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ORIGINAL PAPER

Epigallocatechin-3-gallate Sensitizes Human 786-O Renal Cell Carcinoma Cells to TRAIL-Induced Apoptosis

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Abstract Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a promising anticancer agent. Epigallocatechin-3-gallate (EGCG) is a polyphenolic constituent of green tea. In this study, potentiating effect of EGCG on TRAIL-induced apoptosis human renal carcinoma cell line 786-O which is relatively resistant to TRAIL was examined, and the possible mechanism was investigated. Here, we show that co-treatment with EGCG and TRAIL induced significantly more profound apoptosis in 786-O cells. Treatment of 786-O cells with EGCG and TRAIL downregulated c-FLIP, Mcl-1, and Bcl-2 proteins in a caspase-dependent pathway. Moreover, we found that pretreatment with NAC markedly inhibited the expression levels of c-FLIP, Mcl-1, and Bcl-2 downregulated by the combinatory treatment, suggesting that the regulating effect of EGCG on these above apoptosis-relevant molecules was partially mediated by generation of ROS. Taken together, the present study demonstrates that EGCG sensitizes human 786-O renal cell carcinoma cells to TRAIL-induced apoptosis by downregulation of c-FLIP, Mcl-1, and Bcl-2.

Keywords Epigallocatechin-3-gallate · Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) · Apoptosis · Renal cell carcinoma

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Introduction

Renal cell carcinoma (RCC), accounting for 3 % of all malignant neoplasms, is the most lethal urological disease [1]. RCC is a heterogeneous histological disease, and clear cell renal cell carcinoma (CCRCC) is the most common histological subtype, making up approximately 75–80 % of the cases of renal tumors [2]. Along with the advances in the early diagnostic approaches including by ultrasound, computed tomography, and magnetic resonance imaging, the incidence of RCC has been increasing worldwide over the last few decades [3, 4]. However, still 25 % of patients with RCC are diagnosed in the advanced stage of the disease [5]. Furthermore, despite nephrectomy and radiotherapy, 30 % of patients develop metastatic disease after treatment, with a median survival period of one year [2, 6].

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) belongs to the TNF superfamily, which has been considered to be one of the promising anticancer agents because of its ability to induce apoptosis in a variety of human cancers [7]. Previous studies have shown that TRAIL interacts with death receptor 4 (DR4; TRAIL-R1) and death receptor 5 (DR5; TRAIL-R2), which then causes the formation of the death-inducing signal complex (DISC) with binding of caspase-8, leading to apoptosis (extrinsic or death receptor pathway) [7, 8]. In addition, TRAIL also induces apoptosis via intrinsic or mitochondrial pathway, by disruption of the mitochondria membrane permeability, release of cytochrome c into the cytoplasm and activation of caspase-9 [9]. Despite the beneficial effect of TRAIL to selectively kill tumor cells, only a minority of cancer cells undergo apoptosis induced by TRAIL, while most of cancer cells seem to show resistance to TRAIL [8]. Therefore, efforts are being undertaken to understand the molecular

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mechanisms of TRAIL resistance and develop novel regimen to sensitize cancer cells to TRAIL-induced apoptosis.

Epigallocatechin-3-gallate (EGCG), a major polyphenolic constituent of green tea, has been shown to have marked antiproliferative, proapoptotic, antiangiogenic, antimetastatic, and cell cycle regulation activities in various in vitro and in vivo tumor models [10–12]. More importantly, EGCG exerts anticancer effects without harming normal healthy cells [13]. A number of studies have investigated the mechanisms by which EGCG kill cancer cells. Gupta et al. have reported that EGCG treatment resulted in a increases in the expression of p21, p27, p18, and p16, and a corresponding decrease in cyclin D1, D2, and cyclin E as well as CDK 2, 4, and 6. In prostate cancer cells, it has been found that EGCG treatment reduced the expression of antiapoptotic proteins like phospho-PI3K and its substrate AKT, thereby limiting cell growth [14]. In other studies with human colon cancer and prostate cells, EGCG reduces nuclear localization of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), which has significant roles in cell survival and induction of cytokines [15, 16]. In the context of tumor development, EGCG inhibits the production and activation of vascular endothelial growth factor (VEGF) in breast and colon cancer, and leukemia cells, as well as proteolytic activity of matrix metalloproteinases (MMPs) in prostate cancer cells [17–20].

Currently, the outcome of a combined treatment of RCC with TRAIL and EGCG is unknown. Therefore, the aim of the current study was to investigate and compare the potential chemosensitizing effects of EGCG on TRAIL-mediated apoptosis in RCC 786-O cells as well as the possible mechanisms underlying these modulations, particularly their effects on the expression of some proteins involved in the extrinsic apoptotic pathway such as Bcl-2, Mcl-1, and c-FLIP.

Materials and Methods

Cell Cultures

Human RCC cells, 786-O, were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % fetal bovine serum (FBS). When experimenting, cells were switched to DMEM containing 2 % FBS.

Cell Viability Assays

The viability of cells was determined using the CellTiter 96 AQueous Cell Proliferation Assay (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide [MTT] assay; Promega). In brief, 2×10^4 cells were seeded in a 96-well culture plate and treated with EGCG, TRAIL, or both. Following 48-h incubation, MTT reagent was added to each well, and absorbance was measured at 490 nm with a plate reader (Spectra Max 340; Molecular Devices, LLC, Sunnyvale, CA, USA). Cell viability was normalized to untreated cells.

Caspase Activity Assay

Caspase activity assay was carried out using a fluorometric protease assay kit as per the instructions provided by the manufacturer (BioVision, Mountain View, CA). Enzymatic release of free fluorogenic moiety was measured by a fluorometer (Ex 380 and Em 460 nm). The arbitrary unit was expressed as the fluorescent change per amount of protein.

