5	40.0[c]	Bannerman et al. (2011)
Hepatocellular carcinoma cell line						
BEL-7404/DOX	Doxorubicin	100	104.2	74.5	39.4[d]	Liang et al. (2010)
Gastric cancer cell line						
BGC-823	Docetaxel	100	73.9	49.6[e]	37.0	Wu et al. (2012)
Average % of inhibition		0	26.5	33.7	70.3	

[a] GTP: green tea polyphenol (extract), [b] a dose of EGCG was 200 μM, [c] EGCG 50 mg/kg sc biweekly followed 30 min later by bortezomib 0.8 mg/kg iv ($p < 0.001$), [d] three doses of EGCG 40, 80, and 160 μg/ml resulted in 53.2, 42.6, and 39.4 % of tumor volume, respectively, [e] LDM: i.p. injection of 0.5 mg/kg docetaxel three times a week

of casein kinase 2 and protein expression in both human androgen-sensitive (ALVA-41) and androgen-insensitive (PC-3) prostate cancer cell lines, but combination experiments had not yet been conducted at the time of these studies (Ahmad et al. 2007). Treatments with combinations of EGCG and paclitaxel, and EGCG and docetaxel, completely eliminated tumor development of PC-3ML cells in xenograft mouse models (Stearns and Wang 2011) (Fig. 2a, b), and the combination of green tea extract and celecoxib also showed synergistic inhibition of tumor volume in CWR22Rv1 cells in the same model (Adhami et al. 2007) (Table 12).

Human liver cancer cell lines

Table 6 summarizes the results of three in vitro experiments on two human liver cancer cell lines, treated with combinations of EGCG or ECG, and the anticancer drugs doxorubicin and 5-FU. Two human hepatocellular carcinoma cell lines showed varying sensitivity to doxorubicin (DOX): BEL-7404/DOX cells were resistant, and BEL-7404 cells were sensitive. Treatments of BEL-7404/DOX cells with combinations of EGCG and DOX, and ECG and DOX, reduced the IC_{50} values from 36 to 1.9 and 2.3 mg/ml, respectively. And in the sensitive BEL-7404, treatments with combinations of EGCG and DOX, and ECG and DOX, reduced the IC_{50} values from 0.94 to 0.34 and 0.34 mg/ml, respectively. Thus, EGCG and ECG similarly enhanced sensitivity to DOX in both BEL-7404/DOX and BEL-7404 (Liang et al. 2010). The combination of EGCG and 5-FU augmented the anticancer effects of 5-FU on Hep3B cells (Yang et al. 2012), and treatment of BEL-7404/DOX cells with combinations of EGCG and DOX showed synergistic inhibition of tumor volume in xenograft mouse models (Liang et al. 2010) (Table 12).

Human colon cancer cell lines

Table 7 summarizes the results of two in vitro experiments on three human colon cancer cell lines treated with combinations of EGCG and the anticancer compounds sulforaphane and sodium butyrate. A low-dose combination of 20 μM EGCG and 25 μM sulforaphane decreased cell viability of HT-29 to 70 %, whereas the high-dose combination of 100 μM EGCG and 25 μM sulforaphane

