Biochemical and Biophysical Research Communications 421 (2012) 91–97

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Selective death of cancer cells by preferential induction of reactive oxygen species in response to (-)-epigallocatechin-3-gallate

Na Young Min [a,1], Jin-Hong Kim [a,1], Jee-Hye Choi [a,d], Wen Liang [a], Young Jong Ko [a], Sangmyung Rhee [a], Hyoweon Bang [b], Seung Wook Ham [c], Ae Ja Park [d], Kwang-Ho Lee [a,*]

[a] Department of Life Science, College of Natural Science, Chung-Ang University, Seoul 156-756, Republic of Korea
[b] Department of Physiology, College of Medicine, Chung-Ang University, Seoul 156-756, Republic of Korea
[c] Department of Chemistry, College of Natural Science, Chung-Ang University, Seoul 156-756, Republic of Korea
[d] Department of Laboratory Medicine, College of Medicine, Chung-Ang University, Seoul 156-756, Republic of Korea

ARTICLE INFO

Article history:
Received 13 March 2012
Available online 1 April 2012

Keywords:
EGCG
ROS
hTERT
CTCF
DNMT1

ABSTRACT

(-)-Epigallocatechin-3-gallate (EGCG) induces apoptosis in cancer cells without adversely affecting normal cells. Understanding the cancer-specific cytotoxic activity of EGCG is very important in defining the mechanism of tumorigenesis and identifying superb chemotherapeutic agents against cancer. We comparatively assayed human telomerase reverse transcriptase (*hTERT*)-mediated apoptosis by EGCG-induced reactive oxygen species (ROS) in normal cells and cancer cells. EGCG showed differential levels of ROS induction between the cell types; ROS, especially hydrogen peroxide, was highly induced in cancer cells, while it was not in normal cells. In addition, the higher level of ROS down-regulated *hTERT* via binding of CCCTC binding factor (CTCF) to the core promoter region of *hTERT*, which repressed *hTERT* expression. CTCF binding was epigenetically controlled by the demethylation of the previously hypermethylated site for CTCF, which was induced by down-regulation of DNA methyltransferase 1 (*DNMT1*). In contrast, *hTERT* down-regulation was not observed in normal cells. These results suggest that preferential death of cancer cells by EGCG could be caused by the cancer-specific induction of ROS and epigenetic modulation of expression of apoptosis-related genes, such as *hTERT*.

1. Introduction

Recently, natural products such as curcumin, piperlongumine and (-)-epigallocatechin-3-gallate (EGCG) have been suggested as candidates to ensure selective death of cancer cells with no side effects in clinical applications [1]. Among these molecules, EGCG, which is a major component of the polyphenols in green teas, selectively inhibits the growth of cancer cells without adversely affecting normal cells [2].

Human telomerase reverse transcriptase (*hTERT*) is one of the targets in cancer therapy and cancer diagnostic research because it is expressed in over 90% of cancers but not in normal cells [3,4]. It is widely accepted that the down-regulation of *hTERT* serves as a brake against malignancy via induction of apoptosis [5]. EGCG-induced down-regulation of *hTERT* is epigenetically controlled by demethylation of sites on the *hTERT* promoter region where a cytosine nucleotide occurs next to a guanine nucleotide (CpG site), leading to the binding of E2F-1 and/or CCCTC binding factor (CTCF) to sites within the *hTERT* promoter [6,7]. Since the endogenous level of *hTERT* expression is absent from most normal human somatic cells, *hTERT* inhibition by EGCG should affect only cancer cells with little impact on normal cells. However, it still remains to be determined whether EGCG induces cancer-specific cell death through the *hTERT*-mediated pathway.

EGCG is auto-oxidized in both cells and culture media, and the oxidized EGCG induces apoptosis in cells. However, it is still debatable whether reactive oxygen species (ROS) is induced differently between normal cells and cancer cells. Recent results suggested that ROS generated by a therapeutic reagent induces cancer-specific death due to the dysregulation of the redox balance in cancer cells [8]. Thus, it is postulated that the cancer-specific activity of EGCG may be due to the preferential induction of ROS in cancer cells, not in normal cells.

Collectively considering the available data, it cannot be excluded that the cancer-specific apoptosis by EGCG might be induced by the down-regulation of *hTERT*, which could be caused by the preferential induction of ROS in cancer cells. However, epigenetic molecular linkage of ROS with the down-regulation of *hTERT* remains to be defined.

* Corresponding author. Address: Department of Life Science, College of Natural Science, Chung-Ang University, 84 Heuksuk-Ro, Dongjak-Gu, Seoul 156-756, Republic of Korea. Fax: +82 2 825 5206.
E-mail address: leemanse@cau.ac.kr (K.-H. Lee).
[1] The authors contributed equally to the study.

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.03.120

In our previous study, we suggested that *hTERT* is down-regulated by binding of CTCF to its promoter region, which is modulated by DNA methyltransferase 1 (*DNMT1*)-mediated demethylation of CpGs on a CTCF binding site in trichostatin A (TSA)-treated HCT116 cells [7]. Considering the previous data, including ours, we opined that *hTERT* expression would be epigenetically and genetically regulated by EGCG, although the molecular linkage from EGCG-induced ROS to the down-regulation of *hTERT* was not clearly defined.

The present study was undertaken to define the molecular linkage. We first determined if EGCG, as a pro-oxidant, induced ROS and then assessed whether the induced ROS was capable of regulating genes that might directly regulate *hTERT* expression or indirectly regulate *hTERT* expression via a *DNMT1*-mediated pathway. In addition, we investigated whether apoptosis due to the down-regulation of *hTERT* was cancer-specific and by which mechanism EGCG-induced preferential death of cancer cells was induced. The presented findings could spur new anticancer therapeutic approaches by unveiling the molecular basis of the pro-apoptotic and pro-oxidant properties of EGCG.