Flow Cytometry Analysis

For flow cytometry, the cells were resuspended in 100 ml of PBS, and 200 ml of 95 % ethanol was added, while the cells were being vortexed. The cells were then incubated at 4 °C for 1 h, washed with PBS, resuspended in 250 ml of 1.12 % sodium citrate buffer (pH 8.4) with 12.5 mg of RNase, and finally, incubated for an additional 30 min at 37 °C. The cellular DNA was then stained by adding 250 ml of a propidium iodide solution (50 mg/ml) to the cells for 30 min at room temperature. The stained cells were analyzed by fluorescence-activated cell sorting on a FACScan flow cytometer (Becton–Dickinson and Co., Franklin Lakes, NY, USA) to determine the relative DNA content, which was based on the red fluorescence intensity.

Reverse Transcription Polymerase Chain Reaction

Total RNA was isolated using the TriZol reagent (Life Technologies; Gaithersburg, MD, USA), and the cDNA was prepared using M-MLV reverse transcriptase (Gibco-BRL;Gaithersburg, MD, USA) according to the manufacturers' instructions. The following primers were used for the amplification of human c-FLIP, Mcl-1, and Bcl-2: c-FLIP (sense) 5′-CGGACTATAGAGTGCTGATGG-3′ and (antisense)5′-GATTATCAGGCAGATTCCTAG-3′, Mcl-1 (sense) 5′-GCGACTGGCAAAGCTTGGCCTCAA-3′and (antisense) 5′-GTTACAGCTTGGATCCCAACTGCA-3′, and Bcl-2 (sense) 5′-TGTATGAACTGAGCAATGTGCAAGA-3′, and (antisense) 5′-CACCTGGCAGCGTAGGGTAA-3′. The PCR amplification was carried out using the following cycling conditions: 94 °C for 3 min followed by 23 cycles of 94 °C for 45 s; 58 °C for 45 s, 72 °C for 1 min; and a final extension at 72 °C for 10 min. Expression of mRNA was determined by quantitative reverse

transcription polymerase chain reaction (qRT-PCR) using SYBR Green PCR Master Mix (Bio-Rad Laboratories Inc., Hercules, CA, USA) using the gene-specific primers listed above and normalized to GAPDH expression.

Western Blot

The obtained cell extracts were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and electrophoretically transferred to polyvinylidene difluoride membranes. After blocking, the membranes were incubated with the indicated antibodies against PARP, Bcl-2, Mcl-1, and c-FLIP (Santa Cruz Biotechnology, Santa Cruz, CA) and β-actin (R&D Systems, Minneapolis, MN). Then, the membranes were incubated with horseradish peroxidase-labeled IgG. The blots were developed using enhanced chemiluminescence western blotting reagents. The intensity of each signal was determined using a computer image analysis system (IS1000; Alpha Innotech Corporation).

Statistical Analysis

Data are presented as mean ± SD. Multiple comparisons were carried out using one way analysis of variance (ANOVA) followed by either Dunnett or Tukey–Kramer test for post hoc analysis, as appropriate. Individual groups were compared used unpaired Student's t test. Statistical significance was acceptable to a level of $P < 0.05$.

Results

Effects of EGCG on TRAIL Cytotoxic Activity in 786-O Cells

Cell viability was expressed as the survival fraction compared with untreated control cells as assessed with MTT assay. As shown in Fig. 1a, treatment of 786-O cells with TRAIL at a dose up to 1000 ng/ml did not result in marked decrease in survival fraction of cells, which was consistent with the previous study reporting the relative resistance of 786-O cells to TRAIL [21]. Next, we examined the effect of EGCG treatment on cell viability. As shown in Fig. 1b, EGCG treatment did cause a dose-dependent loss of viability of cells, whereas only dose over 100 μg/ml significantly reduced the cell viability. In an attempt to search for novel sensitizer for TRAIL, we investigated the effect of the combined treatment with EGCG and TRAIL. As shown in Fig. 1c, co-treatment of 786-O cells with TRAIL and EGCG resulted in a marked reduction of cell viability, compared to that of cells treated with TRAIL or EGCG alone.

EGCG Treatment Induces Apoptosis in 786-O Cells

To investigate the effect of EGCG-induced apoptosis, human renal carcinoma 786-O cells were treated using EGCG, TRAIL alone, or in combination. Apoptosis was determined using flow cytometry analysis demonstrating hypo-diploid DNA. As shown in Fig. 2a, either treatment with TRAIL or EGCG by itself induced marked increase in apoptosis, whereas combinatory treatment of 786-O cells with EGCG and TRAIL resulted in a significant increase in apoptotic cell population compared to either single agent ($P < 0.05$). Moreover, we found that the loss of viability and increase in apoptosis was accompanied by the proteolytic cleavage of PARP as well as elevated activities of caspase-3, caspase-8, and caspase-9, as shown in Fig. 2b, c.

EGCG Downregulates Mcl-1 and c-FLIP Protein Expressions

To investigate the underlying mechanisms involved in EGCG enhanced TRAIL-induced apoptosis, we analyzed the changes in the expression levels of a number of molecules that played an essential role in apoptosis. As shown in Fig. 3a, Bcl-2, Mcl-1, and c-FLIP protein expressions were dramatically decreased in response to combinatory treatment of TRAIL and EGCG. To further elucidate the mechanism responsible for the changes in proteins level, we determined the levels of Bcl-2, Mcl-1, and c-FLIP mRNAs by RT-PCR. As shown in Fig. 3b, TRAIL treatment at 200 ng/ml alone did not markedly alter the mRNA expression levels of these above molecules, whereas EGCG treatment at 50 μg/ml resulted in a significant decrease in mRNA expressions of these molecules compared with control. In contrast, combinatory treatment with TRAIL and EGCG led to a significantly more profound decrease in the mRNA levels of these apoptosis-relevant molecules, suggesting that EGCG modulates the expression of these molecules at the transcriptional level.