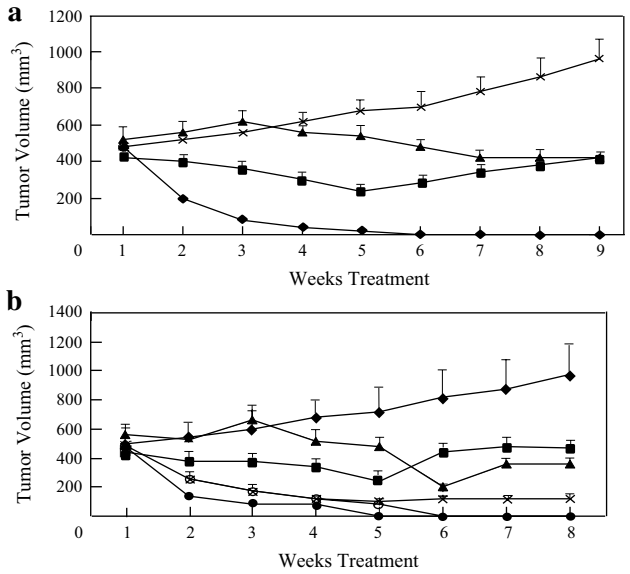

Fig. 2 Development of tumor volume with PC-3ML cells in xenograft in CB17 SCID mice. **a** Inhibition of tumor volume in mice treated with the vehicle (*cross*), EGCG (*triangle*), paclitaxel (*square*), or the combination of EGCG and paclitaxel (*diamond*). **b** Inhibition of tumor volume in mice treated with the vehicle (*diamond*), EGCG (*square*), docetaxel (*triangle*), or the combination of EGCG 100 μM and docetaxel (*cross*), that of EGCG 200 μM and docetaxel (*open circle*), and that of EGCG 300 μM and docetaxel (*filled circle*) (Stearns and Wang 2011, with the permission of Dr. Mark E. Stearns)

decreased it to 40 % at 48 h. The combination dramatically enhanced transcriptional activation of AP-1 reporter in HT-29, in the range of 46- to 175-fold, and attenuated the cellular senescence induced by EGCG alone (Nair et al. 2008). The combination of EGCG and sodium butyrate induced G_2/M arrest in RKO and HCT-116, and G_1 arrest in HT-2 (Saldanha et al. 2014). Synergistic inhibition of tumor growth in xenograft mouse model was not reported with these human colon cancer cell lines.

Human ovarian cancer cell lines

The combinations of EGCG and cisplatin, and EGCG and three *trans*-palladiums (TH5, TH6, and TH7), produced synergistic inhibition of viability for both cisplatin-sensitive A2780 and cisplatin-resistant A2780 (cisR) cell lines, and also showed pronounced cellular accumulation of platinum and a high level of platinum–DNA binding in the cells (Mazumder et al. 2012) (Table 8). The combination of EGCG and sulforaphane was applied to paclitaxel-sensitive SKOV-ip1 and paclitaxel-resistant SKOVTR-ip2 cell lines, resulting in synergistic inhibition of viability for both cell lines. Moreover, the combination significantly increased the apoptosis in paclitaxel-resistant SKOVTR-ip2 and reduced the expression of human telomerase reverse transcriptase (hTERT) (Chen et al. 2013) (Table 8).

Human malignant neuroblastoma

Treatments of SH-SY5Y cells with three retinoids, all-*trans* retinoic acid (ATRA), 13-*cis* retinoic acid, and *N*-(4-hydroxyphenyl)retinamide (4-HPR), followed by EGCG, induced various anticancer effects, including increase in apoptosis of the differentiated cells and down-regulation of telomerase activity (Das et al. 2009) (Table 9). The combination of EGCG and SU5416—an inhibitor of the vascular endothelial growth factor receptor-2 (VEGFR-2)—inhibited survival of SH-SY5Y and SK-N-BE2 cells, which resulted in cell cycle arrest and apoptosis, the latter of which was induced by both down-regulation of Bcl-2 and activation of caspase-3 (Mohan et al. 2011) (Table 9).

Various in vitro combinations of EGCG and anticancer compounds induced the synergistic anticancer effects described above, particularly the inhibition of cell viability and induction of apoptosis: They were effective even though cancer cell lines derived from various human tissues are quite discrete and heterogeneous. In order to study the diversity of anticancer compounds and to avoid repeating of similar anticancer effects, Table 10 summarizes only the combinations of EGCG and anticancer compounds on human leukemia cell lines (Xu and Zhen 2003; Wu et al. 2008; Ghosh et al. 2009; Davenport et al. 2010), and Table 11 shows the diversity of the combination effects on numerous human cancer cell lines derived from various cancer tissues (Tan et al. 2006; Härdtner et al. 2012; Basu and Haldar 2009; Yokoyama et al. 2008; Nihal et al. 2010; Choudhury et al. 2011; Wu et al. 2012). And the treatment for gastric cancer cell line BGC-823 with the combination of EGCG and docetaxel showed synergistic inhibition of tumor volume in xenograft mouse models (Wu et al. 2012) (Table 12).

