Studies of apoptotic signaling pathway in human cancer cells by (-)EGCG of green tea.

Won-Hun Jeong1, Rae-Kil Park2 and Mee-Kyung Shin3

1: Wonkwang Health Science College, Iksan Jeonbuk 570-749, Korea

2 : School of Medicine, Wonkwang University, Iksan Jeonbuk 570-749, Korea

3 : Department of Food & Nutrition, Wonkwang University, Iksan Jeonbuk 570-749, Korea

Summary

Green tea has been recognized as a favorite beverage for centuries in Eastern and Western cultures. Recently, anti-tumor effects of green tea constituents have received increasing attention. However, the mechanism by which catechin-mediated cytotoxicity against tumor cells remains to be elusive.

This study was designed to elucidate the mechanical insights of anti-tumor effects, (-)epigallocatechin gallate (-EGCG) of catechin was applied to lung cancer A549 and prostate cancer DU145 cells.

We here demonstrated that (-)EGCG induces apoptosis of A549 and DU145 cells, which is mediated by signaling pathway involving caspase family cysteine protease, mitochondrial Bcl_2-family proteins, c-Jun N-terminal kinase 1 (JNK1) and transcriptional activation of activating protein-1 (AP-1).

Key words

catechin, (-)EGCG, apoptosis, caspase, JNK1, AP-1

Introduction

The anti-carcinogenic and anti-proliferative effects of green tea have been attributed to the biological activities of its polyphenol components. Green tea extract contains (-)epigallocatechin gallate(-EGCG), (-)epigallo-catechin(-EGC), (-)epicatechin gallate(-ECG), (-)epicatechin(-EC), (Stoner and Mukhtar, 1995). (-)EGCG, the most abundant polyphenol in green tea, has been shown to inhibit cell proliferation (Asano et al., 1997) and induce apoptosis (Hibasami et al., 1998; Paschka et al., 1998) in tumor cells. Other means by which (-)EGCG may prevent tumorigenesis via the inhibition of urokinase activity (Jankun et al., 1997), mitogen-activated protein kinases (MAPKs) activity (Jankun et al., 1997; Ahn et al., 1997; Fujiki et al., 1998), lipoxygenase and cyclooxygenase activities (Stoner and Mukhtar, 1995), and vascular endothelial growth factor (VEGF) induction (Jung et al., 2001). Recently, it is reported that polyphenol (-)EGCG causes a G0/G1-phase cell cycle arrest as well as apoptosis of human epidermoid carcinoma (A431) cells (Ahmad et al., 1997; Ahmad et al., 2000; Fujiki et al., 1998).

Apoptosis is a well-defined physiologic process characterized by many specific features, including DNA fragmentation and protease activation. As apoptosis may play an important role in cancer therapy, factors that modulate sensitivity to apoptosis are of great interest. In this study, we investigated the apoptotic signaling pathway of (-)EGCG, which induces apoptosis in cancer cells.

Materials and Methods

A component of green tea, (-)EGCG was purchased from Sigma Co. Human lung cancer A549 and prostate DU145 cells were treated with (-)EGCG to test genomic DNA fragmentation, Western blotting, measurement of caspase activity, immunoprecipitation and kinase assay.

Results and Discussion

In this study, we investigated the effect of (-)EGCG on cytotoxicity against human cancer cells. We found that (-)EGCG significantly increased the cytotoxic effect of A549 and DU145 cells in a dose-dependent manner (Fig. 1AB, lower panel). Crystal violet staining was parallely performed in 24 well plates (Fig. 1AB, upper panel). To confirm the cytotoxicity by (-)EGCG, genomic DNA was extracted from cells and electrophoresed on agarose gel (Fig. 1C). Concomitant treatment of both cancer cells with (-)EGCG caused the apparent DNA fragmentation, showing distinctive 'ladder pattern' of multiples of approximately 200-base pair fragments, which is regarded as an indication of apoptotic features (Fig. 1C). This result indicates that (-)EGCG induced the apoptotic death in cancer cells.

