Free Radical Biology & Medicine 50 (2011) 1324–1335

Contents lists available at ScienceDirect

Free Radical Biology & Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original Contribution

(−)-Epigallocatechin-3-gallate suppresses growth of AZ521 human gastric cancer cells by targeting the DEAD-box RNA helicase p68

Tomoko Tanaka [a,1], Takeshi Ishii [b], Daisuke Mizuno [a], Taiki Mori [b], Ryoichi Yamaji [a], Yoshimasa Nakamura [c], Shigenori Kumazawa [b], Tsutomu Nakayama [b], Mitsugu Akagawa [a,*,1]

[a] Department of Biological Chemistry, Division of Applied Life Science, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Naka-ku, Sakai 599–8531, Japan
[b] Department of Food and Nutritional Sciences and Global COE Program, University of Shizuoka, Suruga-ku, Shizuoka 422–8526, Japan
[c] Department of Biofunctional Chemistry, Graduate School of Natural Science and Technology, Okayama University, Okayama 700–8530, Japan

ARTICLE INFO

Article history:
Received 27 September 2010
Revised 13 December 2010
Accepted 17 January 2011
Available online 26 January 2011

Keywords:
(−)-Epigallocatechin-3-gallate
Polyphenol
Green tea
p68
Quinone
Cancer
Proteomics
Free radicals

ABSTRACT

(−)-Epigallocatechin-3-gallate (EGCG), the most abundant and biologically active polyphenol in green tea, induces apoptosis and suppresses proliferation of cancer cells by modulating multiple signal transduction pathways. However, the fundamental mechanisms responsible for these cancer-preventive effects have not been clearly elucidated. Recently, we found that EGCG can covalently bind to cysteine residues in proteins through autoxidation and subsequently modulate protein function. In this study, we demonstrate the direct binding of EGCG to cellular proteins in AZ521 human gastric cancer cells by redox-cycle staining. We comprehensively explored the binding targets of EGCG from EGCG-treated AZ521 cells by proteomics techniques combined with the boronate-affinity pull-down method. The DEAD-box RNA helicase p68, which is overexpressed in a variety of tumor cells and plays an important role in cancer development and progression, was identified as a novel EGCG-binding target. Exposure of AZ521 cells to EGCG lowered the p68 level dose dependently. The present findings show that EGCG inhibits AZ521 cell proliferation by preventing β-catenin oncogenic signaling through proteasomal degradation of p68 and provide a new perspective on the molecular mechanism of EGCG action.

Green tea (*Camellia sinensis*) is a widely consumed beverage known to have extensive beneficial health effects, including prevention of cancer and heart disease [1,2]. Extracts of green tea and its polyphenolic components, catechins (Fig. 1), have been shown to inhibit the formation and development of both spontaneous and chemically induced tumors at various organ sites in animal models [1,3,4]. Furthermore, epidemiological studies have revealed that green tea consumption may protect against various cancer types, including prostate, stomach, and breast cancers [1,4]. (−)-Epigallocatechin-3-gallate (EGCG)[2] is the most abundant and biologically active polyphenol in green tea. In various cancer cell lines, there is considerable evidence that EGCG inhibits enzyme activities and modulates multiple signal transduction pathways, resulting in the suppression of cell proliferation and enhancement of apoptosis, as well as cell invasion, angiogenesis, and metastasis [5–8]. Recent accumulating experimental data suggest that EGCG must interact directly with cellular proteins to act on various key proteins involved in cellular proliferation and apoptosis [9,10]. Therefore, the identification of target proteins interacting directly with EGCG is a key step in elucidating the molecular and biochemical mechanisms underlying the anticancer effects of EGCG.

The cell surface catechin receptor 67-kDa laminin receptor (67LR) mediates the EGCG induced-inhibition of cancer cell proliferation [11,12]. Vimentin [13], insulin-like growth factor 1 receptor [14], FYN [15], glucose-regulated protein 78-kDa (GRP78) [16], Ras-GTPase-activating protein SH3 domain-binding protein (G3BP) [17], and ZAP-70 [18] have been shown to be EGCG-binding targets from cultured cell lysates by EGCG–Sepharose 4B affinity chromatography. These proteins are important for the suppression of cell proliferation and enhancement of apoptosis mediated by EGCG in cancer cell lines. Nevertheless, the precise molecular and

