REVIEW

Cancer chemoprevention with green tea catechins by targeting receptor tyrosine kinases

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Recent studies indicate that receptor tyrosine kinases (RTKs), which play important roles in cell proliferation, are one of the possible targets of green tea catechins (GTCs) in cancer cell growth inhibition. (−)-Epigallocatechin-3-gallate (EGCG), the major catechin in green tea, inhibits cell proliferation and induces apoptosis in various types of cancer cells, including colorectal cancer and hepatocellular carcinoma cells, by blocking the activation of the epidermal growth factor receptor (EGFR) family of RTKs. EGCG inhibits the activation of insulin-like growth factor-1 receptor (IGF-1R) and VEGFR2, the other members of the RTK family, and this effect is also associated with the anticancer and chemopreventive properties of this agent. EGCG suppresses the activation of EGFR in part by altering membrane lipid organization and causing the subsequent inhibition of the dimerization and activation of this receptor. Preliminary trials have shown that GTCs successfully prevent the development and progression of precancerous lesions, such as colorectal adenomas, without causing severe adverse effects. The present report reviews evidence indicating that GTCs exert anticancer and chemopreventive effects by inhibiting the activation of specific RTKs, especially EGFR, IGF-1R, and VEGFR2, and concludes that targeting RTKs and their related signaling pathways by using tea catechins could be a promising strategy for the prevention of human cancers.

Keywords:
Colorectal cancer / EGCG / Hepatocellular carcinoma / Lipid rafts / Receptor tyrosine kinase

1 Introduction

Tea is one of the most popular beverages worldwide. In the recent years, tea polyphenols, which are known as catechins, have received great attention for their beneficial effects, in particular their involvement in cancer chemoprevention. Among tea catechins, green tea catechins (GTCs) are best known for their cancer-preventive properties. Rapidly increasing number of studies have reported that (−)-epigallocatechin-3-gallate (EGCG), the major biologically active component in green tea, is one of the most potent catechins capable of inhibiting cell proliferation and inducing apoptosis in cancer cells [1–6]. Recent studies have revealed that GTCs exert cancer chemopreventive and anticarcinogenic effects, at least in part, by modulating the activities of different receptor tyrosine kinases (RTKs) and their multiple downstream signaling pathways, including the Ras/extracellular signal-regulated kinase (ERK) and phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathways [7, 8], which control the expression of the multiple target genes involved in cell proliferation and apoptosis [3–5].

The present report reviews the novel and updated mechanisms by which GTCs prevent carcinogenesis, with a special emphasis on colorectal cancer (CRC) and

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Abbreviations: AP-1, activator protein-1; **CRC,** colorectal cancer; **COX-2,** cyclooxygenase-2; **EGFR,** epidermal growth factor receptor; **EGCG,** (−)-epigallocatechin-3-gallate; **ERK,** extracellular signal-regulated kinase; **GTC,** green tea catechin; **GTE,** green tea extract; **HNSCC,** head and neck squamous cell carcinoma; **HCC,** hepatocellular carcinoma; **HGF,** hepatocyte growth factor; **IGF-1R,** insulin-like growth factor-1 receptor; **LR,** laminin receptor; **MEK,** mitogen-activated protein kinase; **NF-κB,** nuclear factor-κB; **PI3K,** phosphatidylinositol 3-kinase; **PDGF,** platelet-derived growth factor; **PGE_2,** prostaglandin E_2; **RTK,** receptor tyrosine kinase; **VEGF,** vascular endothelial growth factor; **VEGFR,** vascular endothelial growth factor receptor

hepatocellular carcinoma (HCC), and focusing on the effects of these agents on the activity of RTKs. Among the different types of RTKs, attention was particularly given to epidermal growth factor receptor (EGFR), insulin-like growth factor-1 receptor (IGF-1R), and vascular endothelial growth factor receptor-2 (VEGFR2), because alterations in these RTKs and their ligands have been reported to be largely involved in the development of cancer [9–11]. In this review, the ability of GTCs, especially EGCG, to alter the activation status of these RTKs and their downstream signal transduction pathways is discussed. In addition, the potential for the clinical application of GTCs is also examined, particularly in reference to our recent pilot trial showing the preventive effect of GTCs on the recurrence of colorectal adenomas after polypectomy [12].