EGCG Plus TRAIL-Induced Apoptosis was Mediated Via Caspase-Dependent Pathway

Our aforementioned resulted have shown that apoptosis induced by the combination of EGCG and TRAIL correlated with increased activities of caspase-3,-8 and -9. Therefore, we investigated the role of caspase pathway in EGCG plus TRAIL-induced apoptosis by using a general and potent inhibitor of caspases, z-VAD-fmk. As shown in Fig. 4a, b, z-VAD-fmk prevented all these caspase-related events such as cleavage of pro-caspase-3 and PARP. Our results of FACS analysis also revealed that EGCG plus TRAIL-induced apoptosis was almost completely prevented by pretreatment with z-VAD-fmk at 25 μM,

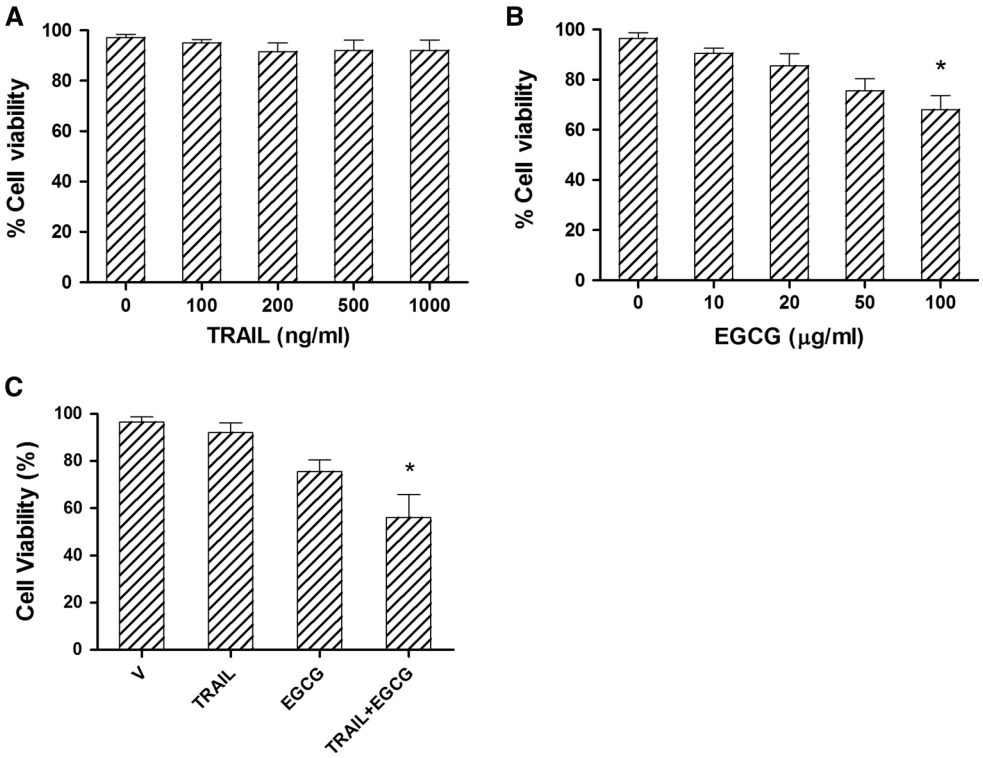

Fig. 1 Effect of EGCG or/and TRAIL on cell viability. Cells were treated with indicated concentration of EGCG, TRAIL, or both (TRAIL 200 ng/ml, EGCG 50 μg/ml) for 48 h. *$P < 0.05$ versus vehicle

