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Inhibitory effect of Epigallocatechin gallate on ovarian cancer cell proliferation associated with aquaporin 5 expression

Chunxiao Yan · Jianhua Yang · Lan Shen · Xuejun Chen

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Abstract
Objective Abnormal expression of aquaporin 5 (AQP5) is associated with ovarian cancer infiltration, metastasis and angiogenesis. AQP 5 expression and apoptosis have been shown to be closely related to nuclear transcription factor NF-κB. In this study, we investigated the inhibition of cell proliferation and the induction of apoptosis by Epigallocatechin gallate (EGCG), a potential anti-cancer drug, in the ovarian cancer cell line SKOV3 as well as the effect of EGCG on AQP5 expression and its possible mechanisms.
Methods SKOV3 cells were treated with different concentrations of EGCG and the NF-κB-specific inhibitor pyrrolidine dithiocarbamate (PDTC) for different times. Cell proliferation was determined using the MTT assay, cell apoptosis was evaluated using the DNA ladder assay, the expression of AQP5, NF-κB p65 and IκBα was detected by immunohistochemistry, western blot analysis and RT-PCR, and the correlation of these protein expression was analyzed.
Results With increasing concentrations of EGCG and prolonged treatment times, the growth inhibition rate of SKOV3 cells gradually increased in a dose- and time-dependent manner. The expression of AQP5 and nuclear p65 and IκBα was significantly decreased ($P < 0.01$). The cytoplasmic expression of IκBα gradually increased ($P < 0.05$), and the apoptosis of SKOV3 cells was induced as evidenced by typical fragmentation pattern in a DNA ladder assay. With increasing concentrations of PDTC and prolonged treatment times, the protein and mRNA levels of AQP5 in SKOV3 cells decreased ($P < 0.01$). In addition, the growth inhibition rate of SKOV3 cells significantly increased in a dose- and time-dependent manner.
Conclusions EGCG inhibited the proliferation and induced the apoptosis of ovarian cancer SKOV3 cells. EGCG also down-regulated expression of AQP5, which may inhibit tumor growth and be associated with nuclear transcription factor NF-κB.

Keywords EGCG · Ovarian cancer · Aquaporin 5 · Cell proliferation · NF-κB

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Introduction

Tea is a popular beverage worldwide, ranking only second to water. Tea polyphenols are extracted from green tea, and their main active ingredient is Epigallocatechin gallate (EGCG). EGCG has many biological activities and pharmacological effects, such as anti-tumor, anti-mutagenic, anti-inflammation and free radical scavenging and antioxidant activities [1]. EGCG has been reported to have anti-cancer biological activity as well; the main mechanism of this activity involves the inhibition of cell proliferation and the induction of apoptosis [2]. EGCG can inhibit the proliferation of cells from skin cancer, lung cancer, oral cancer, gastric cancer, intestinal cancer, colon cancer, hepatocellular carcinoma, pancreatic cancer, rectal cancer, prostate cancer and breast cancer, suggesting that EGCG could be utilized as a potential anti-cancer drug [3–8].

Due to rapid proliferation and division, invasion into the surrounding matrix and vascular access, cancer cells require rapid transmembrane transport of water molecules. Aquaporins (AQPs) are a class of water-specific transmembrane transporter proteins. Aquaporin expression was previously reported to be associated with cancer cell metastasis and angiogenesis and, therefore, a potential drug target of cancer therapy [9–11]. AQP5 protein expression is associated with the carcinogenesis and development of ovarian cancer as well as ascites [12], whereas aquaporin was reported to be regulated by nuclear factor-κB (NF-κB) [13, 14].

NF-κB was identified as immunoglobulin κ light chain gene enhancer-specific binding nuclear factor in lymphocytes by Sen and Bahimore [15]. NF-κB is ubiquitously expressed in eukaryotes as a protein family that consists of complex polypeptide subunits. As the hub of many signal transduction pathways, it is closely related to immunity, tumor occurrence and development, apoptosis regulation and embryonic development [16]. Studies have indicated that the inhibition of the nuclear translocation of NF-κB is expected to be a new target for cancer therapy [17].

In this study, we investigated the ability of EGCG to inhibit the proliferation and induce apoptosis in the ovarian cancer cell line SKOV3, and the effect of EGCG and the NF-κB-specific inhibitor pyrrolidine dithiocarbamate (PDTC) on the expression of NF-κB, IκBα and AQP5 in SKOV3 cells. Finally, we explored the potential role and mechanism of the effect of EGCG in ovarian cancer cells.

