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Mini Review

New insights into the mechanisms of polyphenols beyond antioxidant properties; lessons from the green tea polyphenol, epigallocatechin 3-gallate[☆]Hae-Suk Kim^a, Michael J. Quon^c, Jeong-a Kim^{a,b,*}^a Department of Medicine, Division of Endocrinology, Diabetes, and Metabolism, UAB Comprehensive Diabetes Center, University of Alabama at Birmingham, Birmingham, AL 35294, USA^b Department of Cell, Developmental and Integrative Biology, UAB Comprehensive Diabetes Center, University of Alabama at Birmingham, Birmingham, AL 35294, USA^c Department of Medicine, Division of Endocrinology, Diabetes & Nutrition, University of Maryland School of Medicine, Baltimore, MD 21201, USA

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ABSTRACT

Green tea is rich in polyphenol flavonoids including catechins. Epigallocatechin 3-gallate (EGCG) is the most abundant and potent green tea catechin. EGCG has been extensively studied for its beneficial health effects as a nutraceutical agent. Based upon its chemical structure, EGCG is often classified as an antioxidant. However, treatment of cells with EGCG results in production of hydrogen peroxide and hydroxyl radicals in the presence of Fe (III). Thus, EGCG functions as a pro-oxidant in some cellular contexts. Recent investigations have revealed many other direct actions of EGCG that are independent from anti-oxidative mechanisms. In this review, we discuss these novel molecular mechanisms of action for EGCG. In particular, EGCG directly interacts with proteins and phospholipids in the plasma membrane and regulates signal transduction pathways, transcription factors, DNA methylation, mitochondrial function, and autophagy to exert many of its beneficial biological actions.

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Abbreviations: EGCG, epigallocatechin 3-gallate; ECG, epicatechin gallate; EGC, epigallocatechin; EC, epicatechin

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Introduction

Polyphenols may have therapeutic health effects for a variety of chronic pathological conditions including cancer, neurodegenerative diseases, diabetes, and cardiovascular diseases [16,51,106]. Many polyphenols are derived from natural food products. Thus, they are often considered safer and more easily integrated into lifestyle changes than conventional pharmaceutical drugs. Recently, specific molecular targets for various polyphenols have been discovered. Therefore, scientific interest in polyphenols as therapeutic agents is rapidly increasing. However, many studies often report biological effects of food polyphenols are observed without elucidating the underlying molecular, cellular, and physiological mechanisms. Obstacles to defining mechanistic studies in this field may include non-specific effects due to polyphenols with pleiotropic activities, and complex mechanisms of action. Despite difficulties in defining specific mechanisms, recent work has shed light on more detailed molecular mechanisms underlying bioactive actions of polyphenols. Many polyphenols share beneficial effects against a broad range of pathologies, including cancer, inflammation, diabetes, and cardiovascular diseases. However, individual polyphenols have distinct specific molecular targets in various tissues with different efficacies and bioavailabilities.

Dietary sources of polyphenols have received a great deal of attention. In particular the polyphenolic components in different tea preparations have been examined in detail. Tea is the second most frequently consumed beverage after water. Tea polyphenols have received considerable public attention due to the positive association between tea consumption and beneficial health effects [56]. Epidemiological studies show correlations between tea consumption and decreased risk of cardiometabolic disorders and mortality in a dose-dependent fashion [56]. Green tea extract contains a number of catechins, including epigallocatechin-gallate (EGCG), epigallocatechin (EGC), epicatechin-gallate (ECG), and epicatechin (EC). The profiles of catechins in human plasma and urine after tea consumption have been analyzed [29,61,112]. Although the absorption, excretion, and modification of catechins may affect the bioavailabilities and bioactive potencies, structural characteristics seems to play important roles in differential

bioactivities. It has been proposed that the galloyl moiety of tea catechins may play the critical roles in specific activities of tea catechins, especially in lipid lowering effect [39,40]. Furthermore, the two catechins with galloyl moiety (EGCG and ECG) have the most potent biological activities as listed in Table 1. The most abundant green tea polyphenol, epigallocatechin 3-gallate (EGCG) may be responsible for many of the beneficial effects of green tea in clinical and animal studies as well as in cell culture studies [7,14,47–49,87,101]. One potential mechanism for beneficial health effects of EGCG may be attributable to its anti-oxidative function [9]. However, more recent findings suggest many additional mechanisms of action for EGCG including interactions with plasma membrane proteins, activation of second messengers and signal transduction pathways, modulation of metabolic enzymes, and autophagy [47,49,55,110,111,124]. Furthermore, the biological actions of EGCG are concentration-dependent. Bioavailability studies after tea consumption demonstrate levels of EGCG in human plasma in the low μM range [60]. Circulating levels of EGCG reach about 10 μM in animal studies after oral intake of pure EGCG [57]. In this review, the concentration-dependent biological actions of EGCG (low defined as $\leq 10 \mu\text{M}$ and high defined as $> 10 \mu\text{M}$) and recent progress in elucidation of specific molecular mechanisms of action are discussed (Table 2).