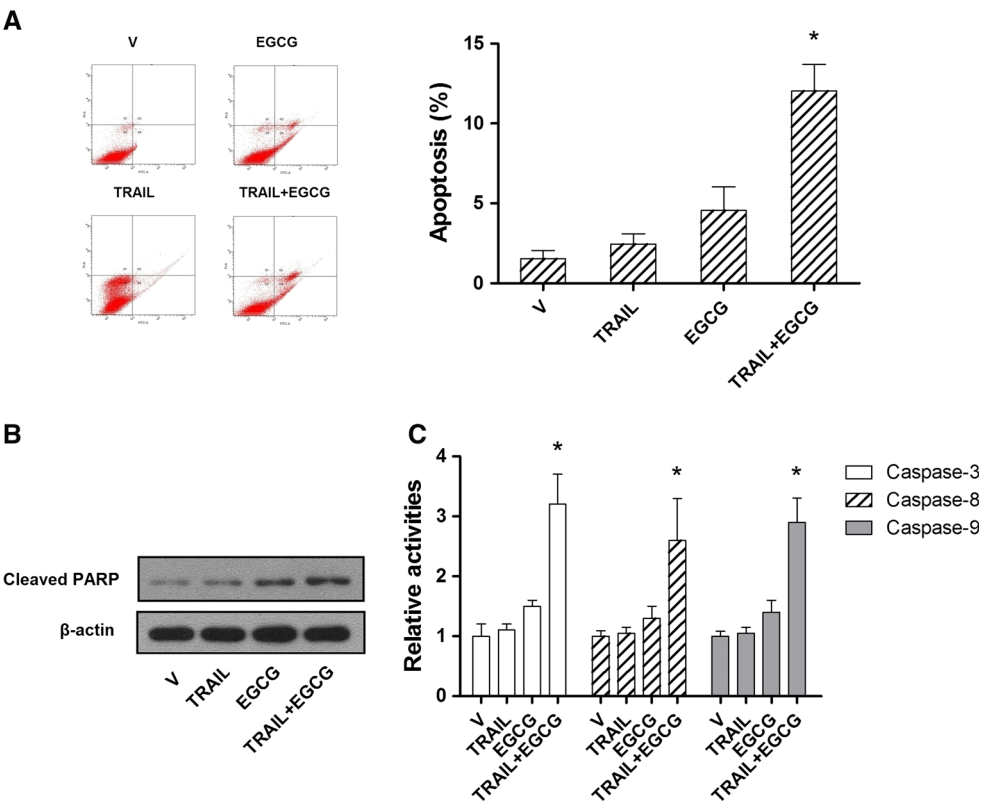

Fig. 2 Cell apoptosis induced by combination of EGCG and TRAIL in 786-O cells. Cells were treated with EGCG, TRAIL, or both (TRAIL 200 ng/ml, EGCG 50 μg/ml) for 48 h. Cell apoptosis was analyzed by flow cytometry, levels of cleaved PARP were determined by western blots, and activities of caspase-3, -8, and -9 were determined using ELISA kit. Flow cytometric graphs and western blots shown were representative of three experiments. *$P < 0.05$ versus vehicle

indicating the combined treatment with EGCG and TRAIL-induced apoptosis was mediated by caspase-dependent apoptosis pathways (Fig. 4c). Moreover, we examined the effect of z-VAD-fmk on the mRNA expression levels of Bcl-2, Mcl-1, and c-FLIP. As shown in Fig. 4d, results from RT-PCR analysis showed that

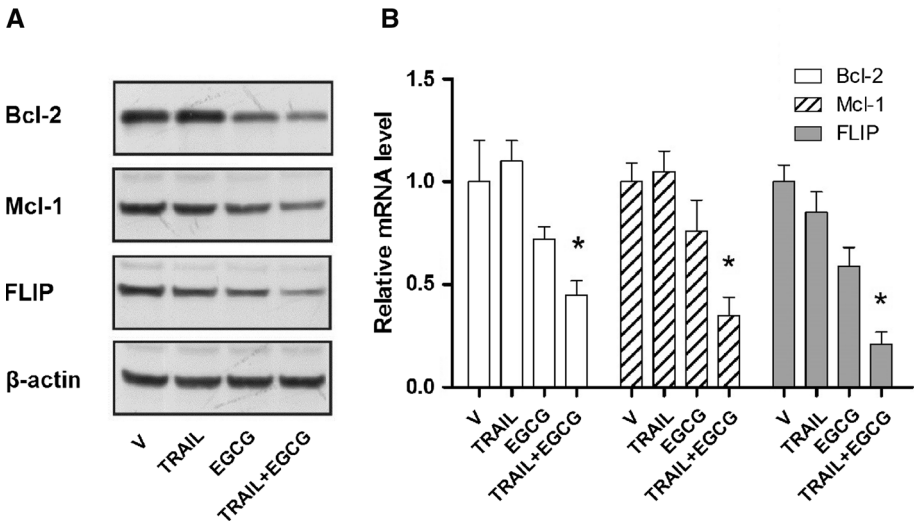

Fig. 3 Effects of TRAIL and EGCG on apoptosis-related molecules in 786-O cells. Cells were treated with EGCG, TRAIL, or both (TRAIL 200 ng/ml, EGCG 50 μg/ml) for 48 h. The protein and mRNA levels of apoptosis-related molecules were assessed by western blot and RT-PCR, respectively. $*P < 0.05$ versus vehicle