Materials and methods

Materials

The human ovarian cancer cell line SKOV3 was purchased from ATCC. EGCG (purity ≥ 95%), PDTC (purity ≥ 99%), RNaseA and proteinase K were purchased from Sigma. A goat anti-human AQP5 polyclonal antibody (sc-9890), mouse anti-human NF-κBp65 monoclonal antibody (sc-8000), rabbit anti-IκBα polyclonal antibody (sc-371), and goat anti-human Actin polyclonal antibody (sc-1616) were purchased from Santa Cruz Biotechnology. A rabbit anti-human Histone H2A.X polyclonal antibody (Ab-139) was purchased from SAB. NE-PER Nuclear and Cytoplasmic Extraction Reagents were purchased from Pierce Biotechnology. A TRIzol kit was purchased from Bio Basic Inc. Moloney murine leukemia virus (M-MuLV) reverse transcriptase was purchased from Fermentas. Primers for AQP5 and β-Actin were synthesized by Sangon, Shanghai. TaKaRa TaqTM was purchased from TaKaRa Biotechnology. An Immunohistochemistry SP Kit was purchased from Beijing Golden Bridge Biotechnology Co., Ltd.

Methods

Cell culture and measurement of cell growth inhibition rate

SKOV3 cells were cultured in RPMI-1640 medium containing 15% FBS in a 5% CO_2 incubator at 37°C. The inhibition of SKOV3 cell growth was examined by MTT assay. SKOV3 cells in logarithmic growth phase were prepared at a density of 1×10^4/ml and inoculated into a 96-well plate. Cells were either treated with various concentrations of EGCG (20, 40, 60, 80 or 100 μg/ml) or PDTC (0.1, 1 or 10 mM) for 24 h or treated for varying time periods with 40 μg/ml EGCG for 6, 12, 24, 48 or 72 h or treated with 1 mmol PDTC for 12, 24 or 48 h. The absorbance (A_{570}) was then measured with a microplate reader. Cell growth inhibition rate = ($1 - A_{570}$ value of experimental group/A_{570} value of control group) × 100%. Cells in the control group were treated with normal RPMI-1640 medium. All experiments were performed in triplicate.

DNA ladder assay

After treatment with various concentrations of EGCG for 24 h or treatment with 40 μg/ml EGCG for the indicated times, 1×10^7 cells were collected by centrifugation, lysed in lysis buffer (10.0 mM Tris–HCl pH 8.0, 10.0 mM NaCl, 10.0 mM EDTA, 100 μg/ml proteinase K) and then centrifuged at 12,000 rpm for 10 min. The supernatants were extracted with an equal volume of phenol/chloroform (1:1), followed by phenol/chloroform/isoamyl alcohol (15:24:1) and chloroform extraction. The supernatants were ethanol precipitated by adding 1/10 volume of 3 M NaOAc and two volumes of ice-cold ethanol at −20°C overnight. The samples were then centrifuged at 12,000 rpm for 10 min at −10°C. The precipitates were dissolved in 20 μl TE buffer containing 1 μl RNase (10 mg/ml) at 37°C for 1 h. These samples were mixed with 4 μl loading buffer, electrophoresed in a 1.8% agarose gel for 40 min and observed under ultraviolet light.

Immunohistochemistry SP assay

SKOV3 cells in logarithmic growth phase were prepared at a density of 1×10^4/ml and inoculated into a 24-well plate with cover slips inside each well. After treatment with 40 μg/ml EGCG, cells were fixed in 4% paraformaldehyde for 30 min for H&E staining and immunohistochemistry. Cells were incubated with goat anti-AQP5, mouse anti-NF-κB p65 or rabbit anti-IκBα (1:50 dilution) at 4°C overnight, followed by staining with 3,3′-diaminobenzidine (DAB) and counterstaining with hematoxylin.

Western blotting

SKOV3 cells in logarithmic growth phase were prepared at a density of 1×10^5/ml and then inoculated into a 50-ml culture flask. Cells were treated with various concentrations of EGCG for the indicated times. After treatment, cells were collected, and the cytoplasmic and nuclear proteins were extracted with NE-PER Nuclear and Cytoplasmic Extraction Reagents according to the manufacturer's instructions. The proteins (50 μg) were electrophoresed, transferred to a membrane and then incubated with goat anti-AQP5 (1:1000 dilution), mouse anti-NF-κBp65 (1:1,000 dilution), rabbit anti-IκBα (1:1,000 dilution), goat anti-β-Actin (1:500 dilution) or rabbit anti-Histone H2A.X (1:500 dilution) at 4°C overnight, followed by incubation with HRP-labeled secondary antibodies (1:5,000 dilution) for 1 h at room temperature. The blots were visualized using a chemiluminescent substrate. Band gray values were measured using Quantity One (Bio-Rad, USA). β-Actin was used as an internal control for cytoplasmic protein, and Histone H2A.X was used as an internal control for nuclear protein.