2 Green tea and cancer chemoprevention

Tea, especially green tea, produced from the leaves of the Camellia sinensis, is one of the most widely consumed beverages in the world. The health benefits of green tea are well documented, including its effect on cancer prevention. Green tea contains several polyphenolic compounds (catechins), including EGCG, (−)-epigallocatechin, epicatechin-3-gallate, and (−)-epicatechin. Among these GTCs, EGCG seems to be the most effective in the suppression of cell proliferation and induction of apoptosis in cancer cells [1–6]. Numerous animal experiments have shown the cancer chemopreventive effects of tea and its components [1, 2]. We recently reported that administration of EGCG through drinking water significantly suppresses chemically induced colonic and hepatic premalignant lesions in obese and diabetic mice [13, 14]. Treatment with EGCG and Polyphenon E, a standardized and well-characterized decaffeinated extract of green tea, significantly suppressed inflammation-related mouse colon carcinogenesis by attenuating the inflammatory reaction on the colonic mucosa [15]. EGCG consumption also significantly inhibited the growth of CRC and HCC xenografts in nude mice [16, 17].

Several properties of GTCs have been implicated in their anticancer and chemopreventive effects, such as their antioxidant [1], pro-oxidant [18], antimutagenic [19], antiinflammatory [15], and antiangiogenic effects [20]. EGCG also serves as a binding partner for many biomolecules, including the 67-kDa laminin receptor (LR) [21, 22] and the Bcl-2 proteins [23], which might be associated with inhibition of the activation of several types of intracellular signaling molecules and the induction of apoptosis [24, 25]. These biological effects exerted by GTCs may act cooperatively in preventing the development of human malignancies (for more details, see References [1, 2]).

Recently, we and other investigators revealed that targeting RTKs and their downstream signaling pathways might be one of the possible mechanisms mediating the effects of GTCs on the prevention of cancers [3–5]. Because abnormalities in the expression and/or function of cell surface RTKs and their specific signaling pathways are widely associated with the development of various types of human malignancies, targeting these aberrant molecules is an effective strategy for the prevention of certain types of cancers, including CRC and HCC. The following section will provide a detailed explanation of the relationship between abnormalities in specific RTKs and the development of CRC and HCC.

3 Abnormalities in RTKs and colorectal and liver cancers

All members of the RTK family show a similar structure consisting of an extracellular ligand-binding domain, a single membrane-spanning region, and a cytoplasmic protein tyrosine kinase domain. The binding of specific ligands (growth factors and cytokines) to the extracellular domain of RTKs stimulates their intrinsic tyrosine kinase activity and triggers autophosphorylation of specific tyrosine residues, thereby resulting in the creation of docking sites for downstream targets. The major signaling pathways activated by RTKs are the Ras/ERK pathway and the PI3K/Akt pathway. In the Ras/ERK pathway, Ras activation by RTKs triggers its interaction with and activation of Raf-1 [7, 8]. The activation of cell surface RTKs and their downstream signalings play an important role in the control of many fundamental cellular processes in normal cells; however, tumor cells often show alterations in the activation of RTKs through several mechanisms, including mutations, overexpression, and the autocrine or paracrine production of their ligands [9–11].

The EGFR family includes four members, namely EGFR (erbB1), HER2 (neu/erbB2), HER3 (neu/erbB3), and HER4 (neu/erbB4), which belong to subclass I of the RTK superfamily. IGF-1R and VEGFR2 belong to a separate family of RTKs. Although approximately 20 different RTK classes have been identified, abnormalities in certain RTKs, especially EGFR, IGF-1R, and VEGFR2, are largely associated with the acquisition of neoplastic properties in various organs, including the colorectum and liver [9–11]. Human CRC often displays an overexpression of EGFR, and the constitutive activation of this receptor and related downstream signaling pathways occurs in the early stages of human colorectal carcinogenesis [26–28]. EGFR is also overexpressed in HCC, and this phenomenon shows significant correlation with the proliferating activity, clinical stage, intrahepatic metastasis, and carcinoma differentiation [29]. Overexpression of IGF-1R is frequently observed in CRC when compared with its expression level in the normal colonic mucosa [30]. IGF-1R, which is expressed at low levels in normal hepatocytes, is also overexpressed in human HCC tissues, whereas the expression levels of IGFBP-3, a negative regulator of the IGF/IGF-1R axis, are

decreased in human HCC samples and this is associated with poor survival of HCC patients [31–33]. In addition, an overexpression and activation of the VEGF/VEGFR axis is observed in both human CRC and HCC, and this has also been shown to correlate with poor prognosis for these malignancies [34–37]. Therefore, targeting these RTKs and their downstream pathways may be a potentially effective strategy for the prevention and, in certain cases, treatment of some types of human malignancies, including CRC and HCC [9–11]. We will mainly discuss the effects of GTCs on EGFR, IGF-1R, and VEGFR in this review.