2. Materials and methods

2.1. Cell culture and drug treatments

HCT116, HEK293 and MRC5 cells were obtained from the Korean Cell Line Bank (Seoul, Korea). The HCT116 human colon cancer cell line was cultured in RPMI 1640 (Sigma–Aldrich, St. Louis, MO, USA) supplemented with 10% (v/v) fetal bovine serum (FBS; WelGENE, Deagu, Korea) at 37 °C under a 5% CO_2 atmosphere. Cells of a normal human embryonal kidney cell line (HEK293) and a normal human lung cell line (MRC5), were grown at 37 °C in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) FBS (WelGENE) in a humidified atmosphere containing 5% CO_2. EGCG (Sigma–Aldrich) was dissolved in dimethylsulfoxide and added to the medium at final concentrations of 50 μM and 100 μM.

2.2. WST-1 cell viability assay

Cytotoxic effects of drugs on cells were determined by the WST-1 assay. Cells (1×10^4) were added to wells of a 96-well plate and incubated with 180 μl of culture medium for 24 h, prior to exposure to the indicated concentrations of EGCG and ROS scavengers. A total of 20 μl of WST-1 solution was added. After 4 h of incubation, the absorbance was measured at 432 nm using an ELISA plate reader.

2.3. Quantitative mRNA analysis of genes using real time-polymerase chain reaction (PCR)

Total RNA was extracted from cultured cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Real-time PCR was performed using a LightCycler faststart DNA master SYBR Green I kit (Roche, Penzberg, Germany) with a LightCycler 2.0 (Roche). Reactions were performed in triplicate using 4 μg of cDNA per reaction. For real time-PCR, primers (forward 5′-GTGGGGGACTGTGTCTCTGT-3′) and (reverse 5′-TGAAA GCTGCATGTCCTCAC-3′) were used for amplification of *DNMT1*. The primers used for *p53* RT-PCR were forward, 5′-GGACAGCCAA GTCTGTGACTTGC-3′ and reverse, 5′-CAGGTCTTGGCCAG TTGGC-3′. For RT-PCR of *p21*, forward, 5′-CTGGAGACTCTCAGGGTCGAA-3′ and reverse, 5′-CGGCGTTTGGAGTGGTAGAA-3′ were used. For RT-PCR of the gene encoding glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), forward, 5′-TGTGGTCATGAGTCCTTCCA-3′ and reverse, 5′-CGAGATCCCTCCAAAATCAA-3′ primers were used. Primers for *hTERT* were designed as previously proposed [9]. The relative expression ratio of each of the genes to GAPDH was calculated.

2.4. Bisulfite modification and PCR-directed sequencing

To analyze the methylation status of the *hTERT* promoter, a previous methodology for bisulfite modification and PCR was used [7]. Following PCR amplification, the purified products were cloned using a TA cloning kit according to the manufacturer's instruction (Real Biotech Corp., Taipei, Taiwan). Plasmid DNAs were isolated using a plasmid mini-prep kit according to the manufacturer's instruction (Real Biotech Corp.) and were sequenced using the M13 universal reverse primer (Macrogen, Seoul, Korea).

2.5. Western blot

Cells were suspended in RIPA buffer (iNtRON Biotechnology, Seongnam, Korea). Protein was quantified using the Bradford assay. The primary antibodies used were anti-*p53* mouse polyclonal IgG (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-*p21* rabbit polyclonal IgG (Santa Cruz Biotechnology), anti-*DNMT1* rabbit IgG (Bethyl, Montgomery, AL, USA) and anti-tubulin mouse monoclonal IgG (Sigma–Aldrich). All antibodies were diluted in Tris-buffered saline (TBS) (1:500–1:1000 dilution) and individually incubated with the membrane overnight at 4 °C. Secondary antibody was diluted in TBS-Tween (TBST) at a dilution of 1/10,000 and incubated with the membrane for 1 h at room temperature. Signals were visualized using enhanced chemiluminescence (Santa Cruz Biotechnology).

2.6. Chromatin immunoprecipitation (ChIP)

Cells (1×10^6) were harvested by centrifugation for 3 min at 2000 g and washed in phosphate buffered saline (PBS) with a protease inhibitor cocktail (Roche). ChIP analysis was performed using a ChIP assay kit (Millipore, Billerica, MA, USA) according to the manufacturer's instructions. For PCR amplification of the *hTERT* promoter region, recommended the primers were used [10]. H19 and dihydrofolate reductase (DHFR) were used as positive controls for CTCF and SP1 binding, respectively, and PCR amplification was performed using previously described primers [11,12].

2.7. Measurement of intracellular ROS

The cells were seeded on poly-D-lysine coated cover-slips and grown for 1 day to achieve 70% confluence prior to drug treatment. The cells were then treated with 100 μM EGCG in the absence or presence of superoxide dismutase; (SOD; 5 U/ml), catalase (30 U/ml) and N-acetyl-cysteine (NAC; 5 mM) for 6 and 12 h at 37 °C. To measure intracellular ROS, cells were incubated with 5 μM of 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA; Molecular Probes, Eugene, OR, USA) for 15 min at 37 °C. Signals were detected using a fluorescence microscopy examination at 100× magnification.