Pro-oxidant or anti-oxidant?

Oxidant properties of polyphenols may be both anti-oxidant and/or pro-oxidant based upon the structure of the particular polyphenol and the cellular redox context that may include increased levels of oxidant scavenging proteins or decreased levels of oxidized proteins and lipids [11,76,122]. Thus, some studies claim EGCG is an anti-oxidant [7,44,83,118]. For example, mitochondrial function is improved by anti-oxidative action of EGCG [72]. Moreover, EGCG ameliorates lipid infusion-mediated insulin resistance, which is associated with increased expression of anti-oxidant enzymes including superoxide dismutase (SOD) and glutathione peroxidase by EGCG *in vivo* [66]. However, these reports are associative and do not directly demonstrate that a

Table 1
Relative biological potency of green tea polyphenols.

Biological actions	EGCG	ECG	EGC	EC	Ref.
Inhibition of histamine release	Strong	Moderate	Moderate	No effect	[70]
Inhibition of leukotriene B4 release	Strong	Moderate	Moderate	No effect	[70]
Angiogenesis	Strong	Strong	Weak	Weak	[53]
RyR1 activation	EGCG >	ECG >	EGC >	EC	[27]
Cytotoxicity in oral cavity	EGCG >	ECG >	EGC >	EC	[5]
FAS inhibition	EGCG <	ECG			[105]
SIRT1 activation	1.75 fold	1.85 fold			[19]
Na/H exchanger inhibition	EGCG <	ECG <	EGC <	EC	[92]

Table 2
Effects of EGCG treatment on various cellular responses.

Cellular function	Cell types	Con. EGCG (μM)	Mechanism	Reference
Glucose uptake	3T3-L1	< 5	Anti-oxidant	[118]
Adipocyte differentiation	3T3-L1	5–10	Up-regulate Adipogenic Genes	[98]
Inhibition of gluconeogenesis	Hepatocyte	< 1	CaMKK β /AMPK pro-oxidant	[15]
Autophagy	Endothelial cells	10	Ca ²⁺ +/CaMKK β	[47]
Autophagy/Anti-tumor (cell death)	Cancer	> 50	Pro-oxidant	[63,99,124]
Transformation	Cancer	20	AP-1 inhibition	[21]
Protection	HaCaT	> 20	Pro-oxidant	[22]
Anti-proliferation	Cancer	> 20	Pro-oxidant	[36,64]

decrease in oxidative stress by EGCG *per se* is the direct mechanism preventing lipid-induced insulin resistance. On the other hand, other reports suggest that green tea extract and EGCG exert pro-oxidant actions [23,36,64,97,121]. EGCG auto-oxidizes, and produces hydrogen peroxide in cell culture media with and without cells. Addition of SOD and catalase abolishes some cellular actions of EGCG by inhibiting the auto-oxidation and dimerization of EGCG [64]. EGCG works in two ways to promote cytotoxicity in anti-tumor activity; one is directly by producing hydrogen peroxide with its pyrogallol moiety, and the other is reducing Fe (III) to Fe (II) which triggers the Fenton reaction to create more potent reactive oxygen species (ROS) such as hydroxyl radicals [77,78]. The combination of hydroxyl radicals and hydrogen peroxide contributes to the cytotoxic effects of EGCG at high μM concentrations ($> 50 \mu\text{M}$). Another study has shown that N-acetyl cysteine (NAC) is able to protect from hydrogen peroxide-induced cytotoxicity but not EGCG-induced cell death. Thus, the cytotoxic effect of EGCG in tumor cells is not mimicked by hydrogen peroxide alone [117]. This suggests that EGCG has tumor-specific mechanisms for mitochondrial damage other than production of hydrogen peroxide. However, these results are based upon cell culture studies using $> 50 \mu\text{M}$ EGCG ($50 \mu\text{M}$ EGCG generates about $1 \mu\text{M}$ hydrogen peroxide in Jurkat cells) [77]. In fact, more modest nM and biologically realistic concentrations ($1\text{--}2 \mu\text{M}$ up to $10 \mu\text{M}$) of EGCG produce lower levels of intracellular reactive oxygen species that stimulate multiple signal transduction pathways to promote cellular protective mechanisms [15,22]. This suggests that EGCG-mediated production of reactive oxygen species contributes to the beneficial biological actions of EGCG. It is possible that different amount of anti-oxidants, including glutathione, thioredoxin, catalase and SOD in various tissues and serum components in animal models may underlie discrepancies in the activities of EGCG under *in vitro* and *in vivo* conditions. Moreover, expression levels of modifying enzymes and their stability may also be factors determining bioavailability of EGCG. EGCG is converted to dimer and multimer, and modified to glucuronated and/or methylated forms. Since many cellular actions of EGCG are acute and occur within minutes, it is likely that EGCG has direct cellular actions that are independent of its metabolites. However, the various metabolites of EGCG may also have some bioactivity, particularly with respect to chronic actions of green tea extracts. Thus, distinct actions and roles of these derivatives may also contribute to pleiotropic biological effects of EGCG [107]. Although it has not been proven that oxidation of EGCG occurs *in vivo*, all of the forms, including intact, oxidized or modified EGCG are potentially bioactive substances. Therefore, the combination of direct and indirect effects of EGCG may create synergistic or additive mechanisms for various actions of green tea.

