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Original article

Epigallocatechin-3-gallate promotes apoptosis and reversal of multidrug resistance in esophageal cancer cells

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ABSTRACT

Evidence for demonstrating the role of the green tea component epigallocatechin-3-gallate (EGCG) in esophageal squamous cell carcinoma cells is limited. In this study, we investigated apoptosis induced by EGCG and the underlying molecular mechanisms in human esophageal squamous cell carcinoma cells. The growth-inhibitory effects of EGCG on esophageal cancer cell (Eca109 and Ec9706) were detected by MTT. Using flow cytometry, we determined the cellular apoptosis, bcl-2, bax and caspase-3 protein expression in Eca109 and Ec9706 cells following treatment with EGCG for 24 h. After treatment of Eca109/ABCG2 (an esophageal cancer multidrug resistance cell line) cells with adriamycin (ADM) combined with EGCG for 24 h, the cellular apoptosis, mitochondrial membrane potential, ADM concentration in cells and ABCG2 protein expression were detected by flow cytometry. EGCG inhibited the growth of Eca109 and Ec9706 cells in a dose- and time- dependent manner. EGCG induced apoptosis, decreased the bcl-2 protein expression and increased the expression of bax and caspase-3 protein. The rate of apoptosis and ADM concentration in the Eca109/ABCG2 cells following treatment with ADM and EGCG were higher than that with ADM treatment alone, although the mitochondrial membrane potential was significantly lower ($P < 0.01$). EGCG reduced the ABCG2 expression of Eca109/ABCG2 cells. Our data indicated that EGCG inhibited cell growth and induced esophageal cancer cell apoptosis. It reduced the bcl-2 protein expression and increased the bax and caspase-3 protein expression. EGCG reversed multi-drug resistance by reducing ABCG2 expression and increasing the anticancer drug concentration in cancer cells.

1. Introduction

Esophageal squamous cell carcinoma is a common malignancy [4,5,20,26]. Initiation and progression of esophageal cancer is a complex process associated with the loss of normal regulatory pathways involving cell proliferation, differentiation and apoptosis. However, current strategies involving chemotherapy have low efficacy [16], as well as drug resistance and side effects are the major challenges associated with chemotherapy [2,25]. Therefore, development of new drugs for esophageal squamous cell carcinoma is imperative [11]. Natural products have been shown as effective agents for cancer prevention, including epigallocatechin-3-gallate (EGCG). EGCG is a major catechin in green tea with anticancer, antioxidant and immunomodulatory effects [3,13,23,24,27]. However, studies investigating the therapeutic mechanisms of EGCG in esophageal cancer are limited, and the role of EGCG in esophageal cancer therapy is poorly understood. Herein, the anticancer effects of EGCG in esophageal squamous cell carcinoma and the underlying molecular mechanisms were studied. The reversal of multidrug resistance by EGCG in Eca109/ABCG2 cells was detected. EGCG enhanced apoptosis and ADM concentration in Eca109/ABCG2 cells induced by ADM. Mitochondrial membrane potential and ABCG2 protein expression in Eca109/ABCG2 cells were reduced by EGCG. Role of EGCG in promoting cellular apoptosis in esophageal cancer cells was investigated and effect in reversing multidrug resistance at dosages lower than the cytotoxic levels was determined.

2. Materials and methods

2.1. Cancer cell lines and culture

Human esophageal cancer cells Eca109 were obtained from the Cancer Institution of the Fourth Hospital of Hebei Medical University, Shijiazhuang City, Hebei Province, China. Human esophageal cancer cells Ec9706 were obtained from Molecular Oncology State Key Laboratory, Cancer Institute and Hospital, Chinese Academy of Medical Sciences, Beijing, China. The Eca109/ABCG2 cell line was established by transfecting the *ABCG2* gene into Eca109 cells [9].

Cells were cultured in RPMI1640 medium supplemented with 10%

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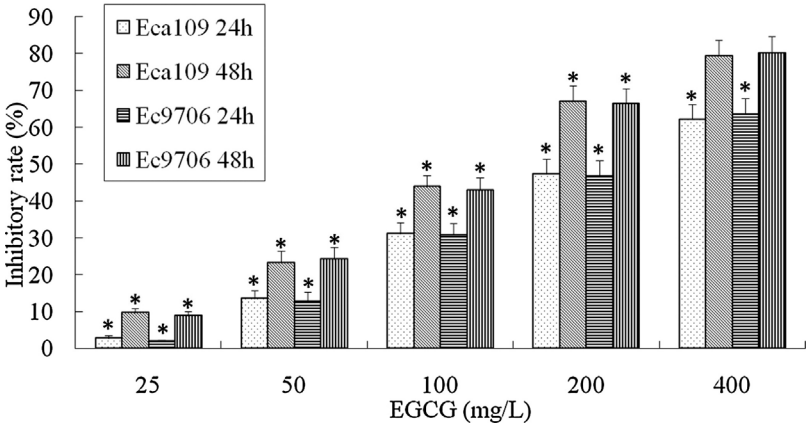
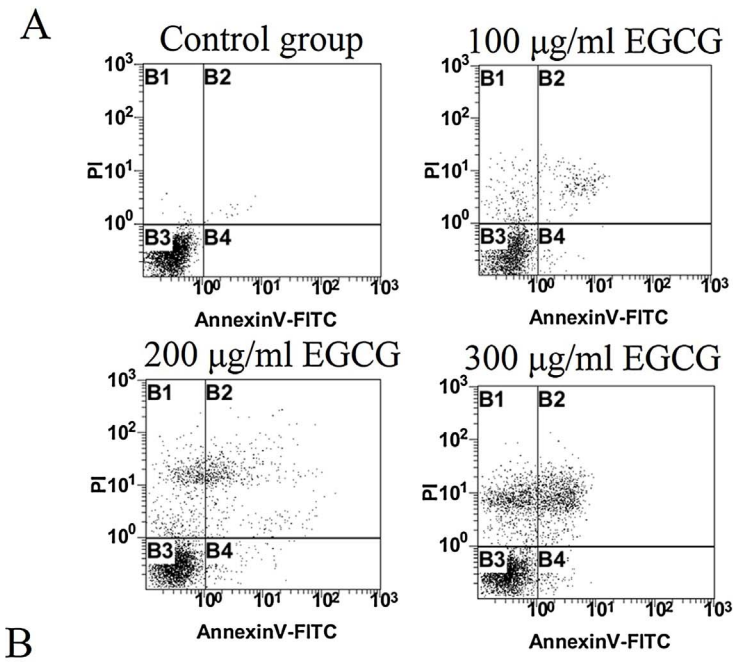