Abbreviations: CBB, Coomassie Brilliant Blue G-250; Chaps, 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate; CHX, cycloheximide; DAPI, 4′,6-diamino-2-phenylindole; DTT, dithiothreitol; ECL, enhanced chemiluminescence; EC, (−)-epicatechin; ECG, (−)-epicatechin gallate; EGC, (−)-epigallocatechin; EGCG, (−)-epigallocatechin-3-gallate; EDTA, ethylenediamine tetraacetic acid; EGTA, ethylene glycol tetraacetic acid; FBS, fetal bovine serum; G3BP, Ras-GTPase-activating protein SH3 domain-binding protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GRP78, glucose-regulated protein 78-kDa; HRP, horseradish peroxidase; Keap1, Kelch-like ECH-associated protein 1;MS, mass spectrometry; 67LR, 67-kDa laminin receptor; MALDI, matrix-assisted laser desorption ionization; NBT, nitroblue tetrazolium; Nrf2, nuclear factor-erythroid 2-related factor 2; PAGE, polyacrylamide gel electrophoresis; PBA, *m*-aminophenylboronic acid–agarose; PBS, phosphate-buffered saline; PMSF, phenylmethylsulfonyl fluoride; RIPA, radioimmunoprecipitation assay; SDS, sodium dodecyl sulfate; TBS-T, Tris-buffered saline containing Tween 20; TCF/LEF, T cell factor/lymphoid enhancing factor; TFA, trifluoroacetic acid; TOF, time-of-flight.

* Corresponding author. Fax: +81 72 254 9460.
E-mail address: akagawa@biochem.osakafu-u.ac.jp (M. Akagawa).

[1] These authors contributed equally to this work.

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doi:10.1016/j.freeradbiomed.2011.01.024

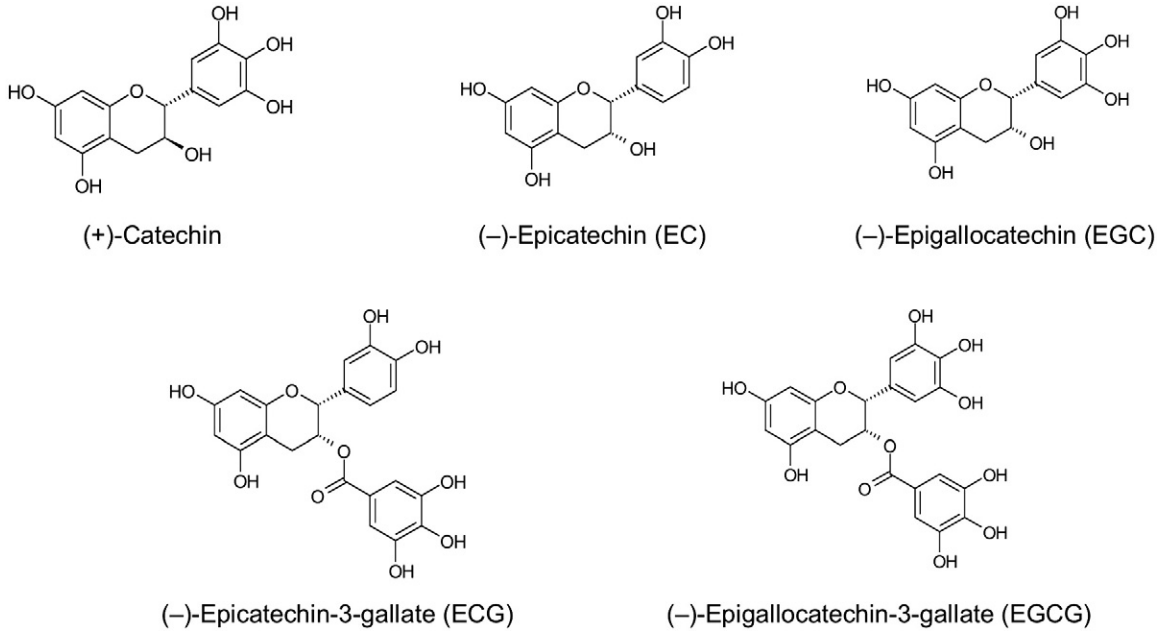

Fig. 1. Structures of major green tea catechins.

biochemical mechanism by which the binding of EGCG to target proteins regulates the protein function remains unknown. The EGCG-binding proteins from EGCG-treated cells have not been analyzed comprehensively by proteomics.