4 Effects of GTCs on the EGF receptor family of RTKs in CRC and HCC cells

Recent studies have revealed that several phytochemicals, including GTCs, exert antitumor activity by suppressing the activation of the EGF receptor family of RTKs and their downstream effectors in cancer cells [3–5]. Liang et al. [38] demonstrated that EGCG directly blocks epidermal growth factor (EGF) binding to the EGFR and thus inhibits the phosphorylation of this receptor and DNA synthesis in human A431 epidermoid carcinoma cells. We have extended this finding and reported that EGCG inhibits the activation of EGFR, HER2, and HER3, and their multiple downstream signaling pathways in human head and neck squamous cell carcinoma (HNSCC), breast cancer, and CRC cell lines [39–43]. EGCG and Polyphenon E preferentially inhibit the growth of CRC cells, which overexpress and activate EGFR and HER2, when compared with a normal human fetal colonic epithelial cell line. Treatment with these agents inhibits the activation of EGFR and HER2, the phosphorylation of Akt and ERK, and also the transcriptional activity of the activator protein-1 (AP-1), c-fos, nuclear factor-κB (NF-κB), and cyclin D1 promoters in the HT29 human CRC cell line [42]. In SW837 human CRC cells, EGCG also inhibits the activation of EGFR, HER2, and HER3, with the subsequent inhibition of the expression of cyclooxygenase-2 (COX-2) at the level of transcription, and it reduces the production of prostaglandin E_2 (PGE_2) by these cells [43]. These findings are of interest because both the EGF receptor family of RTKs and the COX-2/PGE_2 axis are critical targets for CRC chemoprevention and treatment [9, 44].

As described above, EGCG exerts its anticancer and chemopreventive effects in part through the inhibition of the EGFR family of RTKs. Therefore, there is growing interest in preventive and therapeutic strategies involving the combination of EGCG with other agents that inhibit EGFR activation because such a combination treatment targeting the same molecule might provide the potential for synergistic effects on growth inhibition in cancer cells [45]. Indeed, recent in vitro and in vivo studies with HNSCC cells revealed that the combination of EGCG and erlotinib, an EGFR-tyrosine kinase inhibitor, caused synergistic cell growth inhibition by inhibiting EGFR and Akt phosphorylation, inducing apoptosis, and suppressing the NF-κB signaling pathway [46, 47]. The combination of EGCG and erlotinib also resulted in a greater inhibition of both cell proliferation and growth rate of xenografts in non-small cell lung cancer cells than either agent alone [48]. These results suggest the possibility that a combined treatment with EGCG and EGFR-targeting agents provides a promising regimen for future chemoprevention and treatment of human malignancies, owing to the synergistic effects of these compounds.

5 Effects of GTCs on the IGF/IGF-1R axis in CRC and HCC cells

In addition to the EGFR family of RTKs, increasing evidence suggests that GTCs inhibit the tyrosine kinase activities of the other members of the RTK family, such as IGF-1R and VEGFR2. We recently reported that EGCG inhibits the activation of IGF-1R in HepG2 human HCC and SW837 CRC cells that display a constitutive activation of this receptor. In these studies, the inhibition of IGF-1R activation by EGCG was associated with a decrease in the expression levels of IGF-1/2, but an increase in the expression levels of IGFBP-3, which negatively controls the function of IGF-1/2 in these cancer cells [49, 50]. EGCG inhibits the expression of matrix metalloproteinases (MMPs)-7 and -9 in CRC cells and this may play a role in upregulating the expression of IGFBP-3 [49]. Because the IGF/IGF-1R axis, which forms autocrine and paracrine loops in cancer tissues, plays an important role in the development and growth of various types of cancer [10], disruption of these loops by GTCs might be an effective strategy for the prevention and treatment of certain cancers.

Similar effects of EGCG targeting the IGF/IGF-1R axis are also observed in in vivo studies. In an obesity-related colorectal carcinogenesis mice model, EGCG administration through drinking water effectively suppresses the development of premalignant CRC lesions by depressing the IGF/IGF-1R and COX-2/PGE_2 axes. In this study, EGCG caused the inhibition of the expression of COX-2 and the activation of IGF-1R on the colonic mucosa, and decreased the serum IGF-1 levels while increasing the serum IGFBP-3 levels in obese mice [13]. In accordance with this study, administration of EGCG through the drinking water also prevents obesity-related liver tumorigenesis in db/db mice by inhibiting IGF-1R, ERK, Akt, GSK-3β, Stat3, and JNK phosphorylation in the liver and decreasing the levels of insulin, IGF-1, and IGF-2 in the serum [14]. Other investigators have also demonstrated that the oral infusion of GTCs inhibits the development and progression of prostate cancer in mice by reducing the serum IGF-1 levels, inhibiting Akt and ERK activation, and increasing serum IGFBP-3 levels [51, 52]. Drinking EGCG also prevents carbon tetrachloride (CCl_4)-induced rat hepatic fibrosis by inhibiting IGF-1R expression [53]. This finding is significant when considering HCC

chemoprevention because inhibition of hepatic fibrosis, which is a precancerous condition to HCC, might be linked to the prevention of HCC development [54, 55].