Fig. 1. Inhibitory effect of EGCG in Eca109 and Ec9706 cells. The growth of Eca109 and Ec9706 cells was significantly inhibited by EGCG in a dose- and time-dependent manner. *$P < 0.05$, compared with 400 μg/ml EGCG in the 48 h group. EGCG: epigallocatechin-3- gallate.



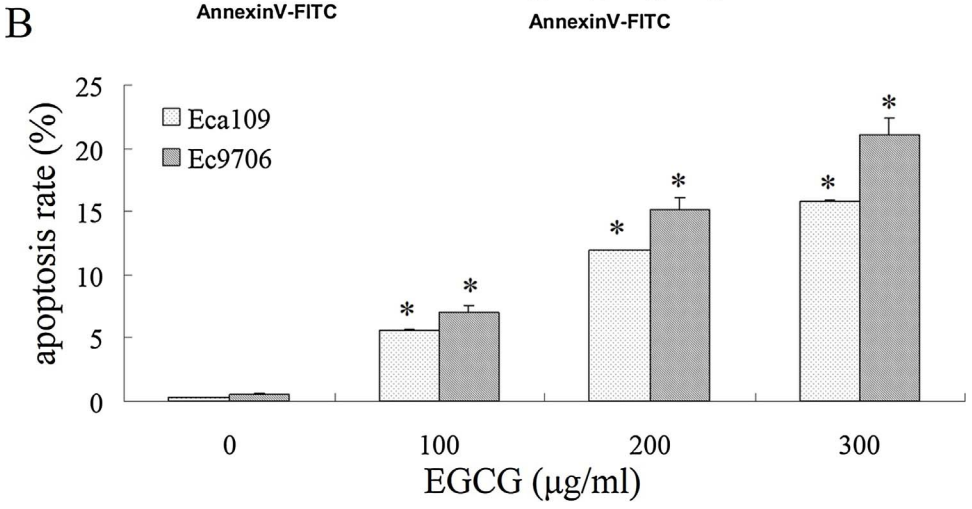


Fig. 2. Apoptosis of Eca109 and Ec9706 cells induced by treatment with various concentrations of EGCG. A, Apoptosis in Ec9706 cells after treatment with EGCG for 24 h was detected by flow cytometry. B, The rate of apoptosis in Eca109 and Ec9706 cells treated with EGCG (100, 200, 300 μg/ml) was significantly higher than that in the control group ($P < 0.01$). The percentages of apoptotic in Eca109 and Ec9706 cells increased with increasing EGCG concentrations. *$P < 0.01$, compared with control group. Normal saline (NS) instead of 0 μg/ml EGCG was used as control group. EGCG,:epigallocatechin-3- gallate.

fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin at 37 °C in a humidified atmosphere of 5% CO_2.

2.2. Chemicals and reagents

EGCG was purchased from Sigma-Aldrich (St. Louis, MO, USA). Annexin V-FITC/PI kit was purchased from Beckman Coulter (Miami, FL, USA); mouse anti-human caspase-3 monoclonal antibody was supplied by Santa Cruz Biotechnology Inc (Santa Cruz, CA, USA); and fluorescein isothiocyanate (FITC)-conjugated ABCG2 antibodies were obtained from Biolegend (San Diego, CA, USA).

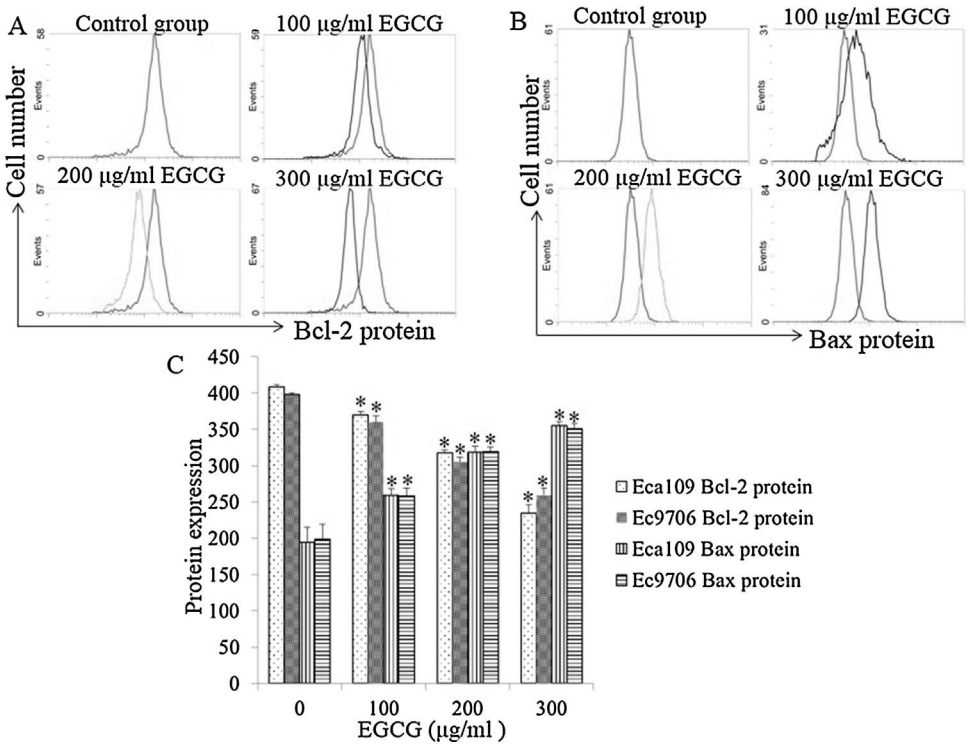


Fig. 3. Bcl-2 and bax protein expression of Eca109 and Ec9706 cells after EGCG treatment. A, B, Bcl-2 and bax protein expression of Ec9706 cells after treatment with various concentrations of EGCG was determined by flow cytometry. C, Bcl-2 protein expression in the EGCG group was significantly lower than that in the control group ($P < 0.01$). Bax protein expression in the EGCG group was significantly higher than that in the control group ($P < 0.01$). *$P < 0.01$, compared with control group. Normal saline (NS) instead of 0 μg/ml EGCG was used as control group. EGCG: epigallocatechin-3- gallate.