3. Results

3.1. Selective death of cancer cells by EGCG treatment

We assayed the endogenous level of *hTERT* expression in human normal (HEK293 and MRC5) and cancer (HCT116) cell lines. As expected, HCT116 highly expressed *hTERT*, whereas MRC5 did not (Fig. 1A). Interestingly, HEK293 also expressed *hTERT*, although the level of its expression was one-third of the level observed in HCT116. Using these three cell lines, which showed different levels

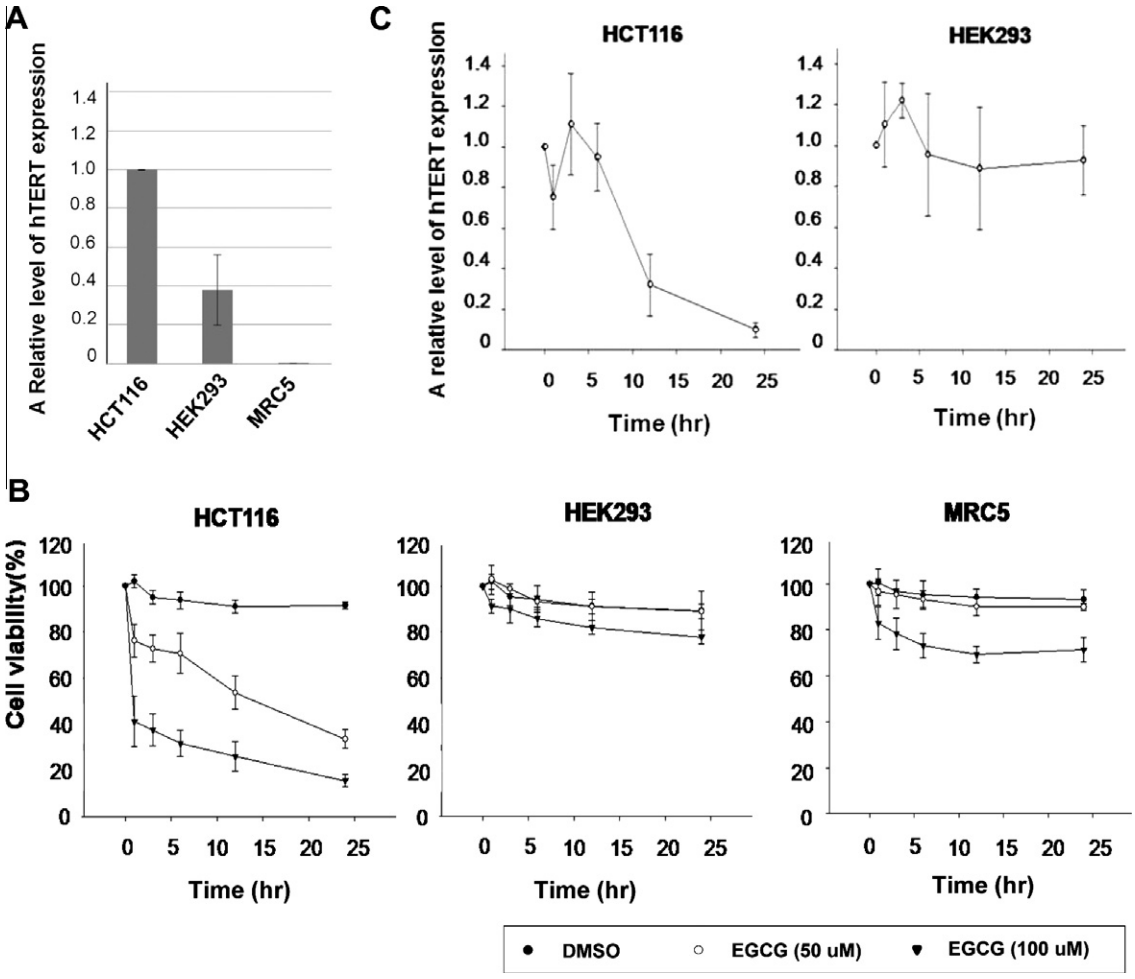

Fig. 1. Assessment of cell viability and *hTERT* expression after EGCG treatment in HCT116, MRC5 and HEK293 cells. A, Real-time PCR showing the relative level of endogenous expression of *hTERT*. B, WST-1 assay to examine cell viability after EGCG treatment. Viable HCT116 cells were markedly decreased up to 17% by EGCG treatment at 100 μM for 24 h, whereas the viability of the MRC5 and HEK293 was 78% and 72%, respectively. C, Real-time PCR showing the relative level of *hTERT* expression after EGCG treatment. HCT116 inhibited *hTERT* expression by up to 88%, whereas inhibition in HEK293 was only 20%.

of *hTERT* expression, we tested the cancer-specific cytotoxic effect of EGCG at concentrations of 50 μM and 100 μM using the WST-1 assay. As shown in Fig. 1B, proliferation of HCT116 cells declined in time-and dose-dependent manners, with about 17% viability evident following 24 h exposure to 100 μM EGCG. In contrast to HCT116 cells, HEK293 and MRC5 cells exhibited less sensitivity towards EGCG, displaying 78% and 72% viability following a 24 h treatment with 100 μM EGCG, respectively (Fig. 1B). These results implied that the inhibitory effect of EGCG on cell proliferation would be restricted to cancer cells, irrespective of the endogenous level of *hTERT* expression.

3.2. Preferential inhibition of hTERT by EGCG in cancer cells

To determine whether EGCG could induce cancer-specific cell death through the down-regulation of *hTERT* expression, we measured *hTERT* expression level after EGCG treatment in HCT116 and HEK293 cells. MRC5 cells were not used for this experiment because they did not express *hTERT* (Fig. 1A). Treatment of HCT116 cells with EGCG markedly decreased *hTERT* mRNA levels from 12 h after EGCG treatment (Fig. 1C). However, no difference in *hTERT* mRNA levels was observed in EGCG-treated HEK293 cells. These results demonstrated down-regulation of *hTERT* only in cancer cells, not in normal cells. HEK293 cells showed no inhibition in the level of *hTERT* expression, whereas a considerable reduction was evident for HCT116 cells, suggesting that EGCG-induced cell death was involved in the reduction of *hTERT* expression, although *hTERT* would not be a direct target of EGCG for cell death.