6 Effects of GTCs on the VEGF/VEGFR axis in CRC and HCC cells

VEGF, which binds to and activates VEGFR, is a mitogen for endothelial cells that is often associated with pathological angiogenesis. Abnormal activation of the VEGF/VEGFR axis is therefore closely associated with tumor growth [11]. EGCG suppresses the growth of xenografts generated from the human HCC cell line Huh7 by decreasing serum VEGF levels and inhibiting the activation of VEGFR2, ERK, and Akt [16]. In CRC cell xenografts, the activation of VEGFR2, ERK, and Akt and the expression of VEGF are also inhibited by EGCG treatment and this might be associated with reduction of the expression of hypoxia-inducible factor (HIF)-1α, which strongly activates VEGF expression [17].

Several in vitro studies have also reported the inhibitory effects of GTCs on the VEGF/VEGFR axis. For instance, work from our group demonstrated that EGCG inhibits the production of VEGF in human HNSCC and breast cancer cells by blocking the activation of Stat3 and NF-κB [40]. EGCG also inhibits the phosphorylation of both VEGFR1 and VEGFR2 and induces apoptosis in chronic lymphocytic leukemia cells [56]. In addition, GTCs significantly inhibit HIF-1α protein accumulation and decrease VEGF expression in HepG2 cells by blocking both the PI3K/Akt and Ras/ERK signaling pathways [57]. EGCG inhibits ERK activation and suppresses the expression and promoter activity of VEGF in HT29 cells [58]. Similar to the findings showing the role of the IGF/IGF-1R axis in mediating the effect of GTCs, the above results suggest that the VEGF/VEGFR axis might also be a promising target of GTCs for the prevention and treatment of some types of human malignancies, including CRC and HCC.

7 Effects of GTCs on the hepatocyte growth factor (HGF)/c-Met and PDGF/PDGFR axes

In this review, we have mainly focused on a discussion of the inhibitory effects of GTCs on the activation of EGFR, IGF-1R, and VEGFR. However, it should be mentioned that GTCs also target other members of the RTK family, such as c-Met and platelet-derived growth factor receptor (PDGFR). c-Met is overexpressed in colon tumors and this is associated with poor prognosis [59, 60]. In human CRC cells, EGCG markedly suppressed the activation of c-Met in the presence of its ligand, HGF [61, 62]. In the liver of CCl_4-injected rats, EGCG significantly decreased the expression of PDGFR and thus attenuated hepatic fibrosis [53]. EGCG also inhibited PDGF-induced cell proliferation and reduced the autophosphorylation of the PDGFR by blocking the binding of PDGF to its receptor in human hepatic stellate cells; this might contribute to the prevention of liver fibrosis progression in patients with chronic liver diseases [63]. These reports suggest the possibility that GTCs can target certain types of RTKs in a variety of cell types; however, the precise mechanisms underlying the GTCs-mediated inhibition of RTKs activation in cancer cells remain to be elucidated.

8 Mechanisms mediating the inhibition of RTKs activation and intracellular signaling pathways by GTCs

One possible mechanism by which the inhibition of RTKs activation by GTCs could be explained is through the ''sealing'' and ''trapping'' effects of GTCs [64]. Namely, EGCG covers the cell surface and directly interrupts the binding of EGF to EGFR [38]. EGCG has also been shown to bind directly to EGF and VEGF, thus preventing these growth factors from interacting with their corresponding receptors and activating downstream signaling cascades [38, 65]. In addition, EGCG may also inhibit the activation of RTKs by affecting the expression levels of their ligands. The expression levels of the EGFR family ligands EGF and heregulin have been shown to be downregulated by EGCG treatment in CRC cells [17]. EGCG also decreases the levels of IGF-1, IGF-2, and VEGF, which might be associated with decreased ERK and Akt activities, in CRC and HCC cells [16, 17, 50]. These findings could partly explain the inhibitory effects of GTCs on the activation of RTKs in various types of cancer cells [16, 17, 38–43, 49, 50].