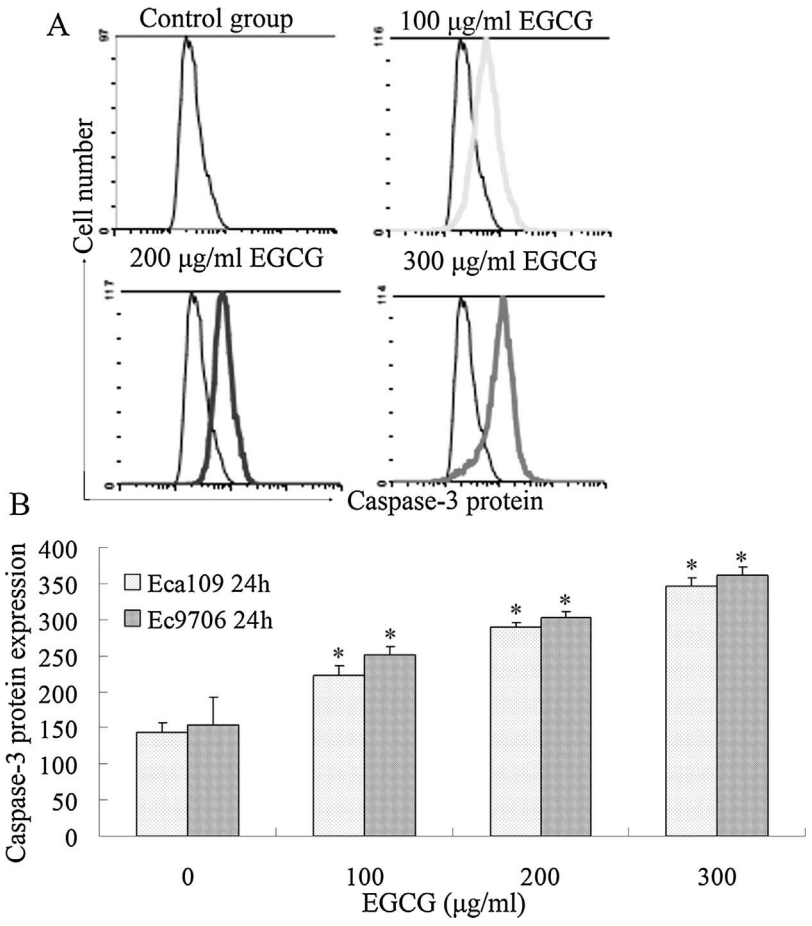


Fig. 4. Caspase-3 protein expression of Eca109 and Ec9706 cells after EGCG treatment. A, Caspase-3 protein expression of Ec9706 cells after treatment with various concentrations of EGCG was determined by flow cytometry. B, Caspase-3 protein expression in the EGCG group was significantly higher than in the control group ($P < 0.01$). *$P < 0.01$, compared with control group. Normal saline (NS) instead of 0 μg/ml EGCG was used as control group. EGCG: epigallocatechin-3- gallate.