Enhanced inhibition of tumor volume in xenograft mouse models

The combination of EGCG, other green tea catechins, and anticancer compounds is now widely accepted by investigators in the world of cancer treatment. Table 12 shows the enhanced inhibition of tumor volume in in vivo xenograft mouse models implanted with nine human cancer cell lines from head, neck, and lungs; breast; prostate; liver; and stomach, after treatments with vehicle, EGCG alone, anticancer compounds alone, or the combination. Tumor volume of each group is shown as % of control. It is important to note that the combinations of EGCG, or green tea

extract, and all tested anticancer compounds synergistically increased anticancer activity an average of 70.3 % (Table 12).

It is striking that the combinations of EGCG and paclitaxel, and EGCG and docetaxel, completely eliminated tumors of human prostate cancer cell line PC-3ML in vivo (Table 12) (Fig. 2a, b), and that the combination of EGCG and docetaxel dose-dependently showed the efficacy of EGCG when it was increased from 100 to 300 μM (Stearns and Wang 2011) (Fig. 2). The amount of EGCG necessary for the elimination of tumors varied from 4.56 to 6.84 mg/day/mouse. If the amount for mice is converted to that for humans, it would be 1.37–2.05 g EGCG/day/person, corresponding to 6–9 Japanese-size-cups of green tea, near the amount previously reported as the effective cancer-preventive amount (Fujiki et al. 2012).

We emphasize here that mice treated with a combination did not show any toxic effects during any of the in vivo experiments. The results strongly indicate that the combination of green tea catechins and anticancer compounds is a new cancer therapeutic strategy. In fact, a poster entitled "Combination of Erlotinib and EGCG Induces Apoptosis of Squamous Cell Carcinoma of the Head and Neck Through Posttranslational Regulation of Bim and Bcl-2" was presented at the Annual Meeting of the American Association for Cancer Research in San Diego in 2014 (Haque et al. 2014). Clinical trials are now going on to try to inhibit or reverse the progression of oral premalignant lesions, at Winship Cancer Institute of Emory University. The combination will surely be welcomed as a new cancer treatment that will result in good prognoses for patients needing tertiary cancer prevention.

Discussion

We discuss here the inhibitory effects of EGCG on self-renewal capacity of cancer stem cells, based on the results showing that the combination of EGCG and quercetin synergistically inhibited stem cell characteristics of human prostate cancer cells (Table 5) (Tang et al. 2010). EGCG also inhibited both viability of human pancreatic cancer stem cells ($CD133^+/CD44^+/CD24^+/ESA^+$) in primary and secondary spheroids in a dose-dependent manner (0–60 μM), and expression of pluripotency maintaining transcription factor genes, *Nanog*, *c-Myc,* and *Oct-4* in cancer stem cells. Since Nanog is a key regulator of self-renewal and pluripotency, it is notable that the expression of *Nanog* gene was more strongly inhibited (with 20 μM EGCG) than any of the other transcription factor genes (Tang et al. 2012). Experiments with SUM-149 cells that showed the presence of a cancer stem-like cell population indicated that formation of primary SUM-149 sphere was completely inhibited by treatment with 80 μg/ml (174.7 μM) EGCG, and that the secondary and tertiary spheres were completely inhibited with 40 μg/ml (87.3 μM) EGCG (Mineva et al. 2013). Treatment for cancer stem K3 cells ($CD44^+$) from head and neck squamous cell carcinoma with 5 μM EGCG reduced the expression of *Oct4*, *Sox2*, *and Nanog* genes, and also the protein levels of Notch1, indicating the suppression of Notch signaling (Lee et al. 2013). All results show that EGCG and other green tea catechins target cancer stem cells in numerous human cancer tissues.

