Abstract. The aim of the present study was to investigate the effects of epigallocatechin-3-gallate (EGCG) on the apoptosis of the human MCF-7 cancer cell line and the underlying mechanism. MCF-7 cells were divided into the control group and EGCG groups. The proliferation of MCF-7 cells in the two groups was determined using MTT and apoptosis was examined using flow cytometry. Western blot analysis and qRT-PCR were used to analyze P53 and Bcl-2 expression levels. The silencing effect of specific siRNA was evaluated using RT-PCR and western blot analysis. P53 and Bcl-2 expression levels were determined using western blot analysis in the si-P53, EGCG and EGCG-combined si-P53 groups. EGCG inhibited the proliferation of MCF-7 cells in a concentration-dependent manner and IC_{50} was 37.681 mol/l. The apoptotic rates were 1.37% and 5.83% (t=8.9, p=0.0124) in the blank control and treatment groups after treatment with 30 µmol/l EGCG. The RT-qPCR and western blot results demonstrated that the effect of siRNA interference was evident. The expression of P53 in the EGCG-combined si-P53 group was higher than that of the si-P53 group, but lower than the EGCG group. The Bcl-2 expression level in the EGCG-combined si-P53 group was lower than that of the si-P53 group and higher than that of the EGCG group. In conclusion, EGCG suppressed the proliferation of human MCF-7 breast cancer cells and promoted apoptosis. In addition, the underlying mechanism may be related to the P53/Bcl-2 signaling pathway.

Introduction

Green tea is one of the main tea species in China. It contains tea polyphenols, catechins, chlorophyll, caffeine, amino acids, vitamins and other active ingredients (1). Epigallocatechin-3-gallate (EGCG) content is very high in green tea (1). EGCG has anti-aging, sterilization, anti-inflammatory and anticancer properties, and it also can inhibit the growth of a variety of malignant cells and induce apoptosis (2-4). It can also inhibit the angiogenesis, invasion and metastasis of malignant tumor cells, in various types of cancer (5).

Breast cancer is one of the common malignancies in women. It is crucial to identify new effective drugs with minimal side effects to treat breast cancer. Liang et al (6) identified that EGCG inhibited the proliferation of breast cancer cells by repressing cyclin-dependent protein activity and also inhibited the growth and metastasis of breast cancer cells by downregulating the expression of VEGF and MMP-9 (7-9). However, the mechanism by which EGCG promoted breast cancer apoptosis has rarely been studied.

The present study provides a theoretical reference for the development of breast cancer target by investigating the mechanism of apoptosis of EGCG on breast cancer.

Materials and methods

Materials. EGCG and thiazolyl blue were purchased from Sigma (St. Louis, MO, USA). RPMI-1640 basal medium and fetal bovine serum (FBS) was produced by HyClone (Logan, UT, USA). RPMI-1640 basal medium and EGCG were used to prepare a 10 mmol/l storage solution. It was sterilized by filtration (0.22 µm) and stored at -80°C in the dark. MTT was dissolved in phosphate-buffered saline (PBS) solution at 5 mg/ml, and was stored at -20°C in the dark after sterilization. Mouse anti-human P53, Bcl-2 and GAPDH were all purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA) and the RT-PCR kit was purchased from Takara (Otsu, Shiga, Japan). P53, Bcl-2 and GAPDH primers were produced by Invitrogen (Carlsbad, CA, USA) (Table I) and si-P53 was supplied by Gemma (Shanghai, China).

Cell line. MCF-7 cells were purchased from the Shanghai ATCC cell bank. The cells were cultured in RPMI-1640 medium containing 10% FBS (with 100 U/ml green-streptomycin) and incubated under 5% CO_2 at 37°C.