Cell surface receptor

One important mechanism frequently overlooked in considering the biological effects of polyphenols is their potential

Table 3
Effects of EGCG on receptor tyrosine kinases.

Biological actions	Signaling molecule	Conc. of EGCG (μM)	Inhibition/Activation	Reference
Atherosclerosis	PDGF-BB	5–100	Inhibition	[1]
Angiogenesis	VEGFR	1.56–100	Inhibition	[53]
Cancer	EGFR	> 10	Inhibition	[67]
Angiogenesis	VEGFR	0.5–10	Inhibition	[93]
Cancer	EGFR	30–50	Inhibition	[96]
Gluconeogenesis	InsR	5–50	Activation	[113]

interaction with receptors capable of initiating cell signaling. Interestingly, when EGCG is incubated with cells, 75% of radioactively labeled EGCG is found in the cytosolic compartment while some radioactivity is found in the membrane fraction [34]. This suggests that EGCG directly binds to membrane components, including proteins and lipids. In fact, EGCG inhibits PDGFR-BB-stimulated signal transduction pathway in vascular smooth muscle cells by direct binding to PDGFR-BB thereby causing inhibition of PDGF-stimulated restenosis [1,95,114]. Moreover, EGCG regulates activities of cell surface growth factor receptors, especially receptor tyrosine kinases (RTK), including epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR), insulin-like growth factor receptor (IGFR), and the insulin receptor (InsR) (Table 3). [1,53,67,93,96,102,113]. Most RTKs are involved in cell proliferation, survival and angiogenesis. In particular, InsR is important for regulation of metabolism and cell survival. It is notable that EGCG inhibits activities of EGFR, VEGFR, and IGFR. By contrast, EGCG mimics or augments InsR signaling [113]. One mechanism proposed for activation of InsR is that EGCG inhibits tyrosine phosphatases through a redox mechanism that results in increased and sustained tyrosine phosphorylation of InsR [113] by increasing the level of hydrogen peroxide. Thus, inhibitory actions of EGCG on proliferation may be specific to abnormally proliferating or cancer cells, but not to normal cells. In fact, by using surface plasmon resonance technique, Tachibana et al. discovered that EGCG, but not other tea catechins, directly binds to the laminin receptor (67LR) ($K_d \sim \text{nanomolar}$) [81]. The binding site in 67LR is located within the peptide LR161–170 and the two basic amino acids K(166) and H(169) are critical for the binding of EGCG [28]. The expression of 67LR is elevated in cancer cells but not in normal cells [71]. This suggests that EGCG may specifically target cancer cells in some contexts. More recently, Kumazoe et al. suggested a mechanism for EGCG-induced apoptosis of cancer cells through 67LR that may provide a potential therapeutic strategy using inhibitors of phosphodiesterase 5 (PDE5) (Fig. 1) [116]. Another role of EGCG-stimulated 67LR is to mediate anti-inflammatory actions. Treatment with EGCG reduces expression of toll-like receptor 4 (TLR4) and increases expression of tollip, a negative regulator of TLRs, through a 67LR-dependent mechanism [33]. Because TLR2 and TLR4 are involved in innate immunity in response to bacterial infection, this activity of EGCG inhibits lipopolysaccharide-stimulated pro-inflammatory responses in macrophages [33]. Identifying the detailed mechanisms for 67LR-mediated reduction of TLR4 and increased tollip expression will provide more detailed mechanisms for anti-inflammatory actions of EGCG. Growing evidence suggests that 67LR plays important roles in biological actions of EGCG (Fig. 2). Despite the importance of 67LR in the activities of EGCG, detailed downstream signaling pathways for 67LR are yet to be elucidated.