RT-PCR

Total tissue mRNA was extracted using TRIzol reagent and 5 μg of total RNA was used for reverse transcription (M-MuLV reverse transcriptase, Fermentas) in a 40 μl reaction volume. β-Actin was used as an internal control for the PCR amplification of AQP5 cDNA. AQP5 primer sequences were as follows: forward primer 5'-CTTCCT CAAGGCCGTGTTC-3' and reverse primer 5′-GCTGGA AGGTCAGAATCAGC-3′. The AQP5 PCR product was 398 bp, and the PCR conditions consisted of 35 amplification cycles of 95°C for 30 s, 62°C for 45 s and 72°C for 50 s. β-Actin primer sequences were as follows: forward primer 5′-GTGGGGCGCCCCAGGCACCA-3′ and reverse primer 5′-CTCCTTAATGTCACGCACGATTTC-3′. The β-Actin PCR product was 534 bp, and the PCR conditions consisted of 23 amplification cycles of 95°C for 30 s, 59°C for 40 s and 72°C for 45 s. The AQP5 and β-Actin PCR products were electrophoresed in a 2% agarose gel and imaged using a Kodak EDAS290 imaging system (Kodak). Band gray values were measured, and the ratio of AQP5/β-Actin was used as the relative abundance of AQP5 mRNA.

Statistical methods

SPSS 13.0 was used for the statistical analyses. All results are presented as the mean ± SD, and $P < 0.05$ was considered as significantly different. One-way ANOVA was used to compare average values (including post-hoc tests). Correlations were analyzed by Pearson correlation analysis.

Results

EGCG-induced apoptosis in SKOV3 cells and its effect on cell growth and AQP5, NF-κB p65 and IκBα expression

EGCG-induced inhibition of SKOV3 cell proliferation

SKOV3 cells were treated with different concentrations of EGCG and cultured for 24 h. With increasing concentrations of EGCG, the inhibition rate increased in a dose-dependent manner ($r = 0.972$, $P < 0.05$). When SKOV3 cells were treated with 40 μg/ml of EGCG for 24 h, the cell proliferation inhibition rate was 50.67 ± 2.40% (Fig. 1).

In addition, SKOV3 cells were treated with 40 μg/ml EGCG for different times. With prolonged treatment times, cell proliferation was inhibited in a time-dependent manner ($r = 0.967$, $P < 0.05$). When treated for 72 h, the cell proliferation inhibition rate was 89.67 ± 7.09% (Fig. 2).

EGCG-induced apoptosis in SKOV3 cells

SKOV3 cells were treated with various concentrations of EGCG for different times. With increasing concentrations and prolonged treatment times, apoptosis was significantly induced as evaluated by a typical DNA ladder assay, which revealed a DNA pattern consisting of fragments in multiples of 180–200 bp (Figs. 3, 4).

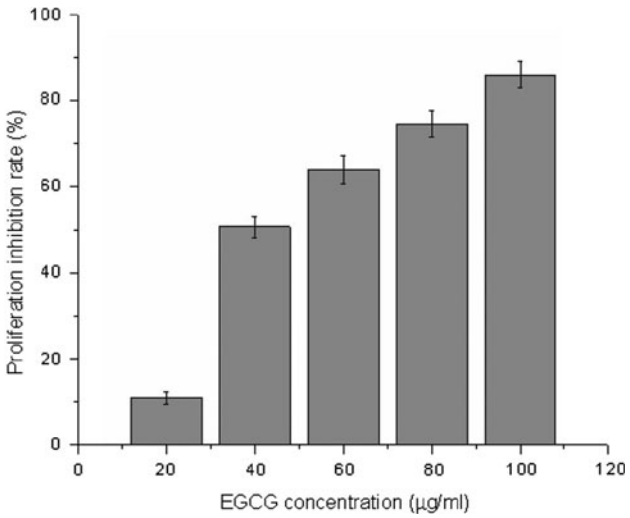

Fig. 1 Proliferation inhibition rates of SKOV3 cells treated with different concentrations of EGCG

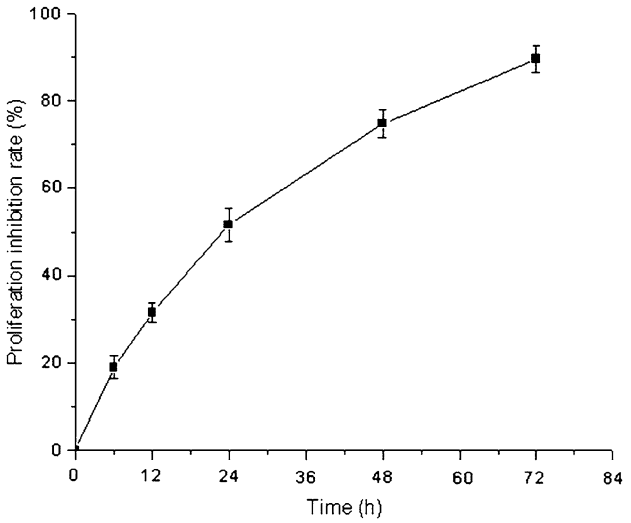

Fig. 2 Proliferation inhibition rates of SKOV3 cells treated with 40 μg/ml EGCG for different times