3.3. EGCG-induced up-regulation of p53 and p21, and concomitant down-regulation of DNMT1 in cancer cells

The increased level of *p53* expression leads to a concomitant increase in the protein level of *p21*, a down-stream transcriptional target of *p53* [13]. Results of a recent study supported the suggestion that *DNMT1* is down-regulated by *p53* via the formation of a complex with specificity protein 1 (SP1) and chromatin modifiers on the *DNMT1* promoter [14]. In addition, another study demonstrated that ectopic expression of *p21* inhibits *DNMT1* at the transcriptional level, implying that *p21* is an upstream regulator of *DNMT1* expression [15]. The collective results support the contention that EGCG is able to indirectly down-regulate *DNMT1* expression through the up-regulation of *p53* and *p21* in cancer cells.

We confirmed the up-regulation of *p53* and *p21* in HCT116 cells treated with EGCG (Fig. 2A). However, HEK293 and MRC5 cells showed no difference in the level of *p53* and *p21* expression before and after treatment with EGCG (Fig. 2A). Moreover, *DNMT1* expression was decreased in HCT116 cells (cancer cells) but not in HEK293 and MRC5 cells (normal cells), as expected from the different pattern of *p53* and *p21* expression (Fig. 2A and B).

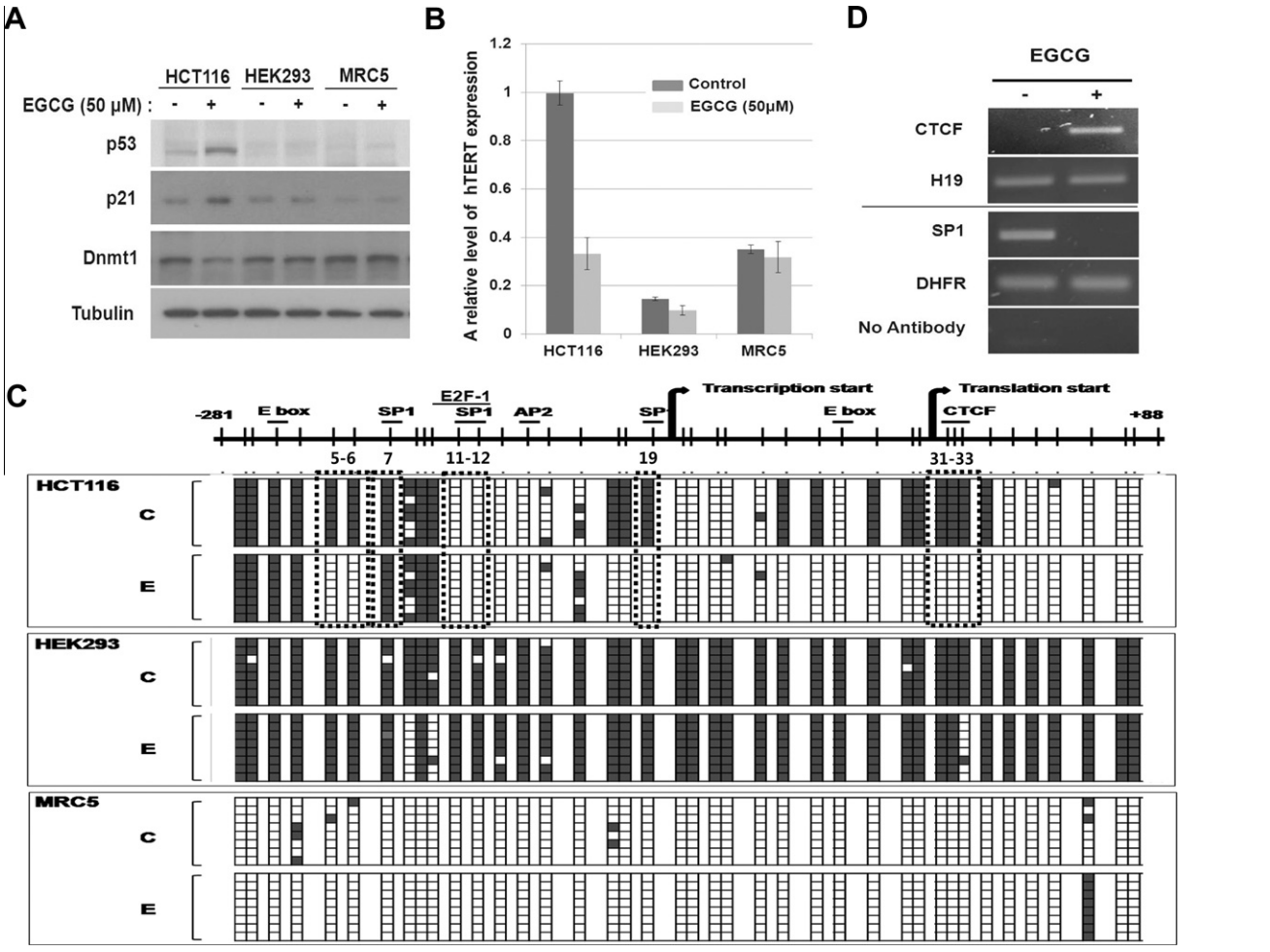

Fig. 2. Expression of *p21*, *p53* and *DNMT1* and *DNMT1*-mediated binding of CTCF and SP1 to the promoter of *hTERT*. A, Western blot assay to assess the expression of *p21*, *p53* and *DNMT1* after EGCG treatment. B, Real-time PCR to assess the expression of *DNMT1* after EGCG treatment. C, Bisulfite sequencing assay to define the site-specific CpG methylation pattern in the promoter region of *hTERT*. D, ChIP assay to test binding of CTCF and SP1 to the promoter region of *hTERT*. H19 and DHFR, which were expected to be bound to their binding sites irrespective of the methylation state, were used as controls to certify the assay. Dotted lines in the square indicate the CpG sites, where methylation states were changed after EGCG treatment.