Intracellular signaling pathways

In cell culture, the majority of [^3H]-EGCG is found in the cytosolic fraction [34]. This suggests that biological actions of EGCG may occur through EGCG metabolites or interaction with intracellular molecules. As mentioned above, EGCG produces low level reactive oxygen species, including hydrogen peroxide that may act as a second messenger for downstream signaling pathways [23,36,64,97,121]. This action may be mediated by direct chemical reactions of EGCG with compounds at the cell surface. However, additional unknown receptor-mediated signaling pathways cannot be excluded. EGCG also increases other intracellular second messengers including Ca^{2+} , cAMP, and cGMP.

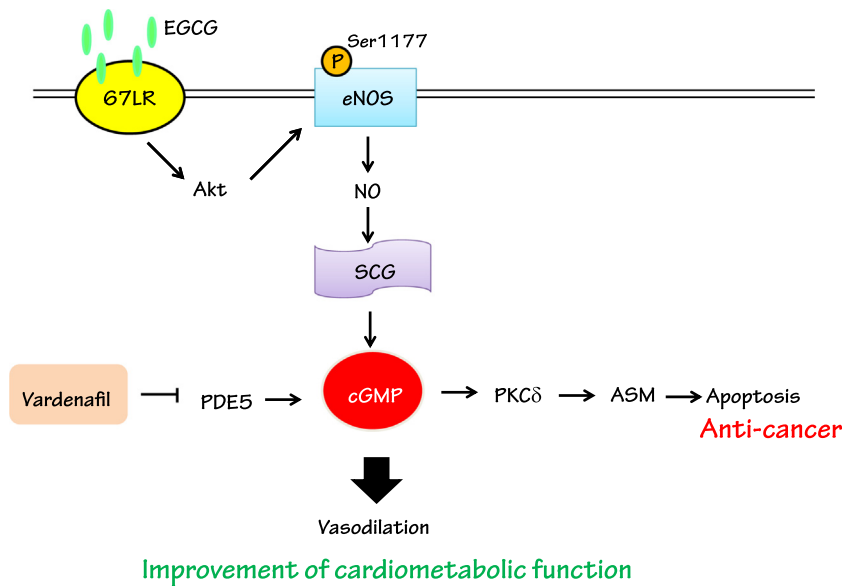


Fig. 1. EGCG-activated eNOS pathways that improve cardiovascular function and anti-cancer effect in the presence of PDE5 inhibitor. EGCG elevates cGMP levels through a specific receptor 67LR that stimulates the Akt/eNOS pathway. This pathway leads to vasodilation which contributes to improvement of cardiovascular function. In cancer cells, inhibition of PDE5 by treatment with Vardenafil leads to sustained elevations of cGMP levels. This elevated cGMP activates PKC δ /acidic sphingomyelinase (ASM) that contributes to apoptosis.