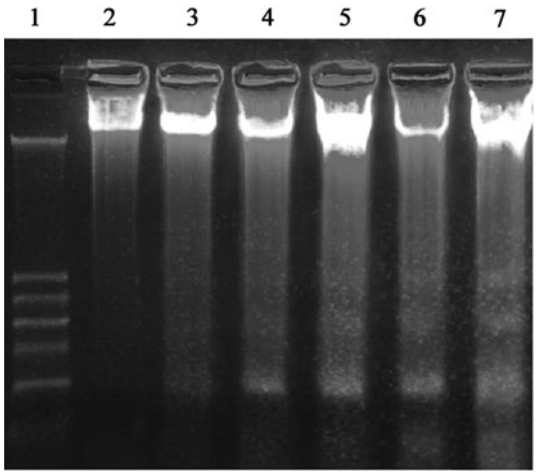

Fig. 3 DNA electrophoretogram of SKOV3 cells treated with different concentrations of EGCG (*lane 1* DNA maker, *lane 2* control, *lane 3–7* SKOV3 cells treated with 20, 40, 60, 80, 100 μg/ml EGCG, respectively)

Effect of EGCG on AQP5, NF-κB p65 and IκB expression in SKOV3 cells

Immunohistochemical staining showed that the AQP5 protein was expressed mainly in the membrane and cytoplasm of SKOV3 cells and that NF-κB p65 and IκBα were predominantly expressed in the cytoplasm. When the cells were treated with 40 μg/ml EGCG for 24 h, the expression of AQP5 in the cell membrane and cytoplasm was reduced. Furthermore, the expression of NF-κB p65 and IκBα also decreased (Fig. 5).

Western blot analysis showed that after incubation with different concentrations of EGCG for 24 h, the expression of AQP5 and nuclear NF-κB p65 and IκBα decreased in

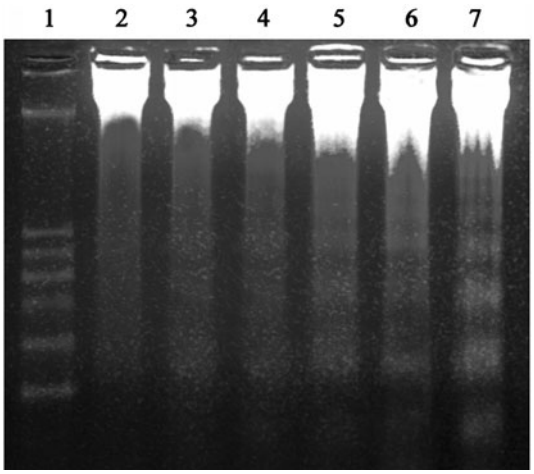

Fig. 4 DNA electrophoretogram of SKOV3 cells treated with 40 μg/ml EGCG for different times (*lane 1* DNA maker, *lane 2* control, *lane 3–7* SKOV3 cells treated with 40 μg/ml EGCG for 6, 12, 24, 48, 72 h, respectively)

SKOV3 cells as the concentrations of EGCG increased ($P < 0.01$). When treated with 100 μg/ml of EGCG, the expression of AQP5 decreased by 44.1%. At the same time, the cytoplasmic expression of IκBα gradually increased ($P < 0.05$), and the expression of NF-κB p65 did not change significantly ($P > 0.05$) (Table 1, Fig. 6).

Western blot analysis also showed that when SKOV3 cells were treated with 40 μg/ml of EGCG, the expression of AQP5 and nuclear NF-κB p65 and IκBα decreased with prolonged treatment times; this decrease was maintained up to 72 h ($P < 0.01$). When the cells were treated with EGCG for 72 h, AQP5 expression was inhibited by 30.9%. At the same time, the expression of cytoplasmic NF-κB p65 significantly decreased ($P < 0.05$), but the expression of IκBα gradually increased ($P < 0.01$) (Table 2, Fig. 7).

Effect of the nuclear transcription factor inhibitor PDTC on cell proliferation and the expression of AQP5, NF-κB p65 and IκB in SKOV3 cells

PDTC-induced inhibition of SKOV3 cell proliferation

SKOV3 cells were treated with different concentrations of PDTC for 24 h. With increasing concentrations, the inhibition rate increased in a dose-dependent manner ($r = 0.931$, $P < 0.05$). When SKOV3 cells were treated with 1 mM PDTC for 24 h, the cell proliferation inhibition rate was 40.00 ± 3.35% (Table 3).