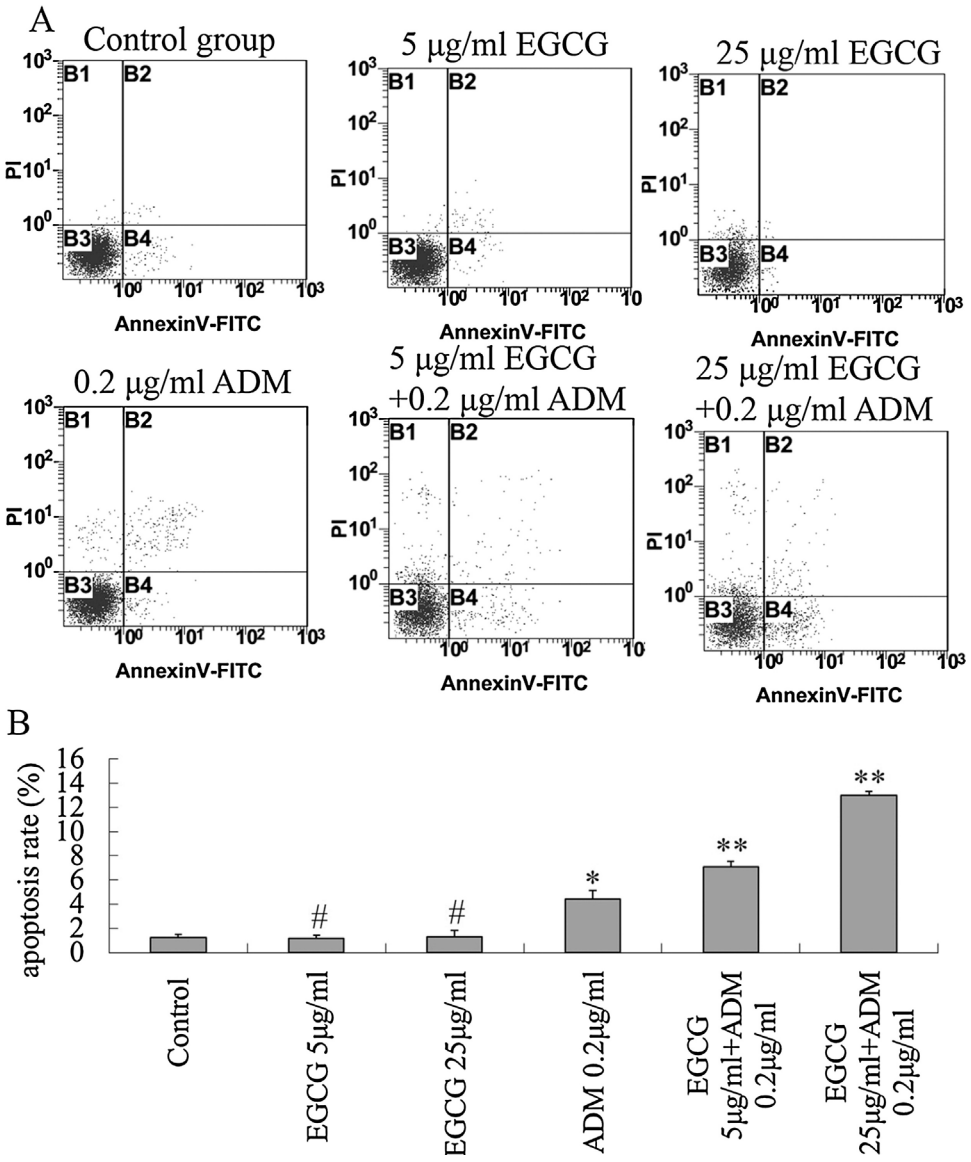


Fig. 5. EGCG enhanced the apoptosis of Eca109/ABCG2 cells induced by ADM. A, Cellular apoptosis was detected by flow cytometry after treatment with different concentrations of ADM and EGCG for 24 h. B, Apoptosis in Eca109/ABCG2 cells was significantly higher after treatment with 25 μg/ml EGCG combined with 0.2 μg/ml ADM than in the other groups ($P < 0.01$). *$P < 0.05$, compared with control group, **$P < 0.01$, compared with control group and 0.2 μg/ml ADM group, #$P > 0.05$, compared with control group. NS served as control group. EGCG,:epigallocatechin-3-gallate.

2.3. Cytotoxicity assay

The sensitivity of Eca109 and Ec9706 cells to EGCG was determined using the MTT assay. It is based on the capacity of viable cells to metabolize (via mitochondrial succinate dehydrogenase) a yellow tetrazolium salt (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide, MTT) to purple formazan crystals, dissolved in acidified propan-2-ol. The resulting purple solution was spectrophotometrically measured at 490 nm. Cells were seeded into 96-well culture plates at a density of 5×10^4 cells/ml. Serial concentrations of EGCG (0, 25, 50, 100, 200, and 400 μg/ml) were added to a final volume of 200 μl per well. After drug treatment for 24 h and 48 h, the medium was replaced with an equal volume of fresh 0.5 mg/ml MTT and incubated for 4 h. The medium was replaced with 180 μl of DMSO and incubated for 10 min at room temperature. The cytotoxic effects of drugs were determined according to the OD values using a microplate reader at 490 nm. Cell viability was expressed as the relative synthesis of formazan in treated samples when compared with control cells [(A490 treated cells/A490 control cells) × 100%]. Normal saline (NS) instead of 0 μg/ml EGCG was used as control group.

2.4. Analysis of cell apoptosis

Cultured tumor cells (Eca109 and Ec9706 cells) treated with various concentration of EGCG (0, 100, 200, and 300 μg/ml) for 24 h were harvested routinely. Normal saline (NS) instead of 0 μg/ml EGCG was used as control group.

Eca109/ABCG2 cells (esophageal cancer multidrug resistance cell lines) were treated with normal saline (NS), 5, 25 μg/ml EGCG, and 0.2 μg/ml ADM, (5, 25 μg/ml EGCG + 0.2 μg/ml ADM) for 24 h and cells were harvested routinely. NS served as control group.

The cells were stained with PI and annexin V-fluorescein isothiocyanate (FITC), and analyzed with Beckman Coulter Epics-XL type flow cytometer (Beckman Coulter, Miami, FL, USA). Early apoptotic cells tested positive for annexin V and negative for PI staining, whereas late apoptotic cells undergoing secondary necrosis were positive for both annexin V and PI staining.

2.5. Analysis of mitochondrial membrane potential expression in esophageal cancer cells

The Eca109/ABCG2 cells were treated with a mixture of EGCG (5 and 25 μg/ml) and ADM (0.2 μg/ml), or normal saline (NS) for 24 h.