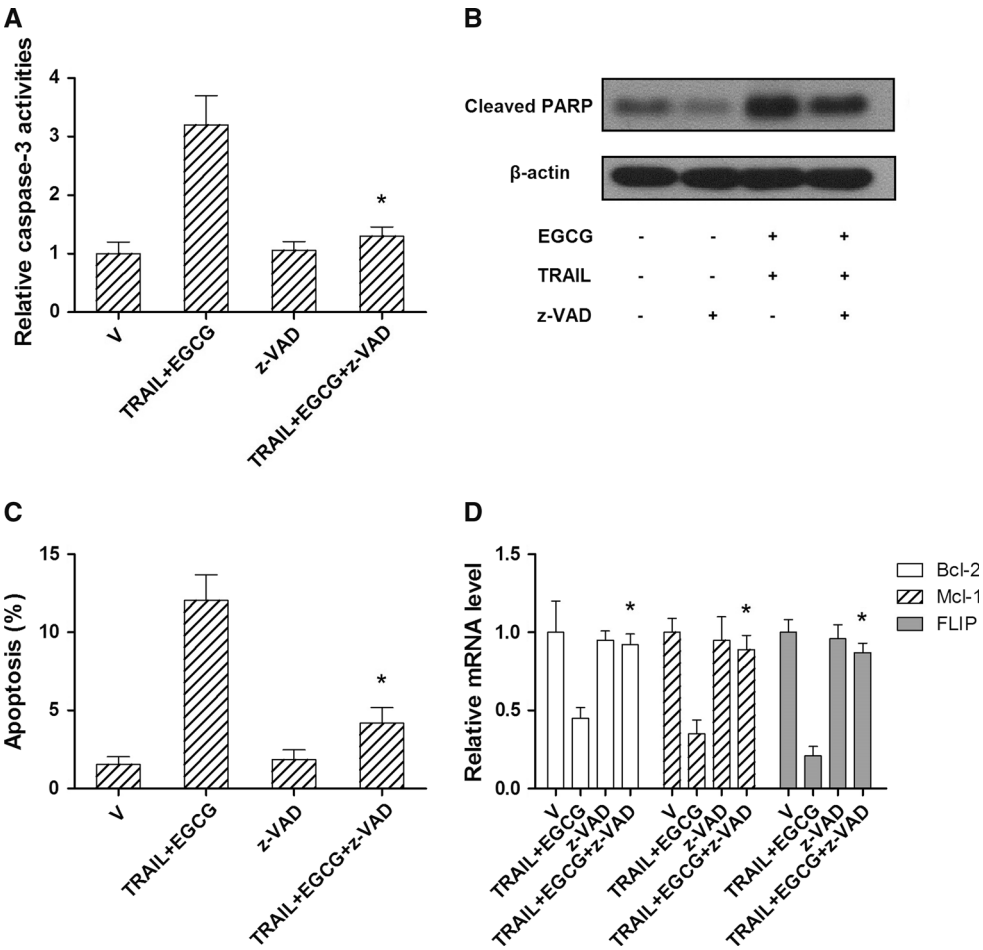

Fig. 4 Combinatory treatment with EGCG plus TRAIL-induced apoptosis was mediated via caspase-dependent pathway. Cells were treated with EGCG, TRAIL, or both (TRAIL 200 ng/ml, EGCG 50 μg/ml) for 48 h with or without pretreatment with 25 μM z-VAD for 1 h. Cell apoptosis was analyzed by flow cytometry, levels of cleaved PARP were determined by western blots, activity of caspase-3 was determined by ELISA kit, and the mRNA levels of apoptosis-related molecules were assessed by RT-PCR analysis. $*P > 0.05$ versus EGCG + TRAIL

pretreatment with z-VAD-fmk partially restored the mRNA level of Bcl-2, Mcl-1, and c-FLIP which were downregulated by combinatory treatment with EGCG plus TRAIL, indicating that the combinatory treatment modulates the expression of these apoptosis-relevant molecules caspase-dependent pathway.

Enhanced TRAIL-Induced Apoptosis Appears to be Partially Dependent on the Formation of Reactive Oxygen Species (ROS)

The role of ROS in apoptosis has been well established and it has been reported that EGCG increases ROS production

in a variety of cancer cells [22–24]. Therefore, we investigated whether ROS generation is directly associated with EGCG plus TRAIL-induced apoptosis. As shown in Fig. 5a, EGCG plus TRAIL-induced apoptosis was significantly abrogated by pretreatment with NAC, as determined by FACS analysis. Furthermore, we found that pretreatment with NAC decreased the elevated mRNA levels of Bcl-2, Mcl-1, and c-FLIP resulting from combinatory treatment (Fig. 5b). Taken together, these results suggested that EGCG-stimulated TRAIL-induced apoptosis appears to be partially dependent on the formation of ROS via downregulation of apoptosis-related molecules.

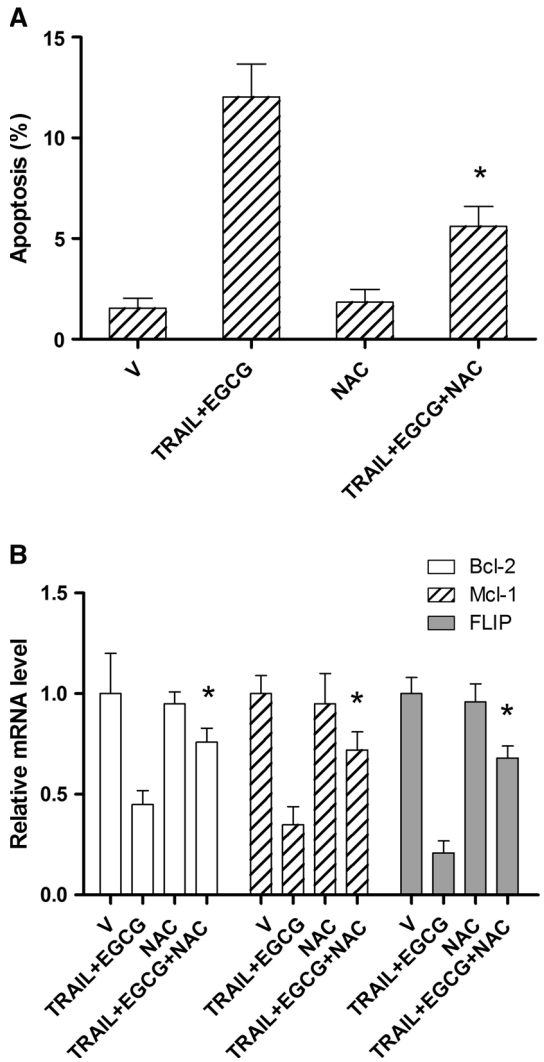

Fig. 5 EGCG-stimulated TRAIL-induced apoptosis appears to be partially dependent on the formation of ROS. Cells were treated with EGCG, TRAIL, or both (TRAIL 200 ng/ml, EGCG 50 μg/ml) for 48 h with or without pretreatment with 5 mM NAC for 1 h. Cell apoptosis was analyzed by flow cytometry, and the mRNA levels of apoptosis-related molecules were assessed by RT-PCR analysis. *$P > 0.05$ versus EGCG + TRAIL