3.4. Negative regulation of hTERT by CTCF

We next assayed the change of *DNMT1*-mediated methylation status on the promoter region of *hTERT* after EGCG treatment. As shown in Fig. 2C, bisulfite modification sequencing assay on the core promoter region of *hTERT* demonstrated that the site-specific methylation patterns were quite different between cancer cells and normal cells. More specifically, several genomic sites including CTCF and SP1 binding sites, which are important regulatory elements of *hTERT* expression, were demethylated after the treatment of EGCG in HCT116. By comparison, there was no considerable difference in the methylation pattern of the entire core promoter region before and after EGCG treatment of HEK293 and MRC5 cells (Fig. 2C). These results implied that demethylation of certain sites of the *hTERT* promoter by EGCG could induce the binding of regulatory proteins to the demethylated sites to regulate *hTERT* expression in HCT116. The ChIP assay indicated that CTCF was bound to the site (CpGs 31st–33rd) demethylated by the treatment of EGCG, which had previously been highly methylated, whereas SP1 was detached from the site (19th CpG) after the EGCG treatment (Fig. 2D). These findings suggested that the difference in the levels of EGCG-induced *hTERT* expression between cancer cells and normal cells would be derived from the preferential binding of CTCF to the *hTERT* promoter in cancer cells, resulting from the reduction of *DNMT1* expression.

3.5. Differential level of inducible ROS by EGCG between cancer cells and normal cells

We regarded ROS as the upstream regulator of *p53*. To determine if this explanation was suitable to describe the cancer-specific up-regulation of *p53* and *p21*, we measured the intracellular ROS level in cells treated with DCFH-DA (Fig. 3A and B). Endogenous levels of ROS were too low to measure the intracellular ROS level in all cells examined by DCFH-DA staining. However, the ROS level was considerably increased in HCT116 cells treated with EGCG, but not in normal counterparts, HEK293 and MRC5 cells (Fig. 3A). These results reflected the considerable difference in the level of inducible ROS between cancer cells and normal cells when they are treated with EGCG.

3.6. Regulation of p53, p21 and DNMT1 by EGCG-induced ROS and subsequent selective death of cancer cells

We found that the elevated level of ROS by EGCG was almost completely abolished by the treatment of various ROS scavengers, including SOD for decomposition of O_2^-, catalase for hydrogen peroxide (H_2O_2) and NAC for both O_2^- and H_2O_2. Especially, catalase was more effective in scavenging ROS induced by EGCG than SOD, indicating that ROS induced by EGCG was mainly H_2O_2. When HCT116 cells were treated simultaneously with catalase and NAC,