Considering the inhibitory effects of EGCG on cancer stem cells, the combination of EGCG and anticancer compounds will increase the effectiveness of conventional chemotherapy and radiotherapy on differentiated progenitor cells, within the framework of targeted cancer therapy. To improve the quality of life for cancer patients, we strongly recommend the combination of green tea catechins and anticancer compounds for their long life. As mentioned before, Professor Tomizo Yoshida, a mentor of Japanese pathology, presented his medical concept: "The final goal of cancer therapy was the survival of cancer patients with coexistence of cancer cells," which was based on his pathological studies of cancer tissues (Yoshida 1992). When we consider Yoshida's concept together with the synergistic anticancer effects induced by the combination, we feel certain that most cancer patients will be able to achieve improved quality of life without suffering the side effects of medicines. The combination is a beneficial cancer treatment within the framework of personalized cancer therapy.

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Conflict of interest Here, we declare that we have no conflicts of interest.

References

Adams J (2004) The development of proteasome inhibitors as anticancer drugs. Cancer Cell 5:417–421

Adhami VM et al (2007) Combined inhibitory effects of green tea polyphenols and selective cyclooxygenase-2 inhibitors on the growth of human prostate cancer cells both in vitro and in vivo. Clin Cancer Res 13:1611–1619

Ahmad KA, Harris NH, Johnson AD, Lindvall HC, Wang G, Ahmed K (2007) Protein kinase CK2 modulates apoptosis induced by resveratrol and epigallocatechin-3-gallate in prostate cancer cells. Mol Cancer Ther 6:1006–1012

Amin AR et al (2010) Enhanced anti-tumor activity by the combination of the natural compounds (−)-epigallocatechin-3-gallate and luteolin: potential role of p53. J Biol Chem 285:34557–34565

Bannerman B et al (2011) Preclinical evaluation of the antitumor activity of bortezomib in combination with vitamin C or with epigallocatechin gallate, a component of green tea. Cancer Chemother Pharmacol 68:1145–1154

Basu A, Haldar S (2009) Combinatorial effect of epigallocatechin-3-gallate and TRAIL on pancreatic cancer cell death. Int J Oncol 34:281–286

Chen H, Landen CN, Li Y, Alvarez RD, Tollefsbol TO (2013) Epigallocatechin gallate and sulforaphane combination treatment induce apoptosis in paclitaxel-resistant ovarian cancer cells through hTERT and Bcl-2 down-regulation. Exp Cell Res 319:697–706

Chisholm K, Bray BJ, Rosengren RJ (2004) Tamoxifen and epigallocatechin gallate are synergistically cytotoxic to MDA-MB-231 human breast cancer cells. Anticancer Drugs 15:889–897

Choudhury SR, Balasubramanian S, Chew YC, Han B, Marquez VE, Eckert RL (2011) (−)-Epigallocatechin-3-gallate and DZNep reduce polycomb protein level via a proteasome-dependent mechanism in skin cancer cells. Carcinogenesis 32:1525–1532

Das A, Banik NL, Ray SK (2009) Retinoids induce differentiation and downregulate telomerase activity and N-Myc to increase sensitivity to flavonoids for apoptosis in human malignant neuroblastoma SH-SY5Y cells. Int J Oncol 34:757–765

Davenport A, Frezza M, Shen M, Ge Y, Huo C, Chan TH, Dou QP (2010) Celastrol and an EGCG pro-drug exhibit potent chemosensitizing activity in human leukemia cells. Int J Mol Med 25:465–470

Fujiki H, Suganuma M (2012) Green tea: an effective synergist with anticancer drugs for tertiary cancer prevention. Cancer Lett 324:119–125

Fujiki H et al (2001) Cancer prevention with green tea and monitoring by a new biomarker, hnRNP B1. Mutat Res 480–481:299–304