Discussions

TRAIL has gained attention as a promising candidate for cancer therapy because it induces apoptosis preferentially in cancer rather than normal cells [25], which has been be explained by the differential expressions of agonistic receptors in cancer and dominant-negative antagonistic receptors in normal cells. It has been proposed that TRAIL transmits a pro-apoptotic signal to the caspase-signaling cascade via agonistic receptors death receptors 4 (DR4) and 5 (DR5) (also known as TRAIL-R1 and TRAIL-R2, respectively), leading to cell death [26, 27]. In contrast, the binding of TRAIL to decoy receptors 1 (DcR1) and 2 (DcR2) (also known as TRAIL-R3 and TRAIL-R4, respectively), which are known as antagonistic receptors for TRAIL, does not induce apoptosis due to the lack of an intracellular death domain in these two receptors [27, 28]. Recently, the prognostic values of TRAIL and its receptors have been noted by a couple of studies. Toiyama et al. have reported that serum TRAIL level may be useful as a prognostic biomarker in RCC patients and TRAIL might contribute to the lymphocyte-mediated cytotoxicity against human RCC cell lines [29]. Macher-Goeppinger et al. have also found that high TRAIL-R2, high TRAIL, and low TRAIL-R4 expression levels are associated with a worse disease-specific survival in patients with RCCs [30]. However, the application of TRAIL in clinical setting has been limited due to the short half-life and intrinsic resistance. Therefore, a lot of efforts have been made to enhance the therapeutic effect of TRAIL in RCC, and a combinatory therapeutic regimen with TRAIL and other agents has provided a most feasible solution.

During the last decade, natural compounds have received considerable attention of research as potential chemopreventive agents that may sensitize cancer cells to TRAIL-induced apoptosis [31, 32]. EGCG, the main active flavonoid compound of green tea, has been known to provide multiple beneficial health effects [33]. Epidemiological studies also proved that the green tea consumption is associated with lower risk of several types of human cancers [34]. In the context of RCC, it has been reported that EGCG inhibits growth and induces apoptosis in vitro [35]. In this study, we show that EGCG could sensitize TRAIL-resistant 786-O cells to TRAIL-induced apoptosis. The ability of EGCG to overcome TRAIL resistance was accompanied by the downregulation of Mcl-1 Bcl-2 and c-FLIP.

The extrinsic apoptotic pathway can be triggered by TRAIL via binding to death receptors on the cell surface to activate caspase-8. Meanwhile, TRAIL-induced apoptosis can be amplified by activation of the mitochondrial apoptotic pathway. Generally, these processes are augmented by overexpression of death receptors, but are negatively regulated by c-FLIP and antiapoptotic Bcl-2 family proteins

[7]. Moreover, Wu et al. have established the association between TRAIL resistance with overexpression of antiapoptosis including FADD-like apoptosis regulator (c-FLIP), antiapoptotic Bcl-2 family proteins (e.g., Bcl-2 and Bcl-xL) and inhibitor of apoptosis proteins (IAPs) [8], indicating that TRAIL-resistant cancer cells can be sensitized by TRAIL sensitizer such as chemotherapeutic drugs and biochemical inhibitors that suppress the expression of antiapoptosis-associated proteins including Bcl-2, c-FLIP or XIAP. A number of natural compounds, including dioscin [36], silibinin [37] and luteolin [38], has been shown to downregualate c-FLIP and subsequence sensitize to TRAIL-induced apoptosis in different cancer cell lines. In consistent with their studies, we found that the potentiating effect of EGCG on TRAIL-induced apoptosis in 786-O cells was also mediated by downregulation of apoptosis-related molecules Mcl-1, Bcl-2 and c-FLIP. It is well established that c-FLIP protein levels can be regulated by ubiquitin/proteasome-mediated degradation [39] or by their transcriptional control through the NF-κB or c-Fos pathway [40, 41]. However, further research is needed for the mechanistic study to elucidate how EGCG regulates the level of c-FLIP levels.

Recently, several studies have shown that ROS downregulates c-FLIP levels and increases the sensitivity to apoptotic stimuli [42, 43]. On the other hand, mounting evidence has shown that EGCG can exert antitumor effect via ROS-dependent pathway and hence activate a variety of downstream signaling [22, 23, 44]. Therefore, we investigated whether downregulations of c-FLIP by EGCG was actually mediated by ROS signaling pathway. In the presence of NAC, the decreased levels of c-FLIP caused by EGCG were partially restored. Taken together, EGCG-stimulated TRAIL-induced apoptosis appears to be dependent on the formation of ROS for downregulations of c-FLIP.

In summary, herein we report one antineoplastic mechanism of EGCG against human RCCs. We have shown that EGCG can sensitize RCC cells to TRAIL-induced apoptosis by downregulating c-FLIP via ROS-dependent pathway, indicating the potential of using EGCG as a therapeutic approach for enhancing sensitivity to TRAIL in the treatment of RCC.