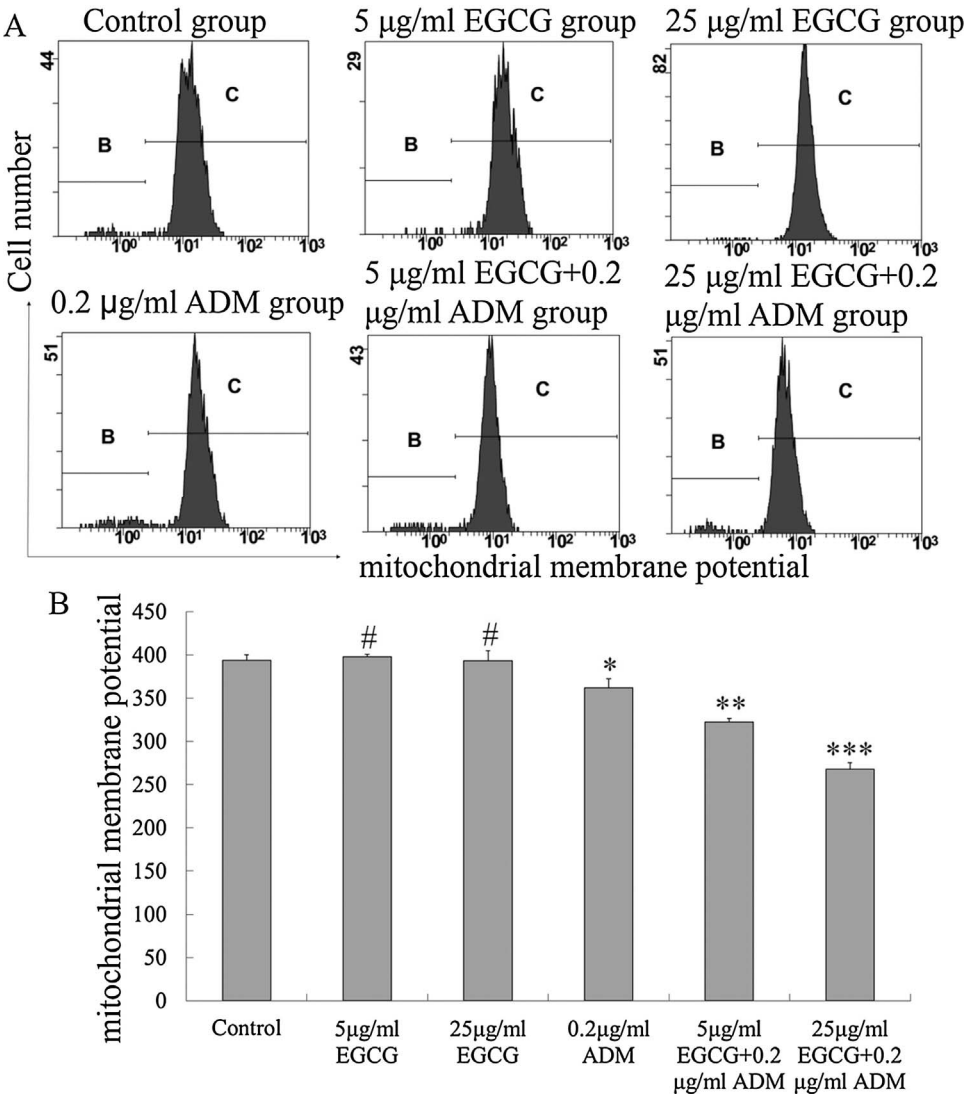


Fig. 6. EGCG modulates mitochondrial membrane potential in Eca109/ABCG2 cells. A, The mitochondrial membrane potential was detected by flow cytometry after treatment with different concentrations of ADM and EGCG for 24 h. B, The mitochondrial membrane potential in cells exposed to 25 μg/ml EGCG combined with 0.2 μg/ml ADM group was significantly lower than that in the other group ($P < 0.01$). #$P > 0.05$, compared with control group, *$P < 0.01$, compared with control group, **$P < 0.05$, compared with 0.2 μg/ml ADM group, ***$P < 0.01$, compared with 0.2 μg/ml ADM group. NS served as control group. EGCG,:epigallocatechin-3- gallate.

Cells were harvested routinely. NS served as control group.

After washing with ice-cold PBS twice, the cells were dyed with 1 ml of flourescence liquor containing 10 μg/ml of Rhodamine 123. Following incubation for 30 min in the dark at 37 °C and washing twice with ice-cold PBS, the stained cells were re-suspended in 1 ml of PBS. The stained cells were analyzed using Beckman Coulter Epics-XL type flow cytometer (Beckman Coulter, Miami, FL, USA).

2.6. Analysis of caspase-3, bcl-2, bax and ABCG2 expression

Cultured tumor cells (Eca109 and Ec9706 cells) were harvested routinely after treatment with various concentrations of EGCG (0, 100, 200, and 300 μg/ml) for 24 h. Normal saline (NS) instead of 0 μg/ml EGCG was used as control group. Cells were fixed overnight with 70% ice-cold ethanol. After washing twice with ice-cold PBS, the fixed cells were re-suspended in 1 ml of PBS containing anti-caspase-3 antibody, anti-bcl-2 antibody, anti-bax antibody respectively, following incubation for 30 min in the dark at room temperature. After washing twice with PBS, cells were re-suspended in 1 ml of PBS containing antibody II (IgG-FITC) and incubated for 30 min in the dark at room temperature. After washing twice with PBS, cells were re-suspended in 1 ml of PBS. The stained cells were analyzed with Beckman Coulter Epics-XL type flow cytometer (Beckman Coulter, Miami, FL, USA).

Eca109/ABCG2 cells were treated with normal saline (NS), EGCG (5 and 25 μg/ml), and ADM (0.2 μg/ml) for 24 h and cells were harvested routinely. NS served as control group. The cells were harvested with trypsin-EDTA (1:20), washed with phosphate-buffered saline (PBS) and centrifuged for 5 min at 1200 × *g*. The FITC-conjugated anti-ABCG2 antibody was added and the cells were incubated at room temperature for 30 min in the dark. The labeled cells were washed in PBS, centrifuged for 5 min at 1200 × *g* and stored at 4° C until use. The stained cells were analyzed using Beckman Coulter Epics-XL type flow cytometer (Beckman Coulter, Miami, FL, USA).

The mean fluorescence intensity represents the expression of caspase-3, bcl-2, bax and ABCG2 protein.

2.7. Analysis of ADM concentration in Eca109/ABCG2 cells

Eca109/ABCG2 cells were treated with normal saline (NS), EGCG (5 and 25 μg/ml), and ADM (0.2 μg/ml) for 24 h, and cells were harvested routinely. NS served as control group. Cells were analyzed using Beckman Coulter Epics-XL type flow cytometer (Beckman Coulter, Miami, FL, USA). The mean fluorescence intensity represents the ADM concentration in Eca109/ABCG2 cells.