Several studies have also provided evidence that GTCs can directly target the kinase activity of RTKs and their intracellular signaling pathways and transcription factors. EGCG was shown to competitively bind to the ATP binding site of IGF-1R and block downstream signaling [66]. Sah et al. [67] demonstrated that EGCG directly inhibits ERK and Akt kinases in immortalized human cervical cells. In addition, EGCG was shown to play a role in the direct inhibition of the activation of ERK and mitogen-activated protein kinase kinase-1 (MEK1) and of the association with Raf-1 with MEK1, and in the inhibition of AP-1 activity in H-ras-transformed mouse epidermal cells [24, 25]. EGCG also exerted antiproliferative effects on H-ras-transformed rat intestinal epithelial cells [68]. These reports seem to be significant when considering the prevention of CRC by GTCs because Ras (KRAS) gene mutations occur frequently in this malignancy [69]. Moreover, administration of EGCG through the drinking water significantly decreased small intestinal tumor formation in $Apc^{Min/+}$ mice, a recognized mouse model for human intestinal cancer, by reducing the expression of the phosphorylated form of Akt and ERK proteins in small intestinal tumors [70]. Administration of EGCG through the drinking water also suppressed tumor

formation in $APC^{Min/+}$ mice by decreasing the levels of basic fibroblast growth factor in small intestinal tissue samples [71]. These reports are important because the mutation of the APC gene, a tumor suppressor gene, is critically implicated in human colorectal carcinogenesis [72]. Altogether, these results suggest that GTCs might exert antitumor and chemopreventive effects by binding, probably with relatively low affinity, to multiple cellular targets (Fig. 1). Moreover, these results also demonstrate the potential of GTCs as an effective chemopreventive agent against CRC in patients bearing APC and/or Ras gene mutations.

9 Lipid rafts: a promising target of EGCG

Evidence exists that several plasma membrane-associated RTKs, including EGFR, IGF-1R, and VEGFR2, are closely associated with the detergent-insoluble ordered membrane domains called ''lipid rafts,'' which play a critical role as signal processing hubs. The localization of RTKs to lipid rafts appears to modulate both their ligand binding and tyrosine kinase activities [73–76]. Lipid organization is also considered to play a fundamental role in receptor internalization [77]. Recent studies show that lipid rafts provide a platform for a 67-kDa LR that binds EGCG, thus affecting the uptake of EGCG [21, 22, 78, 79]. The expression of the 67-kDa LR is found to be upregulated in various types of human cancers, including CRC [80], and to directly correlate with the malignant potential via activation of multiple signal transduction pathways such as MAPK [81, 82]. Therefore, EGCG may presumably mediate its cancer-preventive activity by targeting the 67-kDa LR [83].

In addition, on the basis of our recent series of studies [84–86], we presume that targeting lipid rafts is one of the most relevant mechanisms of EGCG in exerting its anticancer and chemopreventive properties (Fig. 2). EGFR activation was shown to only occur in the lipid raft fraction, whereas total cellular EGFR is present in the non-raft membrane fraction in HT29 cells. In these cells, EGCG

