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Research Article

Epigallocatechin gallate and sulforaphane combination treatment induce apoptosis in paclitaxel-resistant ovarian cancer cells through hTERT and Bcl-2 down-regulation

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ABSTRACT

The cellular development of resistance to chemotherapy contributes to the high mortality noted in patients affected by ovarian cancer. Novel compounds that specifically target cellular drug resistance in ovarian cancer are therefore highly desired. Previous epidemiological studies indicate that consumption of green tea and cruciferous vegetables is inversely associated with occurrence of ovarian cancer. Therefore revealing the effects and mechanisms of major components of green tea (epigallocatechin gallate, EGCG) and cruciferous vegetables (sulforaphane, SFN) on ovarian cancer cells will provide necessary knowledge for developing potential novel treatments for the disease. In this study, EGCG or SFN was used to treat both paclitaxel-sensitive (SKOV3-ip1) and -resistant (SKOV3TR-ip2) ovarian cancer cell lines alone or in combination. We found that SFN inhibits cell viability of both ovarian cancer cell lines time- and dose-dependently and that EGCG potentiates the inhibiting effect of SFN on ovarian cancer cells. Cell cycle analysis indicates SFN can arrest ovarian cancer cells in G2/M phase, while EGCG and SFN co-treatment can arrest cells in both G2/M and S phase. Combined EGCG and SFN treatment increases apoptosis significantly in paclitaxel-resistant SKOV3TR-ip2 cells after 6 days of treatment, while reducing the expression of hTERT, the main regulatory subunit of telomerase. Western blotting also indicates that SFN can down-regulate Bcl-2 (a gene involved in anti-apoptosis) protein levels in both cell types. Cleaved poly(ADP-ribose) polymerase (PARP) becomes up-regulated by 6 days of treatment with SFN and this is more pronounced for combination treatment indicating induction of apoptosis. Furthermore, phosphorylated H2AX is up-regulated after 6 days of treatment with SFN alone, and EGCG can potentiate this effect,

Abbreviations: EGCG, epigallocatechin gallate; SFN, sulforaphane; PARP, poly(ADP-ribose) polymerase; HDAC, histone deacetylase; DMSO, dimethyl sulfoxide; DNMT1, DNA methyltransferase 1; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PI, propidium iodide; PCR, polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay

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suggesting that DNA damage is a potential cellular mechanism contributing to the inhibiting effect of EGCG and SFN combination treatment. Taken together, these results indicate that EGCG and SFN combination treatment can induce apoptosis by down-regulating of hTERT and Bcl-2 and promote DNA damage response specifically in paclitaxel-resistant ovarian cancer cell lines and suggest the use of these compounds for overcoming paclitaxel resistance in ovarian cancer treatment.

Introduction

Ovarian cancer is the leading cause of death from gynecologic cancers in the United States and is the fifth leading cause of cancer death among American women. Most ovarian cancer patients are diagnosed at advanced stages due to the lack of effective screening strategies and specific symptoms associated with early stages. Currently, the preferred treatment is surgical excision followed by platinum/taxane combination chemotherapy. Although most ovarian cancers respond to first-line chemotherapy, recurrence occurs in up to 75% of ovarian cancer patients, most of whom will ultimately succumb to their disease [1]. Thus, novel therapies that can reverse drug resistance or kill drug resistant ovarian cancer cells directly are highly desired.

Green tea is the most popular beverage next to water worldwide. Epidemiological studies have revealed an inverse correlation between the dietary intake of green tea and the risk for certain types of cancers, including ovarian cancer [2–4]. Epigallocatechin gallate (EGCG), as a major component of green tea, is generally accepted to be the most effective constitute that contributes to the anti-cancer effect of green tea. It has been reported that EGCG can induce apoptosis in breast cancer cells [5], and EGCG can target cancer cells through a variety of mechanisms, including decreasing expression of hTERT, the major catalytic subunit of telomerase [6].

Additionally, consumption of cruciferous vegetables such as broccoli, Brussels sprouts or cabbage has also been linked with low occurrence of lung, stomach, colon, rectal, prostate, endometrial and ovarian cancer [7]. Sulforaphane (SFN), as the major component of these vegetables, has received considerable attention in the past due to its anti-cancer effect in numerous cancer cells including ovarian cancer. Mechanistic studies reveal that SFN can target cancer cells through Nrf2-mediated induction of phase 2 detoxification enzymes which can elevate the cellular defense against oxidative damage and promote the removal of carcinogens [8]. SFN has also been observed to suppress cytochrome P450 enzymes [9], induce apoptotic pathways [10], suppress cell cycle progression [10], and inhibit angiogenesis and inflammatory response [11,12]. More recently, SFN has been shown to inhibit histone deacetylase (HDAC) activity leading to reactivation of tumor suppressor genes and silencing of oncogenes [13].

Late stages of ovarian cancer are characterized by resistance to conventional platinum based chemotherapy as aforementioned. Although drug resistance can be mediated by individual genes, such as overexpression of ABC transporters or enhanced DNA repair genes, or down-regulation of apoptosis-promoting genes, alteration in multiple pathways concurrently are commonly observed. Treatments that can target multiple pathways involved in drug resistance or can target multiple intact cancer cell survival pathways are promising strategies. Combination treatment with different compounds that target several different pathways is therefore a promising direction. One frequent obstacle to this approach, however, is increased toxicity that accompanies targeting multiple genes. Therefore drugs that have minimal side effects are of course preferred. Although the anti-cancer effects of EGCG or SFN alone are well known, and both extracts are well-tolerated, it is not clear whether the combination of these two compounds can exert a stronger anti-cancer effect. Additionally, although several studies have attempted to investigate the effect of EGCG and SFN on ovarian cancer cells, the doses in those studies were too high to be reached physiologically [14]. Therefore revealing the effects and mechanisms of EGCG and SFN on ovarian cancer within a reasonable dose range will provide critical knowledge for developing potential novel treatments for the disease.

The present study was undertaken to evaluate the effect of EGCG and SFN combination treatment on ovarian cancer cells, especially paclitaxel-resistant ovarian cancer cells, and to elucidate the potential mechanisms responsible for the effect. Our results indicate that SFN with EGCG can inhibit both sensitive and resistant ovarian cancer cells while exerting a much stronger inhibiting effect on the paclitaxel-resistant ovarian cancer cells. Mechanistic studies reveal that EGCG and SFN combination treatment can cause DNA damage and decrease hTERT (a protein involved in cancer cell survival) and Bcl-2 (a protein involved in anti-apoptosis) expression in these ovarian cancer cells. These findings reveal for the first time that EGCG and SFN combination treatment can inhibit ovarian cancer cells by creating DNA damage through decreasing hTERT and Bcl-2 expression.

Materials and methods

Cell culture

SKOV3-ip1 and SKOV3TR-ip2 cells were grown in RPMI 1640 medium (Mediatech Inc, Manassas, VA) supplemented with 10% fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA) and 1% penicillin/streptomycin (Mediatech). SKOV3TR-ip2 cells were maintained with the addition of 150 ng/mL of paclitaxel [15]. EGCG (Sigma) or *R,S*-sulforaphane (LKT Laboratories, Minneapolis, MN) was dissolved in DMSO and stored at a stock concentration of 100 mM/L at −20 °C. After seeding the cells and culturing for 24 h, EGCG and/or SFN was added to the culture medium at indicated concentrations and the maximum concentration of DMSO was 0.1% (v/v) in the medium. Cells treated only with DMSO served as a vehicle control. Cells were treated with fresh EGCG and/or SFN every 24 h.