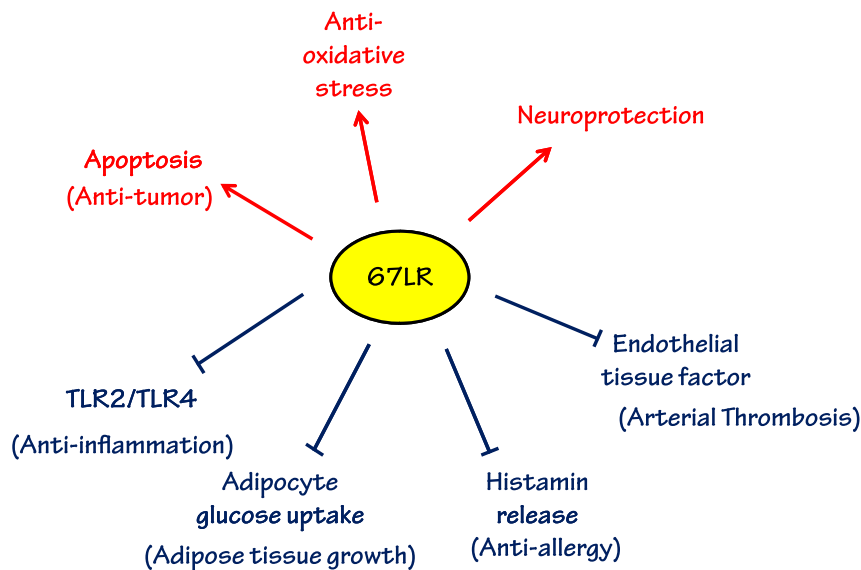


Fig. 2. The roles of 67LR as a specific receptor for EGCG. EGCG has multiple biological actions mediated through 67LR by enhancing (red) and inhibiting (blue) specific pathways.

Calcium

EGCG elevates cytosolic Ca²⁺ levels in excitable and non-excitable cells [13,47,50]. Elevation of Ca²⁺ is achieved after stimulation with nanomolar concentrations of EGCG and ECG, but not by EGC or EC. This increases the sensitivity of the ryanodine receptor (RyR1) in response to extracellular Ca²⁺ inward current or electrical stimulation without changing basal cytosolic Ca²⁺ [27]. This effect is independent of sarcoplasmic/endoplasmic reticulum Ca²⁺-ATPase (SERCA) activity at concentrations of EGCG up to 2 μ M. However, 10 μ M EGCG elevates cytosolic Ca²⁺ without Ca²⁺ challenge or electrical stimulation. This is inhibited by depletion of endoplasmic reticulum Ca²⁺ stores by treatment with cyclopiazonic acid in bovine aortic endothelial cells [47]. This suggests that concentrations of EGCG > 10 μ M elevate cytosolic Ca²⁺ by inhibition of SERCA [43,103]. This elevated

cytosolic Ca²⁺ affects activities of various Ca²⁺ requiring enzymes including calmodulin (CaM)-dependent protein kinase II and CaMKK β [47,50]. CaMKK β is an upstream regulator for AMP-dependent kinase (AMPK), an energy sensing enzyme that plays crucial roles in energy metabolism and cardiovascular functions [79,94,108]. EGCG-stimulated elevation of cytosolic calcium contributes to NO production by binding to calmodulin in the heart and vascular endothelium [32,35,73]. These actions of EGCG are closely related to beneficial cardiovascular actions of EGCG.

Cyclic-nucleotides (cAMP/cGMP)

EGCG treatment increases cAMP in endothelial cells and platelets that participate in phosphorylation of eNOS and vasodilator-stimulated phosphoprotein (VASP) [68,82]. However, this seems to be a cell type-specific response because elevation of intracellular

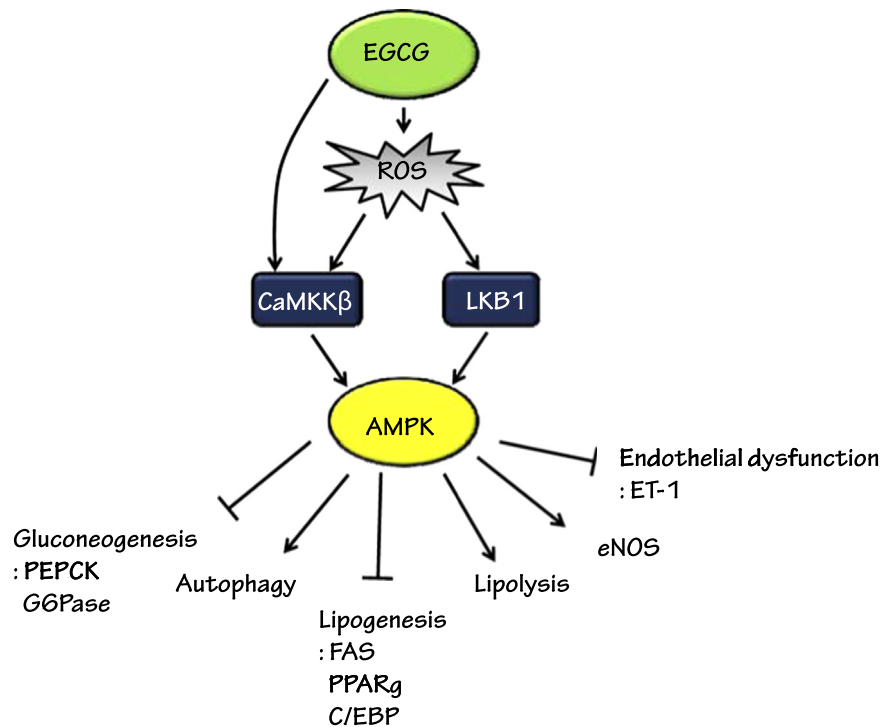


Fig. 3. Metabolic and vascular functions of EGCG. A schematic diagram showing the mechanisms for the activation of AMPK by EGCG. AMPK is a key molecule that regulates enzymes involved with energy metabolism and endothelial functions.