We found that EGCG can covalently bind to cysteinyl thiol residues in proteins through autoxidation (Fig. 2) [19]. EGCG is readily autoxidized to form a semiquinone radical that rearranges to an *o*-quinone at the B ring (gallyl) [20–22]. Thus, the electron-deficient *o*-quinone reacts with the nucleophilic thiol group of cysteine to form an *S*-cysteinyl–EGCG adduct. EGCG irreversibly inhibited glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity by specifically binding to the reactive cysteinyl thiol in the active center. Based on these findings, we proposed a novel signaling pathway by which the proteins are covalently modified by EGCG. Reactive cysteines with a low-pK_a thiol group serve as catalytic intermediates at the active site of many enzymes. Furthermore, reactive cysteinyl thiols present in various transcription factors, such as Kelch-like ECH-associated protein 1/nuclear factor-erythroid 2-related factor 2 (Keap1/Nrf2), nuclear factor-κB, and p53, have been suggested to act as redox sensors for the transcriptional regulation of many genes essential for maintaining cellular homeostasis [23–25]. Therefore, the covalent modification of such thiol groups could critically alter protein function, with potential effects on cell signaling. These events may subsequently lead to inhibition of tumor cell growth, induction of apoptosis, or inhibition of angiogenesis.

In this study, we examined the direct binding of EGCG to cellular proteins in EGCG-treated cells by redox-cycling staining. Furthermore, we used the affinity pull-down assay and proteomics approach to identify EGCG-bound proteins in the EGCG-treated cells. Among the identified proteins, we focused on the ATP-dependent RNA helicase DDX5 (referred to as p68) as a novel EGCG-binding target. Recently, p68 was reported to act as a transcriptional coactivator for a number of highly regulated transcription factors (e.g., β-catenin, estrogen receptor α, and androgen receptor) and to be overexpressed in some cancer cells, including intestinal, breast, and prostate tumor, suggesting that it may play an important role in cancer development and/or progression [26–29]. We investigated the effects of EGCG on the p68-dependent transcriptional regulation.

Materials and methods

Materials

EGCG, (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), (−)-epicatechin (EC), and (+)-catechin were kindly provided by Mitsui Norin Co. Ltd (Shizuoka, Japan). Hybond-P polyvinylidene difluoride (PVDF) membrane was obtained from GE Healthcare UK Ltd. The protease inhibitor cocktail was obtained from Roche Applied Science (Mannheim, Germany). *m*-Aminophenylboronic acid–agarose (PBA) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Fetal bovine

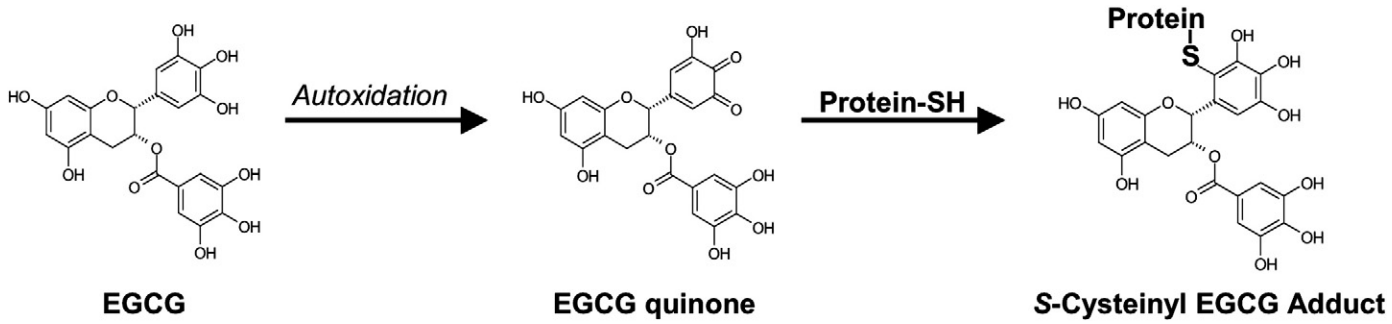

Fig. 2. Putative mechanism for EGCG binding to a protein cysteinyl thiol group through autoxidation. EGCG is oxidized to form EGCG quinone at the B ring by autoxidation. The EGCG quinone can react with the nucleophilic thiol group of a cysteine residue to form EGCG–protein adducts.