References

1. Xue, Y. J., Xiao, R. H., Long, D. Z., Zou, X. F., Wang, X. N., Zhang, G. X., et al. (2012). Overexpression of FoxM1 is associated with tumor progression in patients with clear cell renal cell carcinoma. *Journal of Translational Medicine, 10*, 200.
2. Girgis, A. H., Iakovlev, V. V., Beheshti, B., Bayani, J., Squire, J. A., Bui, A., et al. (2012). Multilevel whole-genome analysis reveals candidate biomarkers in clear cell renal cell carcinoma. *Cancer Research, 72*, 5273–5284.
3. Requena, M. J., Carrasco, J. C., & Alvarez-Kindelan, J. (2008). Role of molecular markers in diagnosis and prognosis of renal cell carcinoma. *Analytical and Quantitative Cytology and Histology/The International Academy of Cytology [and] American Society of Cytology, 30*, 336–337.
4. Lang, K., Danchenko, N., Gondek, K., Schwartz, B., & Thompson, D. (2007). The burden of illness associated with renal cell carcinoma in the United States. *Urologic Oncology, 25*, 368–375.
5. Amen, K. (2007). Managing the complex journey of renal cell carcinoma. *ONS Connect, 22*, 53–54.
6. Takahashi, M., Rhodes, D. R., Furge, K. A., Kanayama, H., Kagawa, S., Haab, B. B., & Teh, B. T. (2001). Gene expression profiling of clear cell renal cell carcinoma: gene identification and prognostic classification. *Proceedings of the National Academy of Sciences of the United States of America, 98*, 9754–9759.
7. Tan, M. L., Ooi, J. P., Ismail, N., Moad, A. I., & Muhammad, T. S. (2009). Programmed cell death pathways and current antitumor targets. *Pharmaceutical Research, 26*, 1547–1560.
8. Wu, G. S. (2009). TRAIL as a target in anti-cancer therapy. *Cancer Letters, 285*, 1–5.
9. Deng, Y., Lin, Y., & Wu, X. (2002). TRAIL-induced apoptosis requires Bax-dependent mitochondrial release of Smac/DIABLO. *Genes & Development, 16*, 33–45.
10. Khan, N., & Mukhtar, H. (2013). Modulation of signaling pathways in prostate cancer by green tea polyphenols. *Biochemical Pharmacology, 85*, 667–672.
11. Katiyar, S. K., Perez, A., & Mukhtar, H. (2000). Green tea polyphenol treatment to human skin prevents formation of ultraviolet light B-induced pyrimidine dimers in DNA. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research, 6*, 3864–3869.
12. Ahmad, N., Gupta, S., & Mukhtar, H. (2000). Green tea polyphenol epigallocatechin-3-gallate differentially modulates nuclear factor kappaB in cancer cells versus normal cells. *Archives of Biochemistry and Biophysics, 376*, 338–346.
13. Ahmad, N., Feyes, D. K., Nieminen, A. L., Agarwal, R., & Mukhtar, H. (1997). Green tea constituent epigallocatechin-3-gallate and induction of apoptosis and cell cycle arrest in human carcinoma cells. *Journal of the National Cancer Institute, 89*, 1881–1886.
14. Siddiqui, I. A., Adhami, V. M., Afaq, F., Ahmad, N., & Mukhtar, H. (2004). Modulation of phosphatidylinositol-3-kinase/protein kinase B- and mitogen-activated protein kinase-pathways by tea polyphenols in human prostate cancer cells. *Journal of Cellular Biochemistry, 91*, 232–242.
15. Hastak, K., Gupta, S., Ahmad, N., Agarwal, M. K., Agarwal, M. L., & Mukhtar, H. (2003). Role of p53 and NF-kappaB in epigallocatechin-3-gallate-induced apoptosis of LNCaP cells. *Oncogene, 22*, 4851–4859.
16. Shimizu, M., Deguchi, A., Lim, J. T., Moriwaki, H., Kopelovich, L., & Weinstein, I. B. (2005). (-)-Epigallocatechin gallate and polyphenon E inhibit growth and activation of the epidermal growth factor receptor and human epidermal growth factor receptor-2 signaling pathways in human colon cancer cells. *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research, 11*, 2735–2746.
17. Jung, Y. D., Kim, M. S., Shin, B. A., Chay, K. O., Ahn, B. W., Liu, W., et al. (2001). EGCG, a major component of green tea, inhibits tumour growth by inhibiting VEGF induction in human colon carcinoma cells. *British Journal of Cancer, 84*, 844–850.
18. Sartippour, M. R., Shao, Z. M., Heber, D., Beatty, P., Zhang, L., Liu, C., et al. (2002). Green tea inhibits vascular endothelial growth factor (VEGF) induction in human breast cancer cells. *The Journal of Nutrition, 132*, 2307–2311.