cAMP by EGCG is not observed in other cell types. Although the detailed molecular mechanism for elevation of cAMP is not known, elevation of cAMP may be achieved through activation of adenylate cyclase, but not through inhibition of phosphodiesterase 4 (PDE4) [82]. This elevated cAMP stimulates protein kinase A that contributes to various biological events [24,68]. By contrast, EGCG stimulates production of NO through a 67LR-mediated mechanism leading to apoptosis in cancer cells and acute myeloid leukemia cells [54,55,119]. This effect is potentiated by the PDE5 inhibitor Vardenafil in cancer cells but not in normal cells. Thus, prolonged elevation of cGMP by EGCG and Vardenafil leads to potentiated cell death. Interestingly, cancer cells express abnormally high levels of 67LR and PDE5. This may aid in cancer-specific targeted treatment. This novel mechanism provides a combinatorial therapeutic strategy to treat cancer patients with EGCG and PDE5 inhibitors [55]. EGCG also stimulates vasorelaxation by increasing both cAMP and cGMP in rat aorta [3]. Thus, EGCG may stimulate production of cyclic nucleotides that may be one of the important mechanisms underlying beneficial biological actions of EGCG in metabolic and cardiovascular physiology and in anti-neoplastic actions [41,49,68] (Fig. 1).

Other signaling pathways

AMPK is an energy sensing molecule that is also activated by EGCG in hepatocytes, adipocytes, cancer cells, and endothelial cells. AMPK contributes to inhibition of gluconeogenesis, stimulation of lipolysis, apoptosis, and reduction of endothelin-1 expression, respectively (Fig. 3) [15,38,74,85,90]. This activity may contribute to improvement of insulin sensitivity and vasodilation [12,37,41]. In fact, EGCG plays an important role in lipid metabolism by regulating lipolytic and lypogenic enzymes [12,62]. EGCG inhibits fatty acid synthase (FAS), PPAR gamma, and C/EBP. Fatty acid binding protein 4 (FABP4) and FAS are reduced by upregulation of nuclear beta-catenin [58]. Knock-down of beta-catenin attenuates inhibition of intracellular lipid accumulation [58]. Upstream kinases for AMPK include

Table 4
Effects of EGCG on transcription factors.

Transcription factor	Cell type	Con. EGCG (μM)	Inhibition/Activation	Reference
Sp1 NF-κB	LNCaP	20	Inhibition	[91]
	HSC	20–100	Inhibition	[9]
	RAW264.7	100	Inhibition	[120]
Nrf2	HAEC	2.5	Activation	[88]
	BAEC	25–100	Activation	[115]
AP-1	HSC	20–100	Inhibition	[9]
	Epidermal cell	5–20	Inhibition	[21]
STAT1	Cardiac myocyte	100	Inhibition	[109]
STAT3	A549, HPAEpic	10	Inhibition	[59]
FOXO1	3T3-L1	100	Inhibition	[45,46]
	BAEC	10	Inhibition	[90]

CaMKKβ and liver kinase B1 (LKB1) that are activated by EGCG [10,15,75]. The previously mentioned Ca²⁺ signaling contributes to stimulation of CaMKKβ in response to EGCG treatment of endothelial cells [47]. The activation of AMPK is dependent on reactive oxygen species because catalase and N-acetyl cysteine suppress this action of EGCG [15,38,74]. This is an example of beneficial pro-oxidant actions of EGCG. In addition to Ca²⁺ and cyclic nucleotides, a number of studies demonstrate that other intracellular signaling pathways are regulated by EGCG. It is difficult to determine unified EGCG-stimulated signal transduction pathways. This seems to be highly dependent on cell type and EGCG concentrations. For example, EGCG stimulates Src-family kinases, including Fyn in endothelial cells [49], while EGCG inhibits concavalin A-stimulated Src in mesenchymal stromal cells [123]. The concentrations of EGCG in both studies are in a

similar range (10–50 μM). Ca^{2+} signaling and Src activation is not observed in NIH-3T3 fibroblast cells (unpublished observation). Furthermore, EGCG-stimulated Fyn/PI 3-kinase/Akt/eNOS pathway contributes to vasorelaxation and protection from ischemia reperfusion injury of cardiac tissues. However, this does not seem to be mediated through a 67LR-dependent mechanism [49,87]. Puzzlingly, a similar pathway in cancer cells is mediated by 67LR and leads to apoptosis [55]. It is conceivable that opposing actions of EGCG may have differential effects depending on whether they are integrated into normal physiology or intervene in dysregulated pathophysiology.