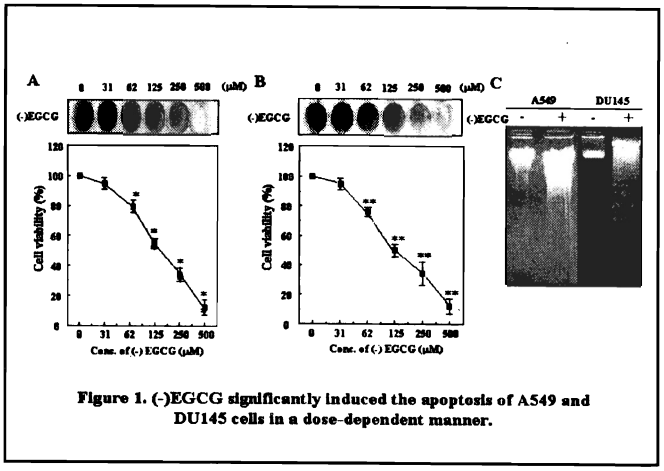

Figure 1. (-)EGCG significantly induced the apoptosis of A549 and DU145 cells in a dose-dependent manner.

Caspases are consisted of different subspecies of protease. It has been demonstrated that induction of cell death is associated with initial activation of several pro-caspases, which lead to sequential activation of down stream caspase family protease. To assess the direct activation of caspase cascade in (-)EGCG-induced apoptotic cell death, both cells were treated with 200 M (-)EGCG for various periods and lysates were used to measure the catalytic activity of caspase protease, respectively (Fig. 2 and 3). We first tested the activation of caspase-8 and caspase-9 protease, which initially involved in apoptotic cell death at indicated periods. The maximal activation of caspase-8 protease was occurred in 30 hr after treatment of (-)EGCG (Fig. 2A). The activation of caspase-8 protease was preceded by activation of caspase-2 and -3 proteases (Fig. 2A and 2B). As shown in Fig. 2C, the activity of caspase 3-like protease was significantly increased at 30 hr after (-)EGCG treatment and reached at a maximal level after 50 hr in a time-dependent manner. Specificity for caspase 3-like protease activity was confirmed by inhibition with 100 μM Ac-DEVD-CHO (Data not shown). Fig. 2D showed that activation of caspase-9 protease was started to increase after stimulation of (-)EGCG for 20 hr in lung cancer A549 cells (Fig. 2D). (-)EGCG also induced the activation of caspase family cysteine proteases including caspase-2, -3, -8 and -9 proteases in prostate cancer DU145 cells (Fig. 3).

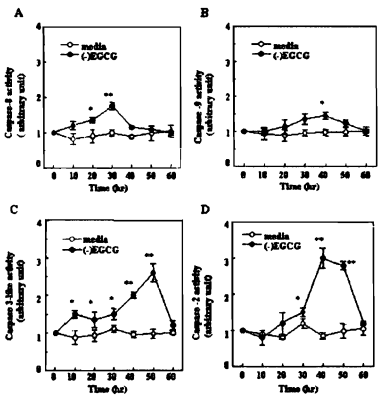

Figure 2. (-)EGCG induced the activation of caspases of A549 cells in a time-dependent manner.

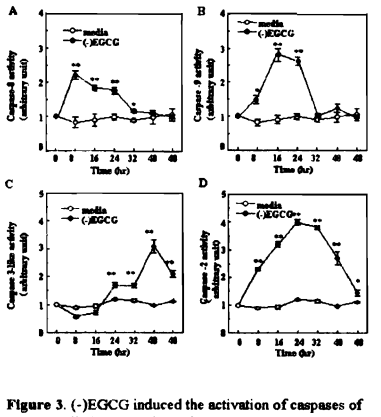

Figure 3. (-)EGCG induced the activation of caspases of DU145 cells in a time-dependent manner.

These results suggest that several proteases, including caspase-8, -2, caspase 3-like protease, and caspase-9 are activated during (-)EGCG-induced apoptosis of human cancer A549 and DU145 cells.