In addition, SKOV3 cells were treated with 1 mM PDTC for different times. As treatment time was lengthened, cell proliferation was inhibited in a time-dependent manner ($r = 0.984$, $P < 0.05$). When treated for 48 h, the cell proliferation inhibition rate was 75.83 ± 5.86% (Table 4).

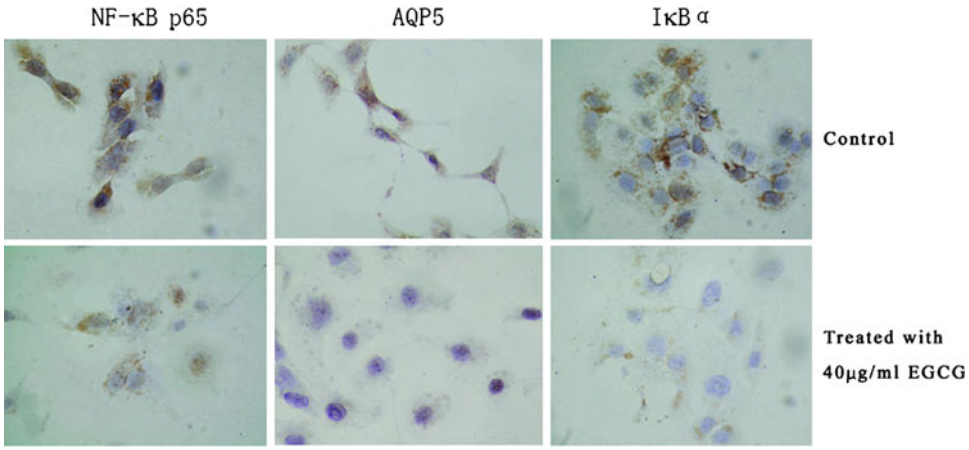

Fig. 5 Expression of AQP5, NF-κB p65 and IκBα in SKOV3 cells before and after treatment with 40 μg/ml EGCG (SP ×400)

Table 1 Expression of AQP5, NF-κB p65 and IκBα in SKOV3 cells treated with different concentrations of EGCG

Group	AQP5	NF-κB(p65) in cytoplasm	IκBα in cytoplasm	NF-κB(p65) in nuclei	IκBα in nuclei
Control	0.68 ± 0.03	1.82 ± 0.09	2.02 ± 0.15	0.81 ± 0.05	1.45 ± 0.03
EGCG 20 μg/ml	0.64 ± 0.03	1.77 ± 0.10	2.10 ± 0.20	0.79 ± 0.02	1.43 ± 0.02
EGCG 40 μg/ml	0.59 ± 0.02*	1.68 ± 0.10	2.28 ± 0.20	0.74 ± 0.03	1.38 ± 0.06
EGCG 60 μg/ml	0.56 ± 0.03*	1.58 ± 0.34	2.41 ± 0.19	0.69 ± 0.02*	1.33 ± 0.01*
EGCG 80 μg/ml	0.45 ± 0.04**	1.48 ± 0.01	2.54 ± 0.17	0.60 ± 0.01**	1.28 ± 0.03**
EGCG 100 μg/ml	0.38 ± 0.01**	1.38 ± 0.02	2.73 ± 0.15*	0.53 ± 0.02**	1.24 ± 0.04**

All values are presented as the mean ± SD of three experiments

* $P < 0.05$, vs. control. **$P < 0.01$, vs. control

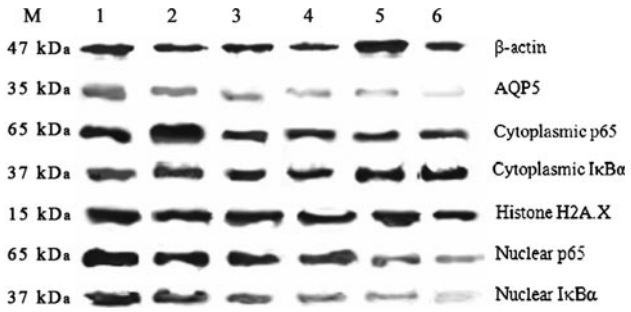

Fig. 6 Expression of AQP5, NF-κB p65 and IκBα in SKOV3 cells after treatment with different concentrations of EGCG as detected by Western blot (*Lane 1* control, *lane 2–6* SKOV3 cells treated with 20, 40, 60, 80, 100 μg/ml EGCG for 24 h, respectively)

Effect of PDTC on AQP5, NF-κB p65 and IκB expression in SKOV3 cells

Western blot and RT-PCR analyses showed that with increasing concentrations of PDTC (0, 0.1, 1 and 10 mM) and prolonged treatment times (0, 12, 24 and 48 h), both AQP5 protein and mRNA expression decreased in SKOV3 cells ($P < 0.01$) (Tables 5, 6; Figs. 8, 9, 10, 11).