Fujiki H, Suganuma M, Imai K, Nakachi K (2002) Green tea: cancer preventive beverage and/or drug. Cancer Lett 188:9–13

Fujiki H, Imai K, Nakachi K, Shimizu M, Moriwaki H, Suganuma M (2012) Challenging the effectiveness of green tea in primary and tertiary cancer prevention. J Cancer Res Clin Oncol 138:1259–1270

Ghosh AK, Kay NE, Secreto CR, Shanafelt TD (2009) Curcumin inhibits prosurvival pathways in chronic lymphocytic leukemia B cells and may overcome their stromal protection in combination with EGCG. Clin Cancer Res 15:1250–1258

Haque A, Rahman MA, Chen ZG, Shin DM, Amin AR (2014) Combination of erlotinib and epigallocatechin-3-gallate induces apoptosis of squamous cell carcinoma of the head and neck through posttranslational regulation of Bim and Bcl-2. Proc Am Assoc Cancer Res 55:548

Härdtner C, Multhoff G, Falk W, Radons J (2012) (−)-Epigallocatechin-3-gallate, a green tea-derived catechin, synergizes with celecoxib to inhibit IL-1-induced tumorigenic mediators by human pancreatic adenocarcinoma cells Colo357. Eur J Pharmacol 684:36–43

Hsieh TC, Wu JM (2008) Suppression of cell proliferation and gene expression by combinatorial synergy of EGCG, resveratrol and gamma-tocotrienol in estrogen receptor-positive MCF-7 breast cancer cells. Int J Oncol 33:851–859

Hsieh TC, Wu JM (2009) Targeting CWR22Rv1 prostate cancer cell proliferation and gene expression by combinations of the phytochemicals EGCG, genistein and quercetin. Anticancer Res 29:4025–4032

Lee SH, Nam HJ, Kang HJ, Kwon HW, Lim YC (2013) Epigallocatechin-3-gallate attenuates head and neck cancer stem cell traits through suppression of Notch pathway. Eur J Cancer 49:3210–3218

Li Y, Yuan YY, Meeran SM, Tollefsbol TO (2010) Synergistic epigenetic reactivation of estrogen receptor-alpha (ERalpha) by combined green tea polyphenol and histone deacetylase inhibitor in ERalpha-negative breast cancer cells. Mol Cancer 9:274

Liang G et al (2010) Green tea catechins augment the antitumor activity of doxorubicin in an in vivo mouse model for chemoresistant liver cancer. Int J Oncol 37:111–123

Masuda M, Suzui M, Weinstein IB (2001) Effects of epigallocatechin-3-gallate on growth, epidermal growth factor receptor signaling pathways, gene expression, and chemosensitivity in human head and neck squamous cell carcinoma cell lines. Clin Cancer Res 7:4220–4229

Maytin EV, Ubeda M, Lin JC, Habener JF (2001) Stress-inducible transcription factor CHOP/gadd153 induces apoptosis in mammalian cells via p38 kinase-dependent and -independent mechanisms. Exp Cell Res 267:193–204

Mazumder ME, Beale P, Chan C, Yu JQ, Huq F (2012) Epigallocatechin gallate acts synergistically in combination with cisplatin and designed trans-palladiums in ovarian cancer cells. Anticancer Res 32:4851–4860

Milligan SA et al (2009) The green tea polyphenol EGCG potentiates the antiproliferative activity of c-Met and epidermal growth factor receptor inhibitors in non-small cell lung cancer cells. Clin Cancer Res 15:4885–4894

Mineva ND, Paulson KE, Naber SP, Yee AS, Sonenshein GE (2013) Epigallocatechin-3-gallate inhibits stem-like inflammatory breast cancer cells. PLoS ONE 8:e73464

Mohan N, Karmakar S, Banik NL, Ray SK (2011) SU5416 and EGCG work synergistically and inhibit angiogenic and survival factors and induce cell cycle arrest to promote apoptosis in human malignant neuroblastoma SH-SY5Y and SK-N-BE2 cells. Neurochem Res 36:1383–1396