2.8. Statistical analysis

All the data were presented as means ± SD and were statistically analyzed using multiple ANOVA followed by Newman-Keuls method of post hoc comparison (SPSS software). $P < 0.05$ was considered

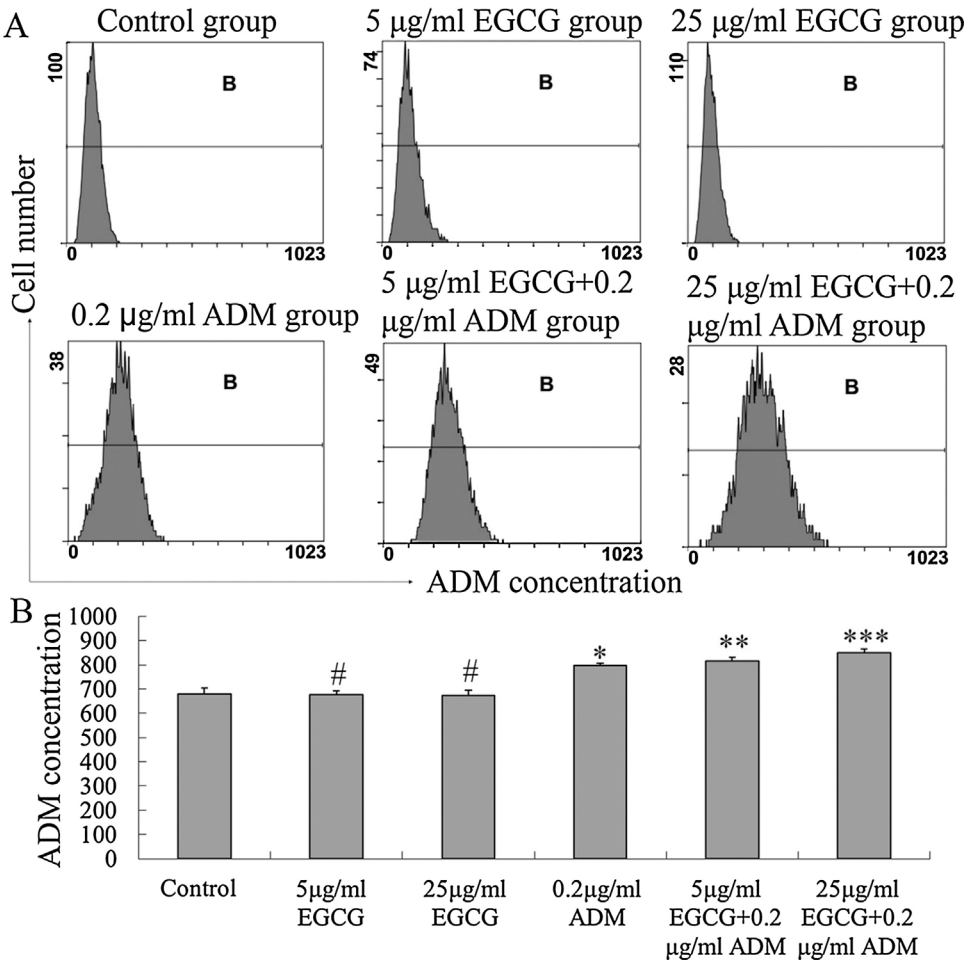


Fig. 7. ADM concentration in Eca109/ABCG2 cells after treatment with EGCG combined with ADM. A, ADM concentration in Eca109/ABCG2 cells was detected by flow cytometry after treatment with different concentrations of ADM and EGCG for 24 h. B, ADM concentration in Eca109/ABCG2 cells was significantly higher after treatment with 25 μg/ml EGCG combined with 0.2 μg/ml ADM than that in the other groups ($P < 0.01$). #$P > 0.05$, compared with control group, *$P < 0.01$, compared with control group, **$P < 0.05$, compared with 0.2 μg/ml ADM group, ***$P < 0.01$, compared with 0.2 μg/ml ADM group. NS served as control group. EGCG,:epigallocatechin-3- gallate.

significant difference.

3. Results

3.1. Inhibitory effect of EGCG on Eca109 and Ec9706 cells

The viability of Eca109 and Ec9706 cells treated with different concentrations of EGCG at a dose range of 25–400 μg/ml for 24 h and 48 h was determined using the MTT analysis. As shown in Fig. 1, the Eca109 and Ec9706 cell survival decreased in a dose- and time-dependent manner after treatment with different concentrations of EGCG ranging from 25 to 400 μg/ml for 24 h and 48 h (Fig. 1).

3.2. EGCG induces esophageal cancer cell apoptosis

Experiments were carried out with Eca109 and Ec9706 human esophageal cancer cells. EGCG exposure for 24 h, Eca109 and Ec9706 cells triggered apoptosis in a dose-dependent manner. In the range of 100–300 μg/ml, EGCG induced apoptosis, as determined by Annexin V/PI staining (Fig. 2).

3.3. EGCG increased the caspase-3 and bax expression and decreased the bcl-2 expression of Eca109 and Ec9706 cells

Eca109 and Ec9706 cells were treated with 0, 100, 200, and 300 μg/ml EGCG for 24 h, washed with cold PBS. Normal saline (NS) instead of 0 μg/ml EGCG was used as control group. The bcl-2, bax and caspase-3 protein expression was analyzed using flow cytometry. The bcl-2 protein expression was significantly down-regulated compared with that of the controls ($P < 0.01$) after treatment with various concentrations of EGCG for 24 h (Fig. 3). The bax protein expression was significantly up-regulated compared with that of the controls ($P < 0.01$) after treatment with various concentrations of EGCG for 24 h (Fig. 3). The caspase-3 protein expression was significantly up-regulated compared with that of the controls ($P < 0.05$) after treatment with various concentrations of EGCG for 24 h (Fig. 4).

3.4. EGCG enhances apoptosis of Eca109/ABCG2 cells induced by ADM

Eca109/ABCG2 cells showed no obvious apoptosis after treatment with 5 and 25 μg/ml EGCG for 24 h. The rate of apoptosis in the 5 and 25 μg/ml EGCG groups was not significantly different from that in control group, $P > 0.05$. The apoptosis in the 0.2 μg/ml ADM group was significantly higher than that in control group ($P < 0.05$). The apoptosis in the Eca109/ABCG2 cells was significantly higher after treatment with 5 and 25 μg/ml EGCG combined with 0.2 μg/ml ADM than that with 0.2 μg/ml ADM alone ($P < 0.01$). The apoptosis in Eca109/ABCG2 cells was significantly higher after treatment with 25 μg/ml EGCG combined with 0.2 μg/ml ADM than that with 5 μg/ml EGCG combined with 0.2 μg/ml ADM ($P < 0.01$) (Fig. 5).