Nuclear function

Cellular responses in intracellular signal transduction pathways generally occur acutely. However, chronic cellular responses often involve nuclear functions that regulate gene expression and chromosomal modifications. In this section, we will discuss the effects of EGCG in nuclear function.

Regulation of transcription factors

EGCG modulates gene expression by inhibiting various transcription factors including Sp1, NF- κB , AP-1, STAT1, STAT3 and FOXO1 (Table 4) [2,9,21,46,59,90,91,109,120]. NF- κB and AP-1 expression are inhibited by EGCG in rats exposed to ischemia-reperfusion (I/R) injury [4]. EGCG inhibits STAT-1 to mediate protective effects of EGCG in myocardial I/R injury [104,109]. Multiple studies show that nuclear activities of EGCG inhibit inflammatory responses that are usually accompanied by increased oxidative stress [6,86]. Thus, one may claim that this anti-inflammatory action is mainly due to direct anti-oxidant activity of EGCG. However, it is not clear whether anti-oxidative effects of EGCG are a major mechanism for anti-inflammatory actions of EGCG. Interestingly, EGCG-stimulated production of ROS that causes activation of NF- κB and NF-E2-related factor 2 (Nrf2) leading to increased expression of HO-1 and glutathione [88,115]. Scavenging ROS by using various anti-oxidants abolishes EGCG-stimulated induction of HO-1 while pretreatment with EGCG has protective effects against hydrogen peroxide-induced cytotoxicity [115]. It is noteworthy that most *in vitro* studies use high concentrations of EGCG to elicit inhibitory actions on transcription factors. However, *in vivo* studies may not achieve the concentrations of EGCG used with *in vitro* studies. These potential differences in conditions and concentrations of EGCG need to be carefully considered when interpreting results of experiments designed to reveal mechanisms for the physiological actions of EGCG *in vivo*. Protective mechanisms that EGCG uses to defend against oxidative stress may be secondary to induction of various endogenous anti-oxidant proteins. Inhibition of the transcription factor FOXO1 by EGCG leads to suppression of basal levels of endothelin-1 (ET-1) and differentiation of adipocytes [45,46]. Both of these mechanisms are linked to activation of Akt that inhibits FOXO1 by direct phosphorylation of FOXO1 and may have cardiometabolic implications [56,80].

DNA methylation

EGCG has epigenetic functions in chromosomes [25]. Aberrant methylation on CpG islands cause gene silencing that leads to altered cellular physiology and cell proliferation. EGCG inhibits DNA methyltransferase (DNMT) which reverses methylation-induced gene silencing by directly binding to DNMT with an IC_{50} of less than 1 μM EGCG [26]. This suggests that EGCG is transported to the nucleus. Although this function of EGCG has been

known for a decade, the specific genes affected by this mechanism are not well defined and this area requires further investigation.

Mitochondrial function

Mitochondria are organelles that play important roles in energy production. EGCG intake reduces obesity and expression of leptin and stearyl-coA desaturase in white adipose cells while increasing fat oxidation [52]. This suggests that EGCG actions enhance mitochondrial fat utilization and reduce adipogenesis in fat tissue. EGCG stimulates mitochondrial biogenesis and promotes oxidative phosphorylation through a cAMP/PKA- and sirtuin-dependent mechanism [111]. With respect to anti-tumor activity, EGCG promotes apoptosis through mitochondrial damage, membrane depolarization, and cytochrome c release [89]. This apoptotic action of EGCG is inhibited by NAC or catalase suggesting that excess hydrogen peroxide may contribute to mitochondrial damage-induced cell death. By contrast, the metabolic function of EGCG with regard to mitochondrial function occurs with much lower concentrations of EGCG. In some animal models, EGCG enhances mitochondrial function that reduces oxidative stress in alcoholic fatty liver or diet-induced obesity [42,52]. In addition, pretreatment with EGCG (30 mg/kg) protects against mitochondrial damage in isoproterenol-induced cardiac toxicity in albino Wistar rats [20]. A study using rat cerebellar granule neurons shows >90% of [^3H]-EGCG is found in the mitochondrial fraction. Moreover, pre-incubation with EGCG protects against mitochondrial damage-caused cell death without changes in SOD, glutathione peroxidase, Nrf2, or Bcl2 expression and oxidative stress. However, toxin-, serum withdrawal-, and proteasome inhibitor-induced cell death are not prevented by EGCG [100]. This effect of EGCG may be considered a direct anti-oxidant property of EGCG. However, it is not completely clear if these effects are due to direct anti-oxidant effects of EGCG alone or additional secondary molecular interactions including changes in mitochondrial transcription activity. These issues require further clarification with specific experiments designed to test well-defined hypotheses.