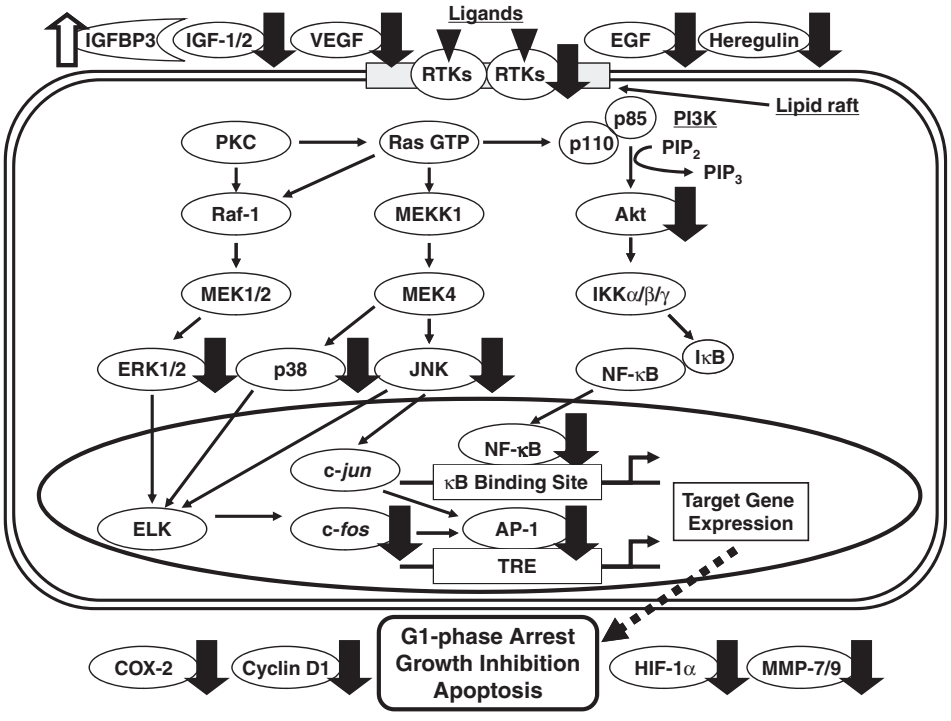

Figure 1. Effects of GTCs on RTKs and their intracellular signaling pathways. Activation of RTKs including EGFR, IGF-1R, and VEGFR2 by specific ligands leads to the induction of their intrinsic tyrosine kinase activities and autophosphorylation of tyrosine residues. The activated RTKs then create docking sites for downstream effector molecules such as Ras, Raf-1, and PI3K, which subsequently stimulate several intracellular processes. Activated Raf-1 stimulates MEK and its signaling cascade, resulting in the phosphorylation of the MAPK protein ERK. In its active state, MAPK activates a variety of transcription factors, including ELK and c-Jun, and subsequently promotes the expression of target genes by stimulating the transcriptional activity of AP-1, a dimeric complex that comprises members of the Jun and Fos families of transcription factors. The activation of PI3K triggers the synthesis of the lipid PIP_3, which activates the downstream pathways that involve Akt. The NF-κB family of transcription factors, which is important in cell survival, is one of the functional targets of Akt. EGCG inhibits the activation of certain RTKs, which takes place in lipid rafts (gray box), as well as the activation of the MAPK cascade, such as Ras/Raf/MEK/ERK/JNK pathways and PI3K pathways. Molecules that appear to be cellular targets for EGCG are indicated by a black arrow (downregulation) or by a white arrow (upregulation), respectively. These multiple effects of EGCG result in the induction of apoptosis and cell cycle arrest in the G_0–G_1 phase, thus inhibiting cell proliferation in cancer cells.

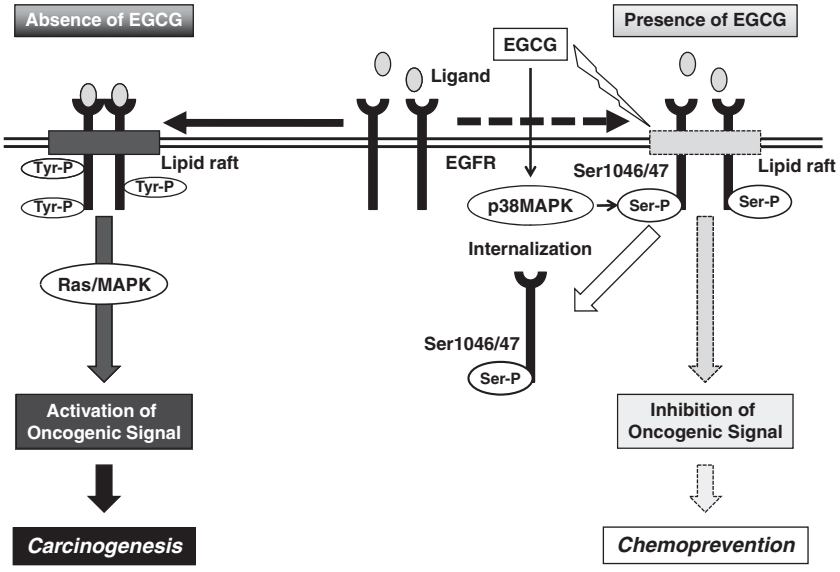

Figure 2. Hypothetical scheme indicating the inhibitory effect of EGCG on the activation of EGFR by inducing the alteration of lipid rafts and internalization of EGFR in CRC cells. EGCG alters the organization of lipid rafts and inhibits the dimerization and activation of EGFR. EGCG also promotes the internalization of nonactivated monomer EGFR into the cytosol through phosphorylation of EGFR at serine 1046/47 by the activation of p38 MAPK. As a result, EGCG decreases the levels of phosphorylated EGFR and inhibits the activation of EGFR signaling, which acts as an oncogenic signal in CRC cells.

inhibits the binding of EGF to EGFR and its subsequent dimerization and activation by reducing the levels of EGFR in lipid rafts [84]. Our group also found that EGCG induces alterations in membrane organization, resulting in the internalization of the inactivated form of EGFR into endosomes and the suppression of CRC cell growth [85]. EGCG also induces the internalization and subsequent degradation of EGFR through the phosphorylation of the receptor, which is associated with the activation of p38 MAPK by EGCG [86]. These findings strongly suggest that EGCG inhibits EGF binding to EGFR and dimerization/activation by causing an alteration in the lipid organization of the plasma membrane [87]. Given the fact that a majority of RTKs function on lipid rafts, this mechanism might explain, at least in part, the ubiquitous inhibitory effects of EGCG on a variety of RTKs. In addition, EGFR internalization triggered by EGCG might also be a possible mechanism mediating the anticancer effect of this agent and other related compounds.