19. Lee, Y. K., Bone, N. D., Strege, A. K., Shanafelt, T. D., Jelinek, D. F., & Kay, N. E. (2004). VEGF receptor phosphorylation status and apoptosis is modulated by a green tea component, epigallocatechin-3-gallate (EGCG) B-cell chronic lymphocytic leukemia. *Blood, 104*, 788–794.
20. Vayalil, P. K., & Katiyar, S. K. (2004). Treatment of epigallocatechin-3-gallate inhibits matrix metalloproteinases-2 and -9 via inhibition of activation of mitogen-activated protein kinases, c-jun and NF-kappaB in human prostate carcinoma DU-145 cells. *The Prostate, 59*, 33–42.
21. Clark, P. E., Polosukhina, D. A., Gyabaah, K., Moses, H. L., Thorburn, A., & Zent, R. (2010). TRAIL and interferon-alpha act synergistically to induce renal cell carcinoma apoptosis. *The Journal of Urology, 184*, 1166–1174.
22. Zhang, Y., Yang, N. D., Zhou, F., Shen, T., Duan, T., Zhou, J., et al. (2012). (-)-Epigallocatechin-3-gallate induces non-apoptotic cell death in human cancer cells via ROS-mediated lysosomal membrane permeabilization. *PLoS One, 7*, e46749.
23. Manohar, M., Fatima, I., Saxena, R., Chandra, V., Sankhwar, P. L., & Dwivedi, A. (2013). (-)-Epigallocatechin-3-gallate induces apoptosis in human endometrial adenocarcinoma cells via ROS generation and p38 MAP kinase activation. *The Journal of Nutritional Biochemistry, 24*, 940–947.
24. Li, W., Nie, S., Yu, Q., & Xie, M. (2009). (-)-Epigallocatechin-3-gallate induces apoptosis of human hepatoma cells by mitochondrial pathways related to reactive oxygen species. *Journal of Agricultural and Food Chemistry, 57*, 6685–6691.
25. Wiley, S. R., Schooley, K., Smolak, P. J., Din, W. S., Huang, C. P., Nicholl, J. K., et al. (1995). Identification and characterization of a new member of the TNF family that induces apoptosis. *Immunity, 3*, 673–682.
26. Pan, G., O'Rourke, K., Chinnaiyan, A. M., Gentz, R., Ebner, R., Ni, J., & Dixit, V. M. (1997). The receptor for the cytotoxic ligand TRAIL. *Science, 276*, 111–113.
27. Sheridan, J. P., Marsters, S. A., Pitti, R. M., Gurney, A., Skubatch, M., Baldwin, D., et al. (1997). Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors. *Science, 277*, 818–821.
28. Pan, G., Ni, J., Wei, Y. F., Yu, G., Gentz, R., & Dixit, V. M. (1997). An antagonist decoy receptor and a death domain-containing receptor for TRAIL. *Science, 277*, 815–818.
29. Toiyama, D., Takaha, N., Shinnoh, M., Ueda, T., Kimura, Y., Nakamura, T., et al. (2013). Significance of serum tumor necrosis factor-related apoptosis-inducing ligand as a prognostic biomarker for renal cell carcinoma. *Molecular and Clinical Oncology, 1*, 69–74.
30. Macher-Goeppinger, S., Aulmann, S., Tagscherer, K. E., Wagener, N., Haferkamp, A., Penzel, R., et al. (2009). Prognostic value of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and TRAIL receptors in renal cell cancer. *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research, 15*, 650–659.
31. Birt, D. F., Hendrich, S., & Wang, W. (2001). Dietary agents in cancer prevention: flavonoids and isoflavonoids. *Pharmacology & Therapeutics, 90*, 157–177.
32. Li, Y., Fang, H., & Xu, W. (2007). Recent advance in the research of flavonoids as anticancer agents. *Mini Reviews in Medicinal Chemistry, 7*, 663–678.
33. Kelemen, K., Kiesecker, C., Zitron, E., Bauer, A., Scholz, E., Bloehs, R., et al. (2007). Green tea flavonoid epigallocatechin-3-gallate (EGCG) inhibits cardiac hERG potassium channels. *Biochemical and Biophysical Research Communications, 364*, 429–435.
34. Yuan, J. M. (2011). Green tea and prevention of esophageal and lung cancers. *Molecular Nutrition & Food Research, 55*, 886–904.
35. Gu, B., Ding, Q., Xia, G., & Fang, Z. (2009). EGCG inhibits growth and induces apoptosis in renal cell carcinoma through TFPI-2 overexpression. *Oncology Reports, 21*, 635–640.
36. Kim, Y. S., Kim, E. A., Park, K. G., Lee, S. J., Kim, M. S., Sohn, H. Y., & Lee, T. J. (2012). Dioscin sensitizes cells to TRAIL-induced apoptosis through downregulation of c-FLIP and Bcl-2. *Oncology Reports, 28*, 1910–1916.
37. Son, Y. G., Kim, E. H., Kim, J. Y., Kim, S. U., Kwon, T. K., Yoon, A. R., et al. (2007). Silibinin sensitizes human glioma cells to TRAIL-mediated apoptosis via DR5 up-regulation and down-regulation of c-FLIP and survivin. *Cancer Research, 67*, 8274–8284.
38. Ou, Y. C., Li, J. R., Kuan, Y. H., Raung, S. L., Wang, C. C., Hung, Y. Y., et al. (2014). Luteolin sensitizes human 786-O renal cell carcinoma cells to TRAIL-induced apoptosis. *Life Sciences, 100*, 110–117.
39. Poukkula, M., Kaunisto, A., Hietakangas, V., Denessiouk, K., Katajamaki, T., Johnson, M. S., et al. (2005). Rapid turnover of c-FLIP short is determined by its unique C-terminal tail. *The Journal of Biological Chemistry, 280*, 27345–27355.
40. Li, W., Zhang, X., & Olumi, A. F. (2007). MG-132 sensitizes TRAIL-resistant prostate cancer cells by activating c-Fos/c-Jun heterodimers and repressing c-FLIP(L). *Cancer Research, 67*, 2247–2255.
41. Benayoun, B., Baghdiguian, S., Lajmanovich, A., Bartoli, M., Daniele, N., Gicquel, E., et al. (2008). NF-kappaB-dependent expression of the antiapoptotic factor c-FLIP is regulated by calpain 3, the protein involved in limb-girdle muscular dystrophy type 2A. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology, 22*, 1521–1529.
42. Nitobe, J., Yamaguchi, S., Okuyama, M., Nozaki, N., Sata, M., Miyamoto, T., et al. (2003). Reactive oxygen species regulate FLICE inhibitory protein (C-FLIP) and susceptibility to Fas-mediated apoptosis in cardiac myocytes. *Cardiovascular Research, 57*, 119–128.
43. Kanayama, A., & Miyamoto, Y. (2007). Apoptosis triggered by phagocytosis-related oxidative stress through C-FLIPS down-regulation and JNK activation. *Journal of Leukocyte Biology, 82*, 1344–1352.
44. Satoh, M., Takemura, Y., Hamada, H., Sekido, Y., & Kubota, S. (2013). EGCG induces human mesothelioma cell death by inducing reactive oxygen species and autophagy. *Cancer Cell International, 13*, 19.