Nair S et al (2008) Synergistic effects of a combination of dietary factors sulforaphane and (−)-epigallocatechin-3-gallate in HT-29 AP-1 human colon carcinoma cells. Pharm Res 25:387–399

Nair S et al (2010) Regulation of Nrf2- and AP-1-mediated gene expression by epigallocatechin-3-gallate and sulforaphane in prostate of Nrf2-knockout or C57BL/6 J mice and PC-3 AP-1 human prostate cancer cells. Acta Pharmacol Sin 31:1223–1240

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

Nakachi K, Matsuyama S, Miyake S, Suganuma M, Imai K (2000) Preventive effects of drinking green tea on cancer and cardiovascular disease: epidemiological evidence for multiple targeting prevention. BioFactors 13:49–54

Nihal M, Roelke CT, Wood GS (2010) Anti-melanoma effects of vorinostat in combination with polyphenolic antioxidant (−)-epigallocatechin-3-gallate (EGCG). Pharm Res 27:1103–1114

Okabe S, Fujimoto N, Sueoka N, Suganuma M, Fujiki H (2001) Modulation of gene expression by (−)-epigallocatechin gallate in PC-9 cells using a cDNA expression array. Biol Pharm Bull 24:883–886

Saha A, Kuzuhara T, Echigo N, Suganuma M, Fujiki H (2010) New role of (−)-epicatechin in enhancing the induction of growth inhibition and apoptosis in human lung cancer cells by curcumin. Cancer Prev Res 3:953–962

Sakata M, Ikeda T, Imoto S, Jinno H, Kitagawa Y (2011) Prevention of mammary carcinogenesis in C3H/OuJ mice by green tea and tamoxifen. Asian Pac J Cancer Prev 12:567–571

Saldanha SN, Kala R, Tollefsbol TO (2014) Molecular mechanisms for inhibition of colon cancer cells by combined epigenetic-modulating epigallocatechin gallate and sodium butyrate. Exp Cell Res 324:40–53

Scandlyn MJ, Stuart EC, Somers-Edgar TJ, Menzies AR, Rosengren RJ (2008) A new role for tamoxifen in oestrogen

receptor-negative breast cancer when it is combined with epigallocatechin gallate. Br J Cancer 99:1056–1063

Shimizu M et al (2008) Green tea extracts for the prevention of metachronous colorectal adenomas: a pilot study. Cancer Epidemiol Biomarkers Prev 17:3020–3025

Shiraishi T et al (2005) Tunicamycin enhances tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis in human prostate cancer cells. Cancer Res 65:6364–6370

Somers-Edgar TJ, Scandlyn MJ, Stuart EC, Le Nedelec MJ, Valentine SP, Rosengren RJ (2008) The combination of epigallocatechin gallate and curcumin suppresses ER alpha-breast cancer cell growth in vitro and in vivo. Int J Cancer 122:1966–1971

Stearns ME, Wang M (2011) Synergistic effects of the green tea extract epigallocatechin-3-gallate and taxane in eradication of malignant human prostate tumors. Transl Oncol 4:147–156

Stearns ME, Amatangelo MD, Varma D, Sell C, Goodyear SM (2010) Combination therapy with epigallocatechin-3-gallate and doxorubicin in human prostate tumor modeling studies: inhibition of metastatic tumor growth in severe combined immunodeficiency mice. Am J Pathol 177:3169–3179

Stuart EC, Rosengren RJ (2008) The combination of raloxifene and epigallocatechin gallate suppresses growth and induces apoptosis in MDA-MB-231 cells. Life Sci 82:943–948

Stuart EC, Larsen L, Rosengren RJ (2007) Potential mechanisms for the synergistic cytotoxicity elicited by 4-hydroxytamoxifen and epigallocatechin gallate in MDA-MB-231 cells. Int J Oncol 30:1407–1412