The relationship among AQP5, cell proliferation of SKOV3, NF-κB p65 and IκBα

Pearson correlation analysis showed that [1] the EGCG-induced cell proliferation inhibition rate was negatively

Table 2 Expression of AQP5, NF-κB p65 and IκBα in SKOV3 cells treated with 40 μg/mL EGCG for different times

Group	AQP5	NF-κB(p65) in cytoplasm	IκBα in cytoplasm	NF-κB(p65) in nuclei	IκBα in nuclei
Control	0.55 ± 0.03	2.80 ± 0.14	1.63 ± 0.06	2.19 ± 0.06	3.23 ± 0.08
EGCG 6 h	0.52 ± 0.01	2.75 ± 0.11	1.67 ± 0.04	2.17 ± 0.05	3.19 ± 0.07
EGCG 12 h	0.50 ± 0.04	2.69 ± 0.14	1.70 ± 0.05	2.12 ± 0.03	3.13 ± 0.08
EGCG 24 h	0.45 ± 0.03*	2.64 ± 0.15	1.73 ± 0.02	2.06 ± 0.07	3.07 ± 0.09
EGCG 48 h	0.43 ± 0.02*	2.34 ± 0.16	1.81 ± 0.04*	1.95 ± 0.08*	2.95 ± 0.04*
EGCG 72 h	0.38 ± 0.03**	2.23 ± 0.18*	1.87 ± 0.03**	1.81 ± 0.11**	2.83 ± 0.03**

All values are presented as the mean ± SD of three experiments

* $P < 0.05$, vs. control. **$P < 0.01$, vs. control

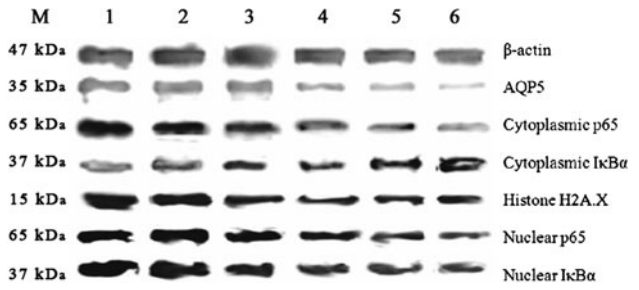

Fig. 7 Western blot analysis of the expression of AQP5, NF-κB p65 and IκBα in SKOV3 cells after treatment with 40 μg/ml EGCG (*Lane 1* control, *lane 2–6* SKOV3 cells treated with 40 μg/ml EGCG for 6, 12, 24, 48, 72 h, respectively)

Table 3 Proliferation inhibition rates of SKOV3 cells treated with different concentrations of PDTC

Group	A570	Proliferation inhibition rate (%)
Control	0.085 ± 0.004	0
PDTC		
0.1 mM	0.072 ± 0.005*	15.27 ± 2.35*
1 mM	0.051 ± 0.002**	40.00 ± 3.35**
10 mM	0.013 ± 0.001**	84.73 ± 6.71**

All values are presented as the mean ± SD of three experiments

* $P < 0.05$ vs. control. **$P < 0.01$ vs. control.

Table 4 Proliferation inhibition rates of SKOV3 cells treated with 1 mM PDTC for different times

Group	A570	Proliferation inhibition rate (%)
Control	0.091 ± 0.006	0
PDTC		
12 h	0.083 ± 0.004*	8.29 ± 2.21*
24 h	0.052 ± 0.002**	42.42 ± 4.37**
48 h	0.021 ± 0.001**	75.83 ± 5.86**

All values are presented as the mean ± SD of three experiments

* $P < 0.05$ vs. control. **$P < 0.01$ vs. control

Table 5 Expression of AQP5 protein and mRNA in SKOV3 cells treated with different concentrations of PDTC

Group	Protein	mRNA
Control	0.58 ± 0.04	0.96 ± 0.08
PDTC		
0.1 mM	0.49 ± 0.02*	0.89 ± 0.08*
1 mM	0.44 ± 0.03**	0.42 ± 0.05**
10 mM	0.25 ± 0.02**	0.30 ± 0.04**

All values are presented as the mean ± SD of three experiments

* $P < 0.05$ vs. control. **$P < 0.01$ vs. control

Table 6 Expression of AQP5 protein and mRNA in SKOV3 cells treated with PDTC for different times

Group	Protein	mRNA
Control	0.69 ± 0.07	0.92 ± 0.09
PDTC		
12 h	0.51 ± 0.03*	0.76 ± 0.07*
24 h	0.10 ± 0.01**	0.50 ± 0.04**
48 h	0.04 ± 0.02**	0.21 ± 0.03**