3.5. EGCG modulates mitochondrial membrane potential in Eca109/ABCG2 cells

The mitochondrial membrane potential in the 5 and 25 μg/ml EGCG groups has no significant difference from that in control group, $P > 0.05$. The mitochondrial membrane potential of the 0.2 μg/ml ADM group was significantly lower than that in control group

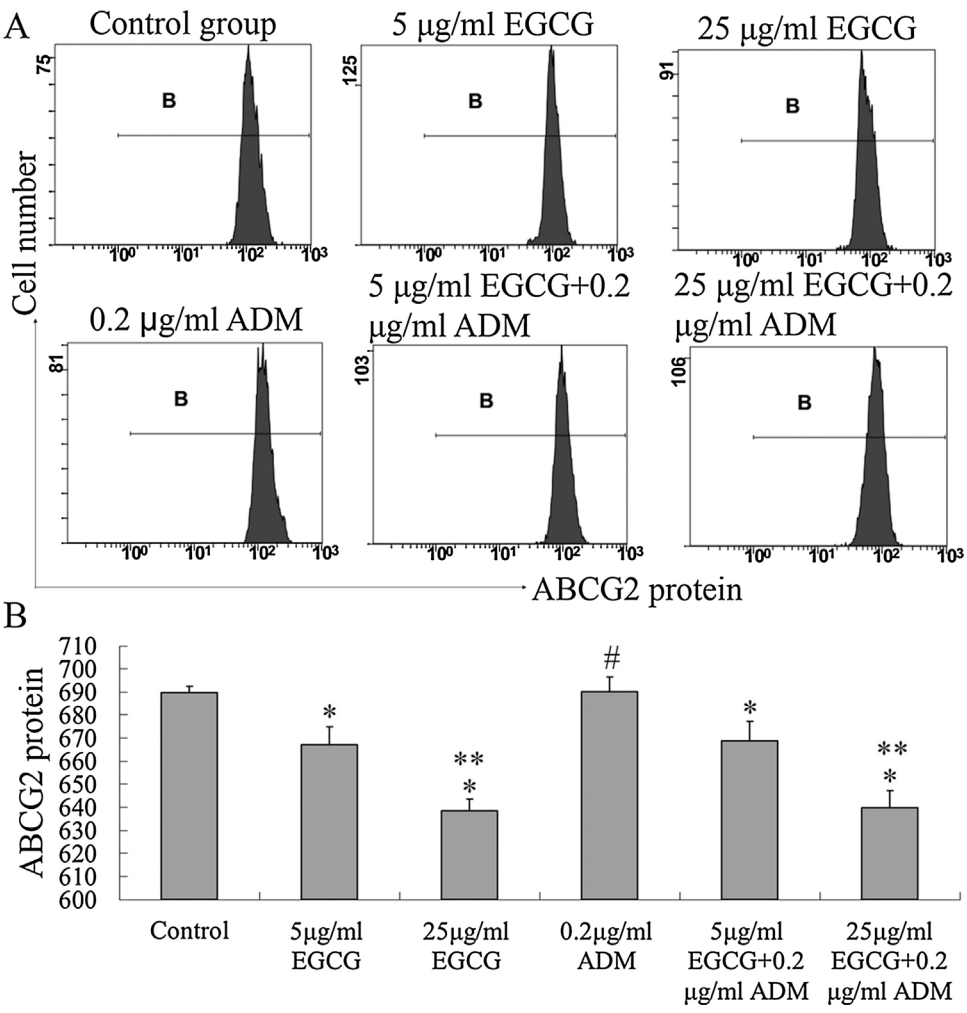


Fig. 8. EGCG modulates ABCG2 protein expression in Eca109/ABCG2 cells. A, ABCG2 protein expression in Eca109/ABCG2 cells was detected by flow cytometry after treatment with different concentrations of ADM and EGCG for 24 h. B, The ABCG2 expression in Eca109/ABCG2 cells of the 25 μg/ml EGCG and (25 μg/ml EGCG + 0.2 μg/ml ADM) groups was significantly lower than that in the other groups ($P < 0.01$). #$P > 0.05$, compared with control group, *$P < 0.01$, compared with control group, **$P < 0.01$, compared with 5 μg/ml EGCG and (5 μg/ml EGCG + 0.2 μg/ml ADM) group. NS served as control group. EGCG,:epigallocatechin-3-gallate.

($P < 0.01$). The mitochondrial membrane potential in cells exposed to 5 and 25 μg/ml EGCG combined with 0.2 μg/ml ADM group was significantly lower than that in the 0.2 μg/ml ADM group ($P < 0.05$). The mitochondrial membrane potential of cells exposed to the combination of 25 μg/ml EGCG and 0.2 μg/ml ADM group was significantly lower than that in the 5 μg/ml EGCG combined with 0.2 μg/ml ADM group ($P < 0.01$), as shown in Fig. 6.

3.6. ADM concentration in Eca109/ABCG2 cells after treatment with EGCG combined with ADM

The ADM concentration in Eca109/ABCG2 cells in the 5 and 25 μg/ml EGCG group was not significantly different from control group ($P > 0.05$). The ADM concentration in the Eca109/ABCG2 cells in the 0.2 μg/ml ADM group was significantly higher than that in control group ($P < 0.01$). The ADM concentration in Eca109/ABCG2 cells treated with 5 and 25 μg/ml EGCG combined with 0.2 μg/ml ADM group was significantly higher than that in the 0.2 μg/ml ADM group ($P < 0.05$). The ADM concentration in the Eca109/ABCG2 cells exposed to 25 μg/ml EGCG combined with 0.2 μg/ml ADM group was significantly higher than that in the group treated with a combination of 5 μg/ml EGCG and 0.2 μg/ml ADM ($P < 0.01$, Fig. 7).