Recent evidence has demonstrated that caspase-8 protease is recruited by the death receptor-signaling complex (Muzio et al., 1996) and FADD. To investigate further the mechanism of (-)EGCG-induced apoptosis, we analyzed its effect on the expression of Fas and FasL in (-)EGCG-induced apoptosis. First, to determine whether (-)EGCG-induced cell death involves the induction of Fas and Fas ligand (FasL) expression, cells were exposed to (-)EGCG for the indicated periods and expression of Fas and FasL was analyzed by Western blotting. Western blot analysis showed that the expression of Fas and FasL was markedly increased following (-)EGCG treatment in A549 and DU145 cells (Fig. 5).

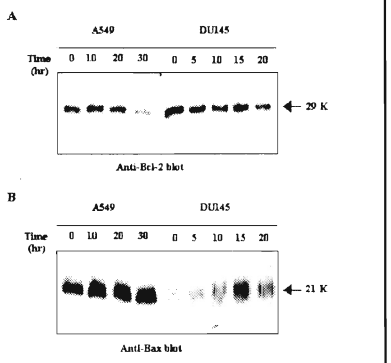

Figure 4. Expression of Bcl2 family proteins including Bcl2 and Bax in (-)EGCG-treated A549 and DU145 cells.

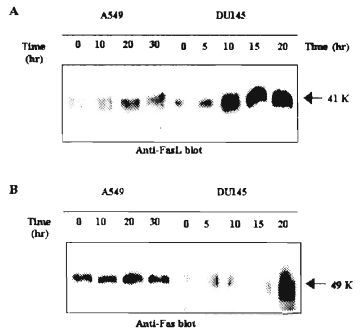

Figure 5. (-)EGCG increased the expression of Fas and FasL protein in A549 and DU145 cells.

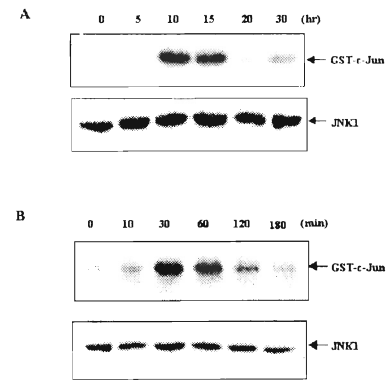

Figure 6. (-)EGCG increased the phosphotransferase activity of JNK1 in A549 (A) and DU145 cells (B) in a time-dependent manner.

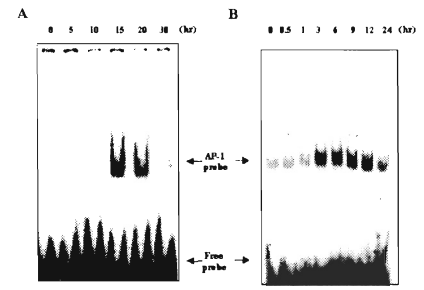

Figure 7. (-)EGCG induced the transcriptional activation of AP-1 in A549 (A) and DU145 (B) cells in a time-dependent manner.

The expression level of Bcl_2 protein was decreased in (-)EGCG-treated DU145 and A549 cells, whereas Bax protein was increased in a time-dependent manner (Fig. 6). Furthermore, (-)EGCG increased the phosphotransferase activity of c-Jun N-terminal kinase1 (JNK1) in a time-dependent manner, which further induced the transcriptional activation of AP-1 in both A549 and DU145 cells (Fig. 7).

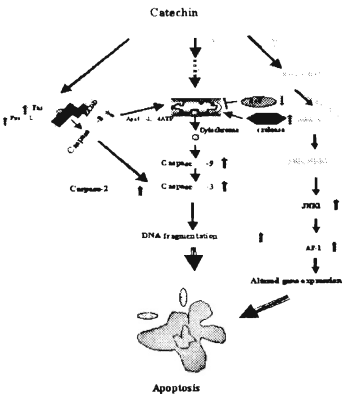

Figure 8. Apoptotic signaling pathway of (-)EGCG

Taken together, these results suggest that (-)EGCG-induced apoptosis of A549 and DU145 cells is mediated by signaling pathway involving caspase family cysteine protease, mitochondrial Bcl_2-family proteins, JNK1 and transcriptional activation of AP-1. We summarized the (-)EGCG-induced signaling pathway in cancer cells (Fig. 8).