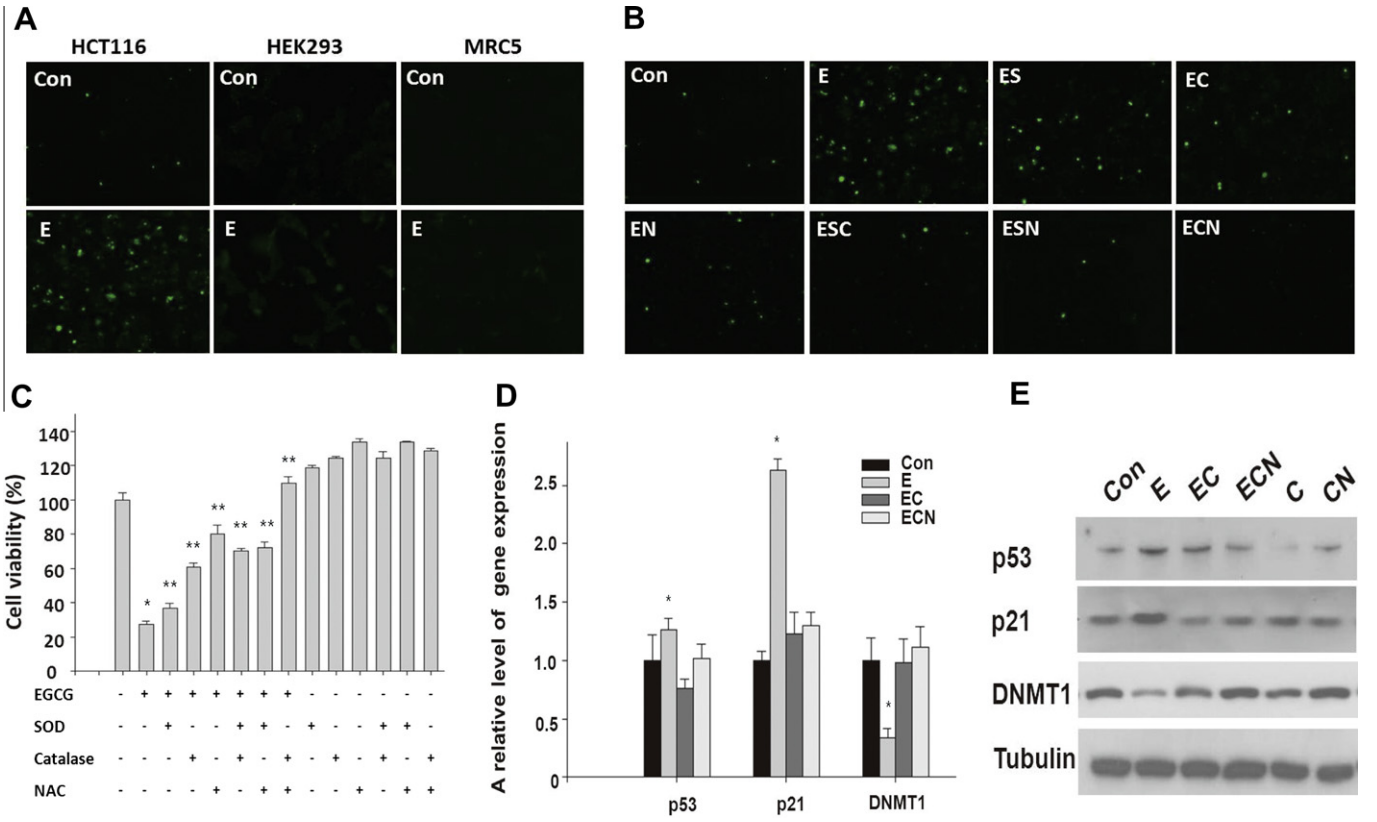

Fig. 3. EGCG-induced anti-proliferation and regulation of genes, including *p21*, *p53* and *DNMT1*. A, Assessment of the level of endogenous and EGCG-induced ROS in cells using DCFH-DA. B, Scavenging effects of anti-oxidants, SOD, catalase and NAC, on EGCG-induced ROS. C, Inhibition of EGCG-induced anti-proliferation by anti-oxidants. D, Effects of antioxidants on regulation of *p21*, *p53* and *DNMT1* expression. E, Western blot assay to assess the effect of anti-oxidants on *p21*, *p53* and *DNMT1* expression induced by EGCG. E, EGCG; S, SOD; C, catalase; N, NAC. $^{*}p < 0.05$ in pairwise *t*-test between control and EGCG-treated cells; $^{**}p < 0.05$ in pairwise *t*-test between cells treated EGCG alone and cells treated with EGCG/one of scavengers. Values of the relative expression level of genes in RT-PCR are indicated as the mean ± S.D. of three independent experiments.

no ROS were observed (Fig. 3B). The cell viability assay also demonstrated that EGCG-induced cytotoxicity was reduced when HCT116 cells were treated with SOD, catalase or NAC, or a combination of two of the three scavengers, showing the highest rate of cell survival in the presence of both catalase and NAC (Fig. 3C). In addition, expression of *p53* and *p21* was also reduced by treatment of catalase or catalase/NAC, suggesting that EGCG-induced ROS was responsible for the increase in the level of *p53* and *p21* expression (Fig. 3D and E). Moreover, *DNMT1* expression, which was decreased in the presence of EGCG, was returned to almost the same expression level as untreated cells when treated with the scavengers, catalase or catalase/NAC. These findings indicated that EGCG-induced ROS could induce the down-regulation of *DNMT1* expression with the up-regulation of *p53* and *p21*, resulting in *hTERT*-mediated cancer cell death.

4. Discussion

Recent studies have attempted to identify clinically available target molecules for cancer chemoprevention and chemotherapy, and to define the mechanism to induce selective death of cancer cells by them without any apparent effect on normal cells. In these studies, the cancer-specific killing capacity of EGCG was examined. The cellular activity of EGCG is selective for cancer cells by virtue of the modulation of the expression of genes related with apoptosis and cell proliferation, including *p21*, *p53* and *hTERT* [6,13]. In addition, EGCG epigenetically altered the expression of tumor suppression genes (TSGs) and apoptosis-related genes by exerting an inhibitory effect on genome-wide DNA methylation by reducing *DNMT1* expression. The results of many clinical studies support the possibility of EGCG that is a potent drug that is capable of inhibiting the initiation, promotion and progression of cancers. However, further studies at the molecular level are needed to fully understand how EGCG promotes cancer prevention and inhibition, and exerts its cancer-specific activity.

EGCG is readily auto-oxidized in cell culture medium to produce ROS, including O_2^- and H_2O_2 [16]. EGCG-induced ROS, mainly H_2O_2, and oxidative stress are involved in DNA oxidative damages, which results in transformation of cells through the subsequent mutation of oncogenes or TSGs [16]. EGCG induces the up-regulation of *p53* and *p21*, which are TSGs and major regulator of apoptosis. The up-regulation of *p21* is achieved through the super-activation of SP1, an ubiquitous transcription factor, by *c-Jun* on the promoter region of *p21* and induces the down-regulation of *DNMT1* [15]. In addition, EGCG induces the down-regulation of *DNMT1* via its direct binding to the catalytic site of *DNMT1* [17]. Presently, EGCG at the high concentration of 200 μM induced apoptosis in various cancer cells through up-regulation of *p21* in a *p53* independent manner (data not shown). As well, EGCG induced up-regulation of *p53* and *p21*, and down-regulation of *DNMT1* in HCT116 cells, whereas there was no difference in the level of expression of two genes in MRC5 cells and HEK293 cells after EGCG treatment (Fig. 2A). These results implied that *DNMT1* could be down-regulated either by direct inhibition of *DNMT1* or by up-regulation of *p53* and *p21* preferentially in cancer cells treated with EGCG.