Methods

MTT detection of the sensitivity of MCF-7 cells to EGCG. The EGCG concentration gradient was 0, 2, 4, 8, 16, 32, 64
and 128 µmol/l. MCF-7 cells (2x10^5) were seeded in a 96-well plate with blank control wells. After cells adhered to the walls, EGCG of the concentration gradient of 0, 2, 4, 8, 16, 32, 64 and 128 µmol/l was added. After incubation for 72 h, the supernatant was discarded and 20 µl of MTT was added to each well. After incubation for 4 h, the liquid was discarded and 150 µl of dimethyl sulfoxide (DMSO) was added to each well of the EGCG. The optical density (OD) of each well at 490 nm was measured by a microplate reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The inhibition rate was calculated as: 

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\text{Inhibition rate} = \frac{\text{control group OD} - \text{experimental group OD}}{\text{control group OD}} \times 100\%
\]

**Effect of EGCG on MCF-7 cell apoptosis measured by flow cytometry (FCM).** MCF-7 cells were seeded in a 6-well cell culture plate. After adherence, 30 µmol/l EGCG was added and the cells were stained after 48 h with Annexin V-FITC kit. The change in apoptotic rate was measured, and the experiment was repeated three times.

**RT-qPCR and western blot analysis determine how EGCG treatment on MCF-7 affects the mRNA and protein levels of P53 and Bcl-2.** The experiment was conducted on the control and EGCG groups. EGCG was added to fresh MCF-7 cells in the experimental group after cell adhesion (EGCG at a final concentration of 30 µmol/l), but the control group was not treated. After 72 h of incubation, total RNA was extracted using TRIzol reagent. The RT-qPCR assay was performed using the Takara kit, and the PCR products were analyzed using a spectrophotometer (Hitachi, Tokyo, Japan). The P53 mRNA expression was evaluated using RT-qPCR, and the P53 protein expression level was measured using western blot analysis. The three groups were compared and the transfection efficiency was calculated.

### Table I. Primers of P53, Bcl-2 and GAPDH used in RT-qPCR.

| Gene   | Forward primer        | Reverse primer         |
|--------|-----------------------|------------------------|
| P53    | 5'-TTCCCTGGATTTGGCCAGACT-3' | 5'-ACCATCGTATCTGACGCAGC-3' |
| Bcl-2  | 5'-CGACTTCGGCGAGATGCCCAGCAG-3' | 5'-ACTTGTGGCCAGATAGGCAACCAGC-3' |
| GAPDH  | 5'-AGAAAGGGTCGGGCTCATTG-3' | 5'-AGGGCCATCCACAGTCTTC-3' |

**Statistical analysis.** Statistical analysis was performed using GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA) and SPSS 20.0 (Chicago, IL, USA) statistical software. The measurement data were presented as mean ± standard deviation. Comparison between two groups was conducted using a t-test. The one-way analysis of variance (ANOVA) test was used for comparison among three groups. P<0.05 was considered to indicate a statistically significant difference.

### Results

**MTT detection of the sensitivity of MCF-7 cells to EGCG.** The sensitivity of EGCG to MCF-7 was evaluated using MTT assay. As shown in Fig. 1, 10 µmol/l EGCG significantly inhibited the proliferation of MCF-7 cells and the inhibition was enhanced with the increase of drug concentration. The IC_{50} was 37.684 µmol/l. We selected 30 µmol/l as the follow-up dose (Table II).

**Effect of EGCG on MCF-7 cell apoptosis measured using FCM.** As shown in Fig. 2, the apoptotic rates of the EGCG...
and control groups were 1.37 and 5.83%, respectively, with a significant difference (t=8.9, p=0.0124). These results suggested that EGCG promoted apoptosis in MCF-7 cells (Table III).

### Effects of EGCG treatment on the expression of P53 and Bcl-2.

After treatment with 30 µmol/l EGCG, P53 mRNA and protein expression levels were significantly higher, while the Bcl-2 mRNA and protein expression levels decreased significantly (Fig. 3; Table IV).

### si-P53 interference effect.

The RNA interference technique was used to silence P53 expression. The interference effect was studied using RT-qPCR and western blot analysis. As shown in Fig. 4, the interference effect was significant and could be further tested.

### Discussion

P53 is an important tumor suppressor gene. It is involved in tumor occurrence and development through DNA repair, cell cycle regulation, angiogenesis inhibition and cell apoptosis (10). The P53 gene has been found to be mutated in more than 50% of malignant tumors (11). Mutations and deletions of the P53 gene have been reported to be closely related to breast cancer (12-15). Apoptosis is a spontaneous cell death process regulated by a series of genes. The proto-oncogene Bcl-2 is a member of the Bcl-2 family. The oncoproteins located in the nuclear membrane, part of the endoplasmic reticulum and mitochondrial outer membrane, can negatively regulate cell apoptosis and prolong cell life (16). Previous findings have shown that P53 can inhibit the expression of Bcl-2 protein (17,18).