All values are presented as the mean ± SD of three experiments

* $P < 0.05$ versus control. **$P < 0.01$ versus control

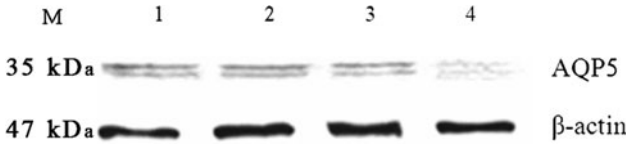

Fig. 8 Western blot analysis of the expression of AQP5 in SKOV3 cells after treatment with different concentrations of PDTC (*Lane 1* control; *lane 2–4* SKOV3 cells treated with 0.1, 1, 10 mM PDTC for 24 h, respectively)

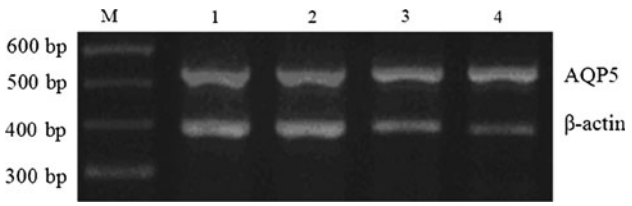

Fig. 9 Electrophoretogram of RT-PCR products of mRNA from SKOV3 cells treated with different concentrations of PDTC for 24 h (*Lane 1* control, *lane 2–4* SKOV3 cells treated with 0.1, 1, 10 mM PDTC for 24 h, respectively)

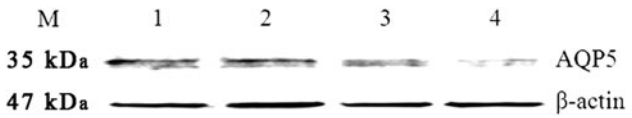

Fig. 10 Western blot analysis of the expression of AQP5 in SKOV3 cells after treatment with 1 mM PDTC (*Lane 1* control, *lane 2–4* SKOV3 cells treated with 1 mM PDTC for 12, 24, 48 h, respectively)

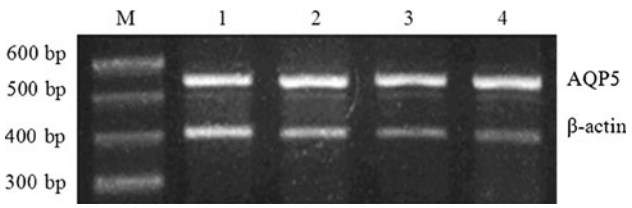

Fig. 11 Electrophoretogram of RT-PCR products of mRNA from SKOV3 cells treated with 1 mM PDTC for different times (*Lane 1* control, *lane 2–4* SKOV3 cells treated with 1 mM PDTC for 12, 24, 48 h, respectively)

correlated to AQP5 expression in SKOV3 cells ($r = -0.927, -0.972$; $P < 0.05$), [2] the PDTC-induced cell proliferation inhibition rate was negatively correlated to AQP5 expression in SKOV3 cells ($r = -0.990, -0.940$; $P < 0.05$) and [3] AQP5 protein expression was positively correlated to the nuclear expression of NF-κB p65 and IκBα protein in SKOV3 cells ($r = 0.968, 0.981, 0.995, 0.971$; $P < 0.05$).

Discussion

Recent studies have shown that tea consumption is associated with a reduced risk of epithelial ovarian cancer in a dose–response manner and increasing the consumption of green tea post-diagnosis may enhance epithelial ovarian cancer survival [16, 17]. EGCG is one of the most abundant catechins with the strongest biological activity found in green tea. Many studies have suggested that EGCG plays an anti-tumor role by inducing tumor cell apoptosis and cell cycle arrest; inhibiting tumor cell invasion, metastasis and angiogenesis; and reducing the chemotherapeutic resistance of tumor cells [18]. However, its molecular mechanisms remain unclear. Our study shows that EGCG inhibited the growth of human ovarian cancer SKOV3 cells in a dose- and time-dependent manner ($r = 0.972$ and 0.967, respectively; $P < 0.05$). EGCG also induced the apoptosis of SKOV3 cells by activating the apoptosis-signaling pathway; DNA outside of the ribosome was found to be degraded by enhanced endonuclease activity to form a DNA ladder consisting of DNA fragments that were multiples of 180–200 bp in length.