10 Possible clinical applications of GTCs

Several studies have used animal models of carcinogenesis to show the significant chemopreventive effects of GTCs. On the contrary, the results of epidemiological studies investigating the effects of tea consumption on the risk of human cancer have been inconclusive [1, 2]. This might be associated with different factors such as human genetic variability, lifestyle, amount and type of tea consumed, and the diversity in cancer etiologies. Among these factors, the quantity and quality of tea consumed appears to be one of the most important variables affecting the relationship between tea consumption and cancer risk reduction. Prospective cohort studies in Japan showed that daily consumption of 10 cups of tea (equivalent to 2.5 g green tea extract (GTE) is required for the cancer-preventive effect [88, 89]. Green tea consumption in specific quantities is also associated with reduced risk of esophageal and breast cancers [90, 91].

On the other hand, some intervention studies provide a clear evidence for the chemopreventive and probable anticancer progression effects of tea preparations. Early double-blind intervention trials showed that oral administration of mixed tea products significantly decreases the size of leukoplakia, an oral precancerous mucosa lesion, suggesting that tea may have a protective effect in oral cancers [92]. A recent double-blind, placebo-controlled study in Italy revealed that the progression of high-grade prostate intraepithelial neoplasia to prostate cancer can be effectively prevented by oral administration of GTCs (600 mg/day for 1 year) [93]. Furthermore, an interesting clinical trial demonstrated that the serum levels of IGF-1, VEGF, and HGF were significantly decreased by the administration of Polyphenon E in prostate cancer patients [94]. These findings support a potential role for GTCs in the prevention and/or treatment of human malignancies.

The successful prevention of the development of colorectal adenomas, the precancerous lesions for CRC, after polypectomy was shown in a pilot study in which the administration of GTE (1.5 g/day for 1 year) in patients who had undergone polypectomy for colorectal adenomas reduced the development of metachronous colorectal adenomas in comparison with patients who did not take this supplement. The size of relapsed adenomas was also significantly smaller in the GTE supplemented group in comparison with the control untreated group (Fig. 3) [12]. The absence of any serious adverse events as a consequence of GTCs administration in these trials [12, 93] is a significant finding for the consideration of the use of GTCs as ''chemopreventive'' in clinical practice. In addition, the results of these clinical trials [12, 92] also suggest the possibility that cancers that develop in the digestive tract,