In addition, we previously suggested that down-regulation of *DNMT1* by TSA treatment was involved in down-regulation of *hTERT* via recruitment of CTCF to the promoter region of *hTERT* in

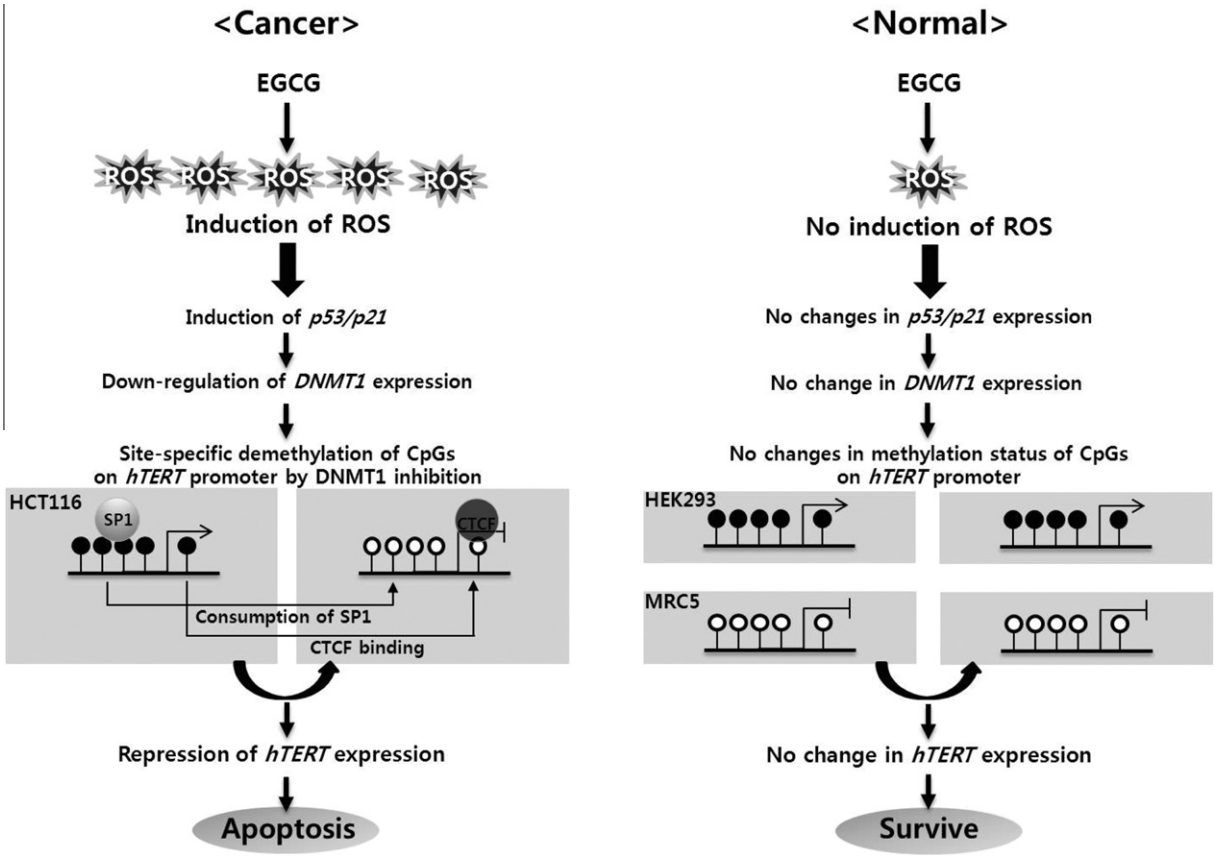

Fig. 4. Schematic presentation showing the preferential increase of ROS and induction of apoptosis by EGCG treatment in cancer cells.

HCT116 [7]. Presently, the site-specific demethylation of the CTCF binding site in the promoter region of *hTERT* recruited CTCF, a negative regulator of *hTERT*, to the site, resulting in down-regulation of *hTERT* (Fig. 2C and D). In contrast to the binding of CTCF for the down-regulation of *hTERT* after EGCG treatment, SP1 was detached from the promoter of *hTERT* by EGCG treatment (Fig. 2D). In the *hTERT* promoter, there are three SP1 sites as well as a CTCF binding site, which are expected to positively regulate *hTERT* expression (Fig. 2C). CpG sites 7th and 11th–12th showed no change in their methylation state after EGCG treatment, but the CpG 19th site was demethylated after EGCG treatment, implying the binding of SP1 to the demethylated site. However, the ChIP result using the SP1 antibody revealed the dissociation of SP1 from the promoter region of *hTERT* rather than the recruitment of SP1 to the site (Fig. 2D). It is possible that the SP1 site of CpG 19th might be vacant since most SP1 proteins were consumed to form *p53*–SP1 complexes with EGCG-induced *p53*. Rather, it seemed that CpGs 11th–12th, which was unmethylated before EGCG treatment, might have been bound to SP1, but became vacant due to the formation of the *p53*-SP1 complex. These findings support the suggestion that *hTERT* was epigenetically down-regulated through the combined effect of entire consumption of SP1, which could cause up-regulation of *p21* and/or *hTERT*, and the negative regulation of CTCF for *hTERT*.

Although a great deal of data on the cancer preventive effects of EGCG have accumulated, the molecular nature of different response to EGCG between cancer cells and normal cells still remains to be defined. It has been demonstrated that piperlongumine, a natural product isolated from the plant species *Piper longum* L., increases the level of ROS and apoptotic cell death in cancer cells but shows no apparent cytotoxicity in normal cells, reflecting its cancer-specific capacity in response to transformation-induced oxidative stress [1]. The latter authors suggested that normal cells have low basal levels of ROS and, therefore, a diminished reliance on the ROS stress-response pathway, whereas cancer cells have high levels of ROS and might, therefore, be expected to have a strong reliance on the ROS stress-response pathway. In contrast to the results of the piperlongumine, our data demonstrated that the differential response to EGCG between cancer cells and normal cells was derived from the differential inducibility of ROS between cancer cells and normal cells, rather than the different basal level of ROS between them. Thus, the difference in the level of inducible ROS between cancer cells and normal cells forms the basis of cancer-specific anti-proliferative and pro-apoptotic property of EGCG. In support of this hypothesis, the basal level of ROS in cancer cells was almost the same as that in normal cells, but EGCG-induced ROS was markedly increased in cancer cells whereas not in normal cells (Fig. 3A).