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 (1999) Synergistic effects of (−)-epigallocatechin gallate with (−)-epicatechin, sulindac, or tamoxifen on cancer-preventive activity in the human lung cancer cell line PC-9. Cancer Res 59:44–47

Suganuma M, Ohkura Y, Okabe S, Fujiki H (2001) Combination cancer chemoprevention with green tea extract and sulindac shown in intestinal tumor formation in Min mice. J Cancer Res Clin Oncol 127:69–72

Suganuma M, Kurusu M, Suzuki K, Tasaki E, Fujiki H (2006) Green tea polyphenol stimulates cancer preventive effects of celecoxib in human lung cancer cells by upregulation of *GADD153* gene. Int J Cancer 119:33–40

Suganuma M, Saha A, Fujiki H (2011) New cancer treatment strategy using combination of green tea catechins and anticancer drugs. Cancer Sci 102:317–323

Tan M, Norwood A, May M, Tucci M, Benghuzzi H (2006) Effects of (−)-epigallocatechin gallate and thymoquinone on proliferation of a PANC-1 cell line in culture. Biomed Sci Instrum 42:363–371

Tang SN, Singh C, Nall D, Meeker D, Shankar S, Srivastava RK (2010) The dietary bioflavonoid quercetin synergizes with epigallocatechin gallate (EGCG) to inhibit prostate cancer stem cell characteristics, invasion, migration and epithelial-mesenchymal transition. J Mol Signal 5:14

Tang SN, Fu J, Nall D, Rodova M, Shankar S, Srivastava RK (2012) Inhibition of sonic hedgehog pathway and pluripotency maintaining factors regulate human pancreatic cancer stem cell characteristics. Int J Cancer 131:30–40

Wu H, Yokoyama T, Zhu B, Shimoishi Y, Murata Y, Nakamura Y (2008) (−)-Epigallocatechin-3-gallate potentiates the cytotoxicity induced by benzyl isothiocyanate and hydrogen peroxide in human Jurkat T lymphocytes. Biosci Biotechnol Biochem 72:3034–3037

Wu H, Xin Y, Xiao Y, Zhao J (2012) Low-dose docetaxel combined with (−)-epigallocatechin-3-gallate inhibits angiogenesis and tumor growth in nude mice with gastric cancer xenografts. Cancer Biother Radiopharm 27:204–209

Xu F, Zhen YS (2003) (−)-Epigallocatechin-3-gallate enhances antitumor effect of cytosine arabinoside on HL-60 cells. Acta Pharmacol Sin 24:163–168

Yang XW et al (2012) Green tea polyphenol epigallocatechin-3-gallate enhances 5-fluorouracil-induced cell growth inhibition of hepatocellular carcinoma cells. Hepatol Res 42:494–501

Yokoyama M, Noguchi M, Nakao Y, Ysunaga M, Yamasaki F, Iwasaka T (2008) Antiproliferative effects of the major tea polyphenol, (−)-epigallocatechin gallate and retinoic acid in cervical adenocarcinoma. Gynecol Oncol 108:326–331

Yoshida N (1992) Cancer cells talked in this way. Bungeishunju, Ltd, Tokyo, p 267 (Japanese)

Yoshida T et al (2005) Proteasome inhibitor MG132 induces death receptor 5 through CCAAT/enhancer-binding protein homologous protein. Cancer Res 65:5662–5667

Zhang X et al (2008) Synergistic inhibition of head and neck tumor growth by green tea (−)-epigallocatechin-3-gallate and EGFR tyrosine kinase inhibitor. Int J Cancer 123:1005–1014

Zhou DH, Wang X, Yang M, Shi X, Huang W, Feng Q (2013) Combination of low concentration of (−)-epigallocatechin gallate (EGCG) and curcumin strongly suppresses the growth of non-small cell lung cancer in vitro and in vivo through causing cell cycle arrest. Int J Mol Sci 14:12023–12036