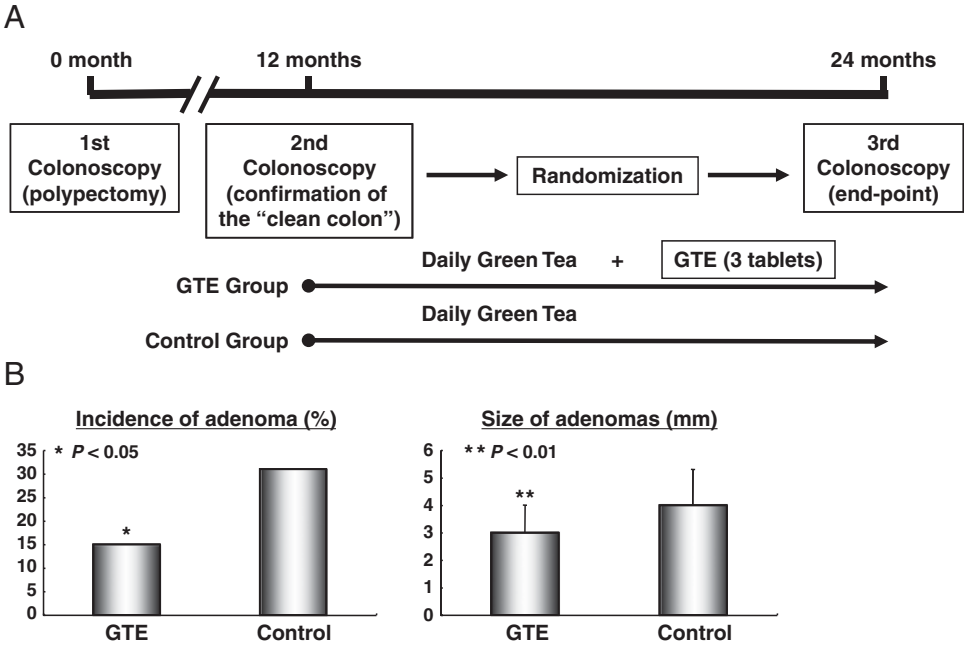

Figure 3. Pilot study revealing the preventive effect of GTE on metachronous adenomas after polypectomy. (A) Study design. The study included 136 participants who underwent endoscopic resection of one or more colorectal adenomas. Twelve months later, the participants received another total colonoscopy to confirm the absence of remaining endoscopically detectable adenoma (confirmation of the ''clean colon''). The participants were then randomized into two groups while maintaining a daily green tea drinking; the GTE group (71 patients) was given three GTE tablets per day for 12 months and the control group (65 patients) received no supplement. After 12 months of GTE supplements, a follow-up (end-point) colonoscopy was conducted in 125 patients (60 in the GTE group and 65 in the control group) to test for the presence of new adenomas. One tablet of GTE (500 mg), which contains 52.5 mg EGCG, 12.3 mg (−)-epicatechin, 34.6 mg (−)-epigallocatechin, 11.1 mg (−)-epicatechin gallate, and 15.7 mg caffeine, is equivalent to approximately two Japanese-size cups of green tea. (B) Effects of the GTE supplement on the incidence and the size of metachronous adenomas at the end-point colonoscopy. Left panel: the incidence of metachronous adenomas was 31% (20 of 65) in the control group and 15% (9 of 60) in the GTE group (relative risk, 0.49; 95% confidence interval, 0.24–0.99: $^{*}p<0.05$). Right panel: the size of relapsed adenomas was 4.0 ± 1.3 mm in the control group and 3.0 ± 1.0 mm in the GTE group ($^{**}p<0.01$).

such as in the oral cavity, esophagus, stomach, and colorectum, might be more effective targets for chemoprevention using GTCs, because direct contact (''exposure'') with the digestive tract seems to be a key factor for the cancer-preventive activity of polyphenolic compounds [95].

An interventional study using GTCs in a high-risk group of individuals for HCC has been conducted in a high aflatoxin exposure area in China. In this double-blinded and placebo-controlled phase IIa chemoprevention trial, administration of GTCs in these individuals, who were seropositive for both HBs-Ag and aflatoxin-albumin adducts, effectively reduced the levels of urinary 8-hydroxydeoxyguanosine, a surrogate marker of oxidative DNA damage [96]. Daily GTCs administration also modulated aflatoxin biomarkers in this trial [97]; however, whether GTCs ultimately prevent the development of HCC has yet to be clarified. In addition, HCC development is frequently associated with chronic inflammation and subsequent cirrhosis of the liver induced by a persistent infection with hepatitis viruses. Increasing evidence also indicates that obesity and related metabolic abnormalities, especially insulin resistance, raise the risk of HCC [98, 99], whereas GTCs seem to have antiobesity and antidiabetic effects [100]. Therefore, well-designed interventional trials should be conducted to examine whether GTCs prevent the development of HCC in high-risk patients with viral liver cirrhosis and obesity. Recent rodent experiments showing the antifibrotic [53] and chemopepreventive effects of EGCG in obesity-related liver tumorigenesis [14] might encourage the use of GTCs for such patients.

11 Concluding remarks

In concluding this review, it should be mentioned that the concentrations of EGCG used in some of the cell culture experiments (20–100 μM) aiming to elucidate the mechanisms of action of this agent are higher than the plasma and tissue concentrations observed in human trials or in mice in cancer chemoprevention experiments [101]. Therefore, it remains unclear and thus requires careful consideration whether the information obtained from in vitro studies with high EGCG concentrations can be directly extrapolated to cancer chemoprevention in animals and humans. On the

other hand, a number of high-affinity EGCG-binding proteins, including IGF-1R, have been revealed by recent affinity chromatography studies [66, 102, 103]. These studies indicate that EGCG does in fact bind to target proteins at low concentrations, although relatively high concentrations are required to exert its physiological functions. Moreover, for consideration of GTCs in the clinical practice, it should be emphasized that EGCG can inhibit the activation of EGFR at low micromolar concentrations [42, 43, 85] that are considered within the physiologically relevant range for human exposure [104]. Furthermore, EGCG preferentially inhibits the growth of cancer cells without affecting the growth of the corresponding normal cells [16, 42, 50, 105].

A possible explanation for these phenomena is the concept of ''oncogene addiction'' according to which cancers associated with multiple genetic, epigenetic, and chromosomal abnormalities are usually dependent on or ''addicted'' to one or a few genes for both maintenance of the malignant phenotype and cell survival and, therefore, targeting only one or a few of these aberrant molecules might be effective to inhibit carcinogenesis and growth of cancer cell [106, 107]. It is likely that EGCG preferentially inhibits growth and induces apoptosis in cancer cells by blocking the activity of one or a few of ''addicted'' oncogenic factors, including abnormalities in RTKs.

Tea is currently considered one of the most promising dietary agents for the prevention and treatment of many diseases, especially cancer. The present review provides evidence that the effects of GTCs on the inhibition of carcinogenesis are mediated, at least in part, by the regulation of the activity of certain RTKs and their related intracellular signaling pathways; this observation does not exclude other mechanisms that may also play critical roles in mediating the anticancer and cancer chemopreventive effects of these agents [1, 2]. The safety and efficacy of GTCs demonstrated in recent intervention studies [12, 93] could be crucial for the clinical application of GTCs as chemopreventive agents.

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