Recent studies have suggested that the anti-cancer role of EGCG may be related to its inhibition of NF-κB activation [19–21]. NF-κB is a group of eukaryotic transcription factors that consists of homo- or heterodimers of NF-κB1 (P50), RelA (p65), NF-κB2 (p52) and RelB. P50/p65 is the most widely distributed dimer and plays an important role. In the cytoplasm, NF-κB binds to its inhibitor IκB to form a trimer, which is inactive. When IκB is activated and degraded, NF-κB is released and translocates to nucleus, where NF-κB can accumulate, specifically bind to the B sequence and activate the transcription of target genes. Down-regulation of NF-κB inhibits the expression of NF-κB target anti-apoptosis genes, such as TNF receptor associated factor 1, thereby promoting apoptosis. In the squamous carcinoma cell line A431, EGCG treatment increased IκB expression in a dose- and time-dependent manner, thus inhibiting the nuclear translocation of NF-κB [22]. NF-κB is constitutively activated in epithelial ovarian cancer, and the tumorigenesis, invasion and metastasis of ovarian cancer has been shown to be related to an imbalance between NF-κB and its inhibitor IκB and the deregulation of NF-κB target genes [23, 24].

Our study demonstrates that EGCG induced SKOV3 cell apoptosis in a time- and dose-dependent manner, as evidenced by a typical DNA fragmentation pattern. At the same time, IκBα expression was increased ($P < 0.05$), leading to inhibition of the nuclear translocation of p65. Therefore, nuclear p65 expression was significantly decreased ($P < 0.01$), suggesting that EGCG-induced apoptosis in SKOV3 ovarian cancer cells may be closely related to the reduced nuclear translocation of p65.

Recent studies have shown that AQPs plays an important role in pathological processes of tumor growth, invasion, and metastasis. Balancing the secretion and absorbance of liquids by regulating the expression of AQPs provides a novel idea and approach for cancer therapy [9–11]. It was reported that the up-regulation of AQP5 and AQP1 expression in ovarian cancer cells is related to the tumorigenesis and development of ovarian cancer as well as ascites production [12, 25, 26]. Further study revealed that ascites of ovarian cancer patients increase AQP5 protein expression in the ovarian cancer cell lines SKOV3 and CAOV3, whereas chemotherapy drugs reduce the expression of the AQP5 protein [27]. In this study, we show that as the concentration of EGCG was increased and treatment time was prolonged, the growth of SKOV3 cells was significantly inhibited and AQP5 expression was gradually decreased. A significant negative correlation ($r = -0.927, -0.972$; $P < 0.05$) was found between AQP5 expression and the EGCG-induced growth inhibition rate in the SKOV3 cells, indicating that AQP5 expression is related to SKOV3 cell growth. EGCG-induced down-regulation of AQP5 expression may inhibit tumor growth.

Currently, the mechanism for the regulation of AQP expression remains unclear. Previous studies have shown that both long-term and short-term regulation exists [28, 29]. Short-term regulation refers to changes in aquaporin activity or water channel numbers under certain conditions. Long-term regulation refers to the increased synthesis of AQP at the transcriptional level, which leads to increased expression of AQP mRNA and protein. Upstream of the AQP5 open reading frame, promoter sequences were found, such as NF-κB, AP1 and AP2 [30, 31]. Towner et al. showed that TNF-α, after binding TNF-α receptor 1, activates the NF-κB signaling pathway and down-regulates the expression of AQP5 protein and mRNA [24]. Ito et al. demonstrated that IL-1β up-regulates AQP4 expression in rat astrocytes through the NF-κB pathway [14]. PDTC is a specific inhibitor of NF-κB, which is a common tool used to inactivate NF-κB in researches. By decreasing peroxidase activity of lipid, it inhibits the degradation of IκB, prevents the dissociation of IκB and NF-κB and reduces the nuclear translocation of NF-κB, therefore blocking the

activation of NF-κB. In addition, PDTC can bind thiol and thus affect the DNA binding activity of NF-κB [32]. In this study, we found that as the concentration of PDTC was increased and treatment time was prolonged, the levels of AQP5 protein and mRNA in SKOV3 cells were gradually decreased and the inhibition rate of SKOV3 cell proliferation was increased. These results demonstrate that blocking NF-κB activation can directly inhibit the expression of the AQP5 protein and the transcription of its gene, suggesting that NF-κB can regulate AQP5 expression in SKOV3 cells.

The mechanism of EGCG-induced down-regulation of AQP5 expression was previously unknown. In this study, EGCG was found to reduce AQP5 expression and at the same time inhibit the nuclear expression of NF-κB p65 and IκB with a positive correlation ($r = 0.968$, 0.981, 0.995, 0.971; $P < 0.05$), suggesting that the EGCG-induced reduction of AQP5 protein expression in SKOV3 cells may be related to NF-κB. The possibility that AQP5 is regulated by NF-κB will be confirmed in future research.

In summary, EGCG inhibits AQP5 expression, induces apoptosis, and prevents tumor growth in ovarian cancer cells. Further research on the role and mechanism of the effect of EGCG in ovarian cancer cells is required. Nagle et al. provided some evidence that women who drink green tea have a lower risk of ovarian cancer [33]. In the future, we hope that EGCG could be used for the treatment of ovarian cancer as a new drug target.

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