In conclusion, the cancer-specific pro-apoptotic property of EGCG might be explained by inducing the preferential genetic and epigenetic regulation of apoptosis-related genes, including *p21*, *p53* and *hTERT*, in cancer cells (Fig. 4). In addition, it seemed that the preferential expression of those genes would be due to the differential inducibility of ROS, especially H_2O_2, by EGCG between cancer cells and normal cells (Fig. 4). That is, increased perturbation of redox and ROS homeostasis by EGCG-induced ROS in cancer cells would be the base for the selectivity of EGCG-induced apoptosis in cancer cells.

Acknowledgments

This Research was supported by the Chung-Ang University Research Grants in 1999.

References

[1] L. Raj, T. Ide, A.U. Gurkar, M. Foley, M. Schenone, X. Li, N.J. Tolliday, T.R. Golub, S.A. Carr, A.F. Shamji, A.M. Stern, A. Mandinova, S.L. Schreiber, S.W. Lee, Selective killing of cancer cells by a small molecule targeting the stress response to ROS, Nature 475 (2011) 231–234.

[2] S. Gupta, T. Hussain, H. Mukhtar, Molecular pathway for (-)-epigallocatechin-3-gallate-induced cell cycle arrest and apoptosis of human prostate carcinoma cells, Arch. Biochem. Biophys. 410 (2003) 177–185.

[3] A. Ahmed, T.O. Tollefsbol, Telomerase, telomerase inhibition, and cancer, J. Anti. Aging Med. 6 (2003) 315–325.

[4] S. Kyo, M. Takakura, T. Fujiwara, M. Inoue, Understanding and exploiting hTERT promoter regulation for diagnosis and treatment of human cancers, Cancer Sci. 99 (2008) 1528–1538.

[5] A. Mittal, M.S. Pate, R.C. Wylie, T.O. Tollefsbol, S.K. Katiyar, EGCG down-regulates telomerase in human breast carcinoma MCF-7 cells, leading to suppression of cell viability and induction of apoptosis, Int. J. Oncol. 24 (2004) 703–710.

[6] J.B. Berletch, C. Liu, W.K. Love, L.G. Andrews, S.K. Katiyar, T.O. Tollefsbol, Epigenetic and genetic mechanisms contribute to telomerase inhibition by EGCG, J. Cell. Biochem. 103 (2008) 509–519.

[7] J.H. Choi, N.Y. Min, J. Park, J.H. Kim, S.H. Park, Y.J. Ko, Y. Kang, Y.J. Moon, S. Rhee, S.W. Ham, A.J. Park, K.H. Lee, TSA-induced DNMT1 down-regulation represses hTERT expression via recruiting CTCF into demethylated core promoter region of hTERT in HCT116, Biochem. Biophys. Res. Commun. 391 (2010) 449–454.

[8] N. Ballatori, S.M. Krance, S. Notenboom, S. Shi, K. Tieu, C.L. Hammond, Glutathione dysregulation and the etiology and progression of human diseases, Biol. Chem. 390 (2009) 191–214.

[9] R.L. Zinn, K. Pruitt, S. Eguchi, S.B. Baylin, J.G. Herman, hTERT is expressed in cancer cell lines despite promoter DNA methylation by preservation of unmethylated DNA and active chromatin around the transcription start site, Cancer Res. 67 (2007) 194–201.

[10] S. Renaud, D. Loukinov, Z. Abdullaev, I. Guilleret, F.T. Bosman, V. Lobanenkov, J. Benhattar, Dual role of DNA methylation inside and outside of CTCF-binding regions in the transcriptional regulation of the telomerase hTERT gene, Nucleic Acids Res. 35 (2007) 1245–1256.

[11] K. Ishihara, H. Sasaki, An evolutionarily conserved putative insulator element near the 3′ boundary of the imprinted Igf2/H19 domain, Hum. Mol. Genet. 11 (2002) 1627–1636.

[12] D.E. Jensen, A.R. Black, A.G. Swick, J.C. Azizkhan, Distinct roles for Sp1 and E2F sites in the growth/cell cycle regulation of the DHFR promoter, J. Cell. Biochem. 67 (1997) 24–31.

[13] K. Hastak, M.K. Agarwal, H. Mukhtar, M.L. Agarwal, Ablation of either p21 or bax prevents p53-dependent apoptosis induced by green tea polyphenol epigallocatechin-3-gallate, FASEB J. 19 (2005) 789–791.

[14] R.K. Lin, C.Y. Wu, J.W. Chang, L.J. Juan, H.S. Hsu, C.Y. Chen, Y.Y. Lu, Y.A. Tang, Y.C. Yang, P.C. Yang, Y.C. Wang, Dysregulation of p53/Sp1 control leads to DNA methyltransferase-1 overexpression in lung cancer, Cancer Res. 70 (2010) 5807–5817.

[15] H.H. Tan, A.G. Porter, P21(WAF1) negatively regulates DNMT1 expression in mammalian cells, Biochem. Biophys. Res. Commun. 382 (2009) 171–176.

[16] G.X. Li, Y.K. Chen, Z. Hou, H. Xiao, H. Jin, G. Lu, M.J. Lee, B. Liu, F. Guan, Z. Yang, A. Yu, C.S. Yang, Pro-oxidative activities and dose-response relationship of (-)-epigallocatechin-3-gallate in the inhibition of lung cancer cell growth: a comparative study in vivo and in vitro, Carcinogenesis 31 (2010) 902–910.

[17] W.J. Lee, J.Y. Shim, B.T. Zhu, Mechanisms for the inhibition of DNA methyltransferases by tea catechins and bioflavonoids, Mol. Pharmacol. 68 (2005) 1018–1030.