3.7. EGCG modulates ABCG2 protein expression in Eca109/ABCG2 cells.

The ABCG2 expression in Eca109/ABCG2 cells of the 0.2 μg/ml ADM group was not significantly different from that in control group ($P > 0.05$). The ABCG2 expression in the Eca109/ABCG2 cells treated with 5 and 25 μg/ml EGCG, and the combination of 5, 25 μg/ml EGCG and 0.2 μg/ml ADM was significantly lower than that in control group ($P < 0.01$). The ABCG2 expression in Eca109/ABCG2 cells of the 25 μg/ml EGCG and (25 μg/ml EGCG + 0.2 μg/ml ADM) groups was significantly lower than that in the 5 μg/ml EGCG and the (5 μg/ml EGCG + 0.2 μg/ml ADM) groups ($P < 0.01$, Fig. 8).

4. Discussion

Epigallocatechin-3- gallate (EGCG) is the most abundant catechin in green tea, showing anti-inflammatory, antioxidant, immunomodulatory and anticancer activities [6,13,14,17,21]. Studies suggested that the potential health benefits of green tea consumption are attributed to the presence of EGCG. The anticancer activity of EGCG has been extensively explored in various tumor cells [8,22,29]. However, there were few studies demonstrating the role of EGCG in esophageal cancer. In our study, we demonstrated that EGCG suppressed the proliferation of esophageal cancer cells Eca109 and Ec9706 via apoptosis induced in a dose-dependent manner. EGCG induces apoptosis of cancer cells via modulation of apoptosis-related genes such as bcl-2, bax and caspase-3. It reduced cell proliferation by modulating cell cycle progression. The reversal of multidrug resistance in esophageal cancer cell line Eca109/ABCG2 by EGCG was also detected.

In this study, two esophageal cancer cell lines, Eca109 and Ec9706, were selected to test the potential effects of EGCG in esophageal squamous cell carcinoma. We investigated the tumor-suppressive effects of EGCG against esophageal cancer cells *in vitro*. MTT assay was adopted to investigate the inhibitory effect of EGCG on Eca109 and

Ec9706 cells. The results showed that EGCG inhibited the growth of Eca109 and Ec9706 cells in a dose- and time-dependent manner. However, the molecular mechanisms underlying EGCG-induced cell death in esophageal cancer cells were not fully clear. In the present study, we reported that EGCG exerted potent cytotoxic effects on human esophageal cancer cell lines Eca109 and Ec9706 *in vitro*. The cytotoxicity of EGCG was mediated by apoptosis, which was further supported by the detection and expression of apoptosis-related proteins. Apoptosis mediates cellular homeostasis. Therefore, dysfunction in apoptotic signaling may lead to serious conditions such as cancer.

Currently, apoptosis is the most studied mechanism in anticancer therapy. Cellular apoptosis is an automated gene-controlled death mediated via complex regulatory mechanisms. In the study, cellular apoptosis was detected with annexin V/PI staining using flow cytometry. Treatment with various concentrations of EGCG induced apoptosis of Eca109 and Ec9706 cells in a dose-dependent manner. Reduced cell proliferation is related to cellular apoptosis. Bcl-2, bax and caspase-3 that were essential effectors of the intrinsic pathway of apoptosis, were modulated by EGCG treatment. Bcl-2 protein expression in Eca109 and Ec9706 cells was down-regulated after EGCG treatment in a dose-dependent manner. Bax and caspase-3 protein expression in Eca109 and Ec9706 cells was up-regulated after EGCG treatment in a dose-dependent manner. In our study, EGCG induced the apoptosis of Eca109 and Ec9706 cells by down-regulating the bcl-2 protein expression and up-regulating the bax and caspase-3 expression.

Chemotherapy is the most common treatment for esophageal carcinoma, particularly for advanced esophageal cancer and recurrent cancer. However, the clinical response remains poor. Drug resistance is a major challenge in chemotherapy and is attributed to several processes that occur in neoplastic cells. For instance, decreased accumulation of anticancer drugs within the cancer cells due to drug efflux, is mediated by ABC transporters [1,15,19,28]. Overexpression of these transporters results in drug resistance in a number of tumors [7,12,18]. ABCG2 is a member of the ABC family and functions as an ABC discharge pump [10]. The Eca109/ABCG2 cell line established by transfecting *ABCG2* gene into Eca109 cells is an esophageal cancer multidrug resistance cell line. The Eca109/ABCG2 cells showed elevated expression of *ABCG2* gene and protein compared with the Eca109 cells [9]. In our study, The reversal of multidrug resistance in esophageal cancer cell line Eca109/ABCG2 by EGCG was detected. The rate of apoptosis in the Eca109/ABCG2 cells induced by ADM was enhanced by combining with EGCG. The EGCG concentration (5 and 25 μg/ml) used in the study was not adequate to induce apoptosis. EGCG reduced the level of ABCG2 expression and inhibited the ADM efflux of the Eca109/ABCG2 cells. The ADM concentration in Eca109/ABCG2 cells was increased after treatment with a combination of ADM and EGCG. Increasing the ADM concentration in Eca109/ABCG2 cells enhances the apoptosis of Eca109/ABCG2 cells.

In summary, the study explored the specific mechanisms of inhibition and reversal of multidrug resistance in esophageal cancer cells by EGCG. EGCG induced esophageal cancer cell apoptosis, reduced the bcl-2 protein expression and increased the bax and caspase-3 expression level. EGCG reversed the drug resistance by reducing *ABCG2* expression and increasing the anticancer drug concentration in the cancer cells. EGCG is expected to be associated with low toxic side-effects and may be a highly effective anticancer agent decreasing resistance to chemotherapy clinically.

5. Conclusion

EGCG induced esophageal cancer cell apoptosis. It reduced the bcl-2 protein expression and increased the bax and caspase-3 protein expression. EGCG reversed multi-drug resistance by reducing ABCG2 expression and increasing the anticancer drug concentration in cancer cells.

Conflict of interest

The authors declared that they have no conflict of interest.

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