(-)EGCG, a major constituent of green tea is known to induce

apoptosis in various types of tumor cells. The fact that tumor cells are much more sensitive to apoptosis induction by (-)EGCG than the normal counterparts offers merit in its potential usage as a chemopreventive agent. Epidemiologic studies have shown that the consumption of green tea lowers the risk of developing gastric and colon cancers. In rodent models, green tea preparations protect against the development of cancers in various tissues including skin, lung, mammary gland and gastrointestinal tract (Rogers et al, 1998). Green tea and black tea catechin compounds, such as (-)EGCG and theaflavin, have been investigated more intensively to reveal the molecular basis for anti-tumor activities. From these findings, we may conclude that (-)EGCG has cytotoxic effects on various cancer cells and is potentially implicated in cancer therapy.

References

1. Stoner GD and Mukhtar H, Polyphenols as cancer chemopreventive agents. J Cell Biochem Suppl 22: 169 180, 1995.

2. Asano Y, Okamura S, Ogo T, Eto T, Otsuka T and Niho Y, Effect of (-)-epigallocatechin gallate on leukemic blast cells from patients with acute myeloblastic leukemia. Life Sci 60(2): 135-142, 1997.

3. Hibasami H, Komiya T, Achiwa Y, Ohnishi K, Kojima T, Nakanishi K, Akashi K and Hara Y, Induction of apoptosis in human stomach cancer cells by green tea catechins. Oncol Rep 5(2): 5 27-529, 1998.

4. Paschka AG, Butler R and Young CY, Induction of apoptosis in prostate cancer cell lines by the green tea component, (-)-epigallocatechin-3-gallate. Cancer Lett 130(1-2): 1-7, 1998.

5. Jankun J, Selman SH, Swiercz R and Skrzypczak-Jankun E, Why drinking green tea could prevent cancer. Nature 387(6633): 561, 1997.

6. Ahn HY, Hadizadeh KR, Seul C, Yun YP, Vetter H and Sachinidis A, Epigallocathechin-3 gallate selectively inhibits the PDGF-BB-induced intracellular signaling transduction pathway in vascular smooth muscle cells and inhibits transformation of sis-transfected NIH 3T3 fibroblasts and human glioblastoma cells (A172). Mol Biol Cell 10(4): 1093-1104, 1999.

7. Fujiki H, Suganuma M, Okabe S, Sueoka N, Komori A, Sueoka E, Kozu T, Tada Y, Suga K, Imai K and Nakachi K, Cancer inhibition by green tea. Mutat Res 402(1-2): 307-310, 1998.

8. Jung YD, Kim MS, Shin BA, Chay KO, Ahn BW, Liu W, Bucana CD, Gallick GE and Ellis LM, (-)EGCG, a major component of green tea, inhibits tumour growth by inhibiting VEGF induction in human colon carcinoma cells. Br J Cancer 84(6): 844-850, 2001.

9. Ahmad N, Feyes DK, Nieminen AL, Agarwal R and Mukhtar H, Green tea constituent epigallocatechin-3-gallate and induction of apoptosis and cell cycle arrest in human carcinoma cells. J Natl Cancer Inst 89(24): 1881-1886, 1997.

10. Ahmad N, Cheng P and Mukhtar H, Cell cycle dysregulation by green tea polyphenol epigallocatechin-3-gallate. Biochem Biophys Res Commun 275(2): 328-334, 2000.

11. Muzio M, Chinnaiyan AM, Kischkel FC, O'Rourke K, Shevchenko A, Ni J, Scaffidi C, Bretz JD, Zhang M, Gentz R, Mann M, Krammer PH, Peter ME and Dixit VM, FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death--inducing signaling complex. Cell 85(6): 817-827, 1996.