EGCG is the most active substance in green tea, which has great developmental value, and its anticancer effect has been widely studied. Previous results have shown that EGCG can promote apoptosis in a variety of malignant tumor cells, including pancreatic and gastric cancer cells (18,19). In the present study, we showed that the apoptotic rate in MCF-7 cells treated with EGCG was significantly higher than that of the control group. In order to explore the mechanism of apoptosis, P53 and Bcl-2 expression levels were determined. The RT-qPCR and western blot results showed that EGCG induced the expression of P53 but inhibited the expression of Bcl-2. The EGCG + si-P53 group had a higher P53 expression level compared with the si-P53 group. The P53 expression level was

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**Table II. Different concentrations of EGCG and the inhibitory rate of MCF-7 cells using MTT.**

| Concentration (mean ± SD) | Inhibition rate (%) |
|---------------------------|---------------------|
| 0                         | 1.187±0.049         |
| 10                        | 0.979±0.035         |
| 20                        | 0.726±0.021         |
| 40                        | 0.589±0.027         |
| 60                        | 0.427±0.012         |
| 80                        | 0.309±0.017         |
| 100                       | 0.219±0.016         |
| 120                       | 0.112±0.011         |

EGCG, epigallocatechin-3-gallate;
NC, negative control.

**Table III. The effect of 30 µmol/l EGCG on the apoptotic rate of MCF-7 after 48 h was detected using a flow cytometer.**

| Group     | Apoptosis rate (%) (mean ± SD) | t     | P-value |
|-----------|-------------------------------|-------|---------|
| NC        | 1.38±0.19                     |       |         |
| EGCG      | 5.65±0.64                     | 8.9   | 0.0124  |

EGCG, epigallocatechin-3-gallate; NC, negative control.

**Table IV. The effect of 30 µmol/l EGCG on the Cq and F value of P53 and Bcl-2 in MCF-7 cells.**

| Gene | 2^(-ΔΔCq) (mean ± SD) | t  | P-value |
|------|------------------------|----|---------|
| P53  | 4.289±0.831            | 9.451 | <0.001  |
| Bcl-2| 12.013±1.082           | 7.985 | <0.001  |

EGCG, epigallocatechin-3-gallate; NC, negative control.

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Figure 2. (A) The effect of 30 µmol/l EGCG on the apoptosis rate of MCF-7 after 48 h was detected by flow cytometer. (B) Statistics of apoptosis rate. *P<0.05, compared to the NC group. EGCG, epigallocatechin-3-gallate; NC, negative control.

Figure 3. (A) The effect of 30 µmol/l EGCG on the mRNA expression of P53 and Bcl-2 in MCF-7 cells. **P<0.001 compared to the NC group. (B) The effect of 30 µmol/l EGCG on the protein expression of P53 and Bcl-2 in MCF-7 cells. EGCG, epigallocatechin-3-gallate; NC, negative control.

Figure 4. (A) The Cq value of P53 in the MCF-7 cells after transfection with si-P53 was measured using RT-qPCR. (B) Protein expression of P53 and Bcl-2 was examined using western blot analysis on MCF-7 cells treated with EGCG and transfected with si-P53. EGCG, epigallocatechin-3-gallate.

Table V. The Cq value of P53 was detected by RT-qPCR after transfection with si-P53.

| Group    | 2^ΔΔCq (mean ± SD) | F     | P-value |
|----------|--------------------|-------|---------|
| NC       | 12.332±1.063       |       |         |
| si-NC    | 11.219±1.097       |       |         |
| si-P53   | 4.329±0.658        | 61.450| <0.001  |

NC, negative control.
lower than that of the EGCG group, while the Bcl-2 expression was lower than that in the si-P53 group but higher than that in the EGCG group. These results suggest that EGCG inhibited the expression of Bcl-2 and promoted the expression of P53.

In conclusion, the findings show that EGCG inhibited the proliferation of MCF-7 cells and promoted apoptosis in these cells. The underlying mechanism involved may be related to the P53/Bcl-2 signaling pathway, which can provide a theoretical basis for EGCG to be used in the new target drug development for breast cancer.

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