Autophagy

Autophagy is a lysosomal catabolic process that degrades accumulated and unnecessary intracellular materials [17,18,69]. Autophagy requires a number of molecules that interact in a highly organized manner to help determine cell survival or death. Most polyphenols, including EGCG, resveratrol, quercetin, and curcumin induce autophagy. This may contribute to anti-aging effects of polyphenols [84]. Anti-aging actions of polyphenols mimic effects of calorie restriction [84]. Some studies show that high concentrations of EGCG (100 μM) inhibit autophagy leading to apoptosis in macrophage cell lines (Raw 264.7 cells) and cancer cells [31,124]. By contrast, low concentrations of EGCG (10 μM) induces autophagy that facilitates degradation of endotoxin-induced aggregation of high mobility group B-1 (HMGB1) leading to anti-inflammatory actions [65]. We recently reported that EGCG (10 μM) stimulates autophagy and autophagic flux in endothelial cells that helps degradation of lipid droplets through a Ca^{2+} /CaMKK β /AMPK dependent mechanism [47]. This may be an additional mechanism for protective effects of EGCG related to inflammation, lipotoxicity, and cell death. Thus, the regulation of autophagy by EGCG is dependent on concentration, stress conditions, and cell types. Further studies elucidating more detailed mechanisms for EGCG or other polyphenols to regulate cell survival, death, and metabolism will shed light on potential novel roles for EGCG in promoting health and preventing chronic diseases characterized by inflammation and oxidative stress.

Summary and perspectives

In this review, we emphasize biological actions of EGCG that do not directly involve anti-oxidant properties (graphic summary). EGCG directly interacts with plasma membrane proteins and phospholipids which stimulates intracellular signaling pathways. In addition, EGCG is transported to intracellular compartments, cytosol, mitochondria, lysosome, and nucleus where it mediates additional biological actions. These various effects are dependent on cell type, stress conditions, and concentrations of EGCG. Two major points should be emphasized: one is that the EGCG effects observed *in vitro* may not be the same as *in vivo*. The major reason for this is that EGCG is readily modified after absorption through the gut which modulates bioavailability. Moreover, the concentrations of EGCG often used in *in vitro* experiments cannot be reached *in vivo* in either plasma or tissues in whole animals. Despite these caveats, we can extend some *in vitro* results to *in vivo* physiology. With moderate and low concentrations of EGCG, low level oxidative stress may be a beneficial cue for the body to initiate induction of protective anti-oxidant systems and boost immune responses. Thus, under pathological conditions with increased oxidative stress (e.g., ischemia/reperfusion, alcoholic fatty liver, and obesity-induced inflammation), supplementation of green tea polyphenol may help cope with these stressful conditions. A second important point is that EGCG has cell type- and environment-specific responses because gene expression and signaling molecules are differentially regulated. One prominent action of EGCG recently discovered is to increase lipolysis through autophagy-dependent and -independent mechanisms [12,47,62]. This suggests that EGCG plays an important role in lipid metabolism in whole body physiology as well as at the cellular level. In the future, it will be helpful to identify more specific biomarkers that respond to physiologically effective concentrations of EGCG so that pharmacodynamic studies may be performed with physiological outcomes. Further investigations are required to understand how EGCG acts differentially in different cell types. Recent molecular, cellular, and animal studies have begun to reveal detailed mechanisms linking drinking green tea and life-style adjustment with prevention of chronic diseases including cancer, diabetes, and cardiovascular disorders. Furthermore, chemical modification of an EGCG pharmacophore may modify relative therapeutic activities so that combinatorial supplementation may synergistically enhance beneficial health effects [8,30].

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