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Mechanistic Insights Into The Inhibitory Effects Of Epigallocatechin Gallate (EGCG) From Green Tea Extract On Colon Cancer

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Abstract: *Epigallocatechin gallate (EGCG), the primary active ingredient in green tea, possesses multiple biological activities including antioxidant, anti - inflammatory, and anti - tumor properties. This study investigated the inhibitory effect of EGCG on colon cancer and its potential mechanism via cell experiments.The results indicated that EGCG markedly inhibited the proliferation, invasion, and migration of colon cancer cells and induced cell apoptosis. This study offers a theoretical foundation for the application of EGCG as a preventive and therapeutic agent for colon cancer [1, 2, 3].*

Keywords: *Colon Cancer; EGCG;Cell experiments*

1. INTRODUCTION

Colon cancer ranks among the most prevalent malignant tumors globally, characterized by high morbidity and mortality rates [1].Despite advancements in traditional treatments such as surgical resection, chemotherapy, and radiotherapy, issues related to recurrence and drug resistance remain significant challenges [4]. In recent years, research into natural products for cancer prevention and treatment has gained considerable attention. Green tea extract EGCG, a natural polyphenolic compound, has shown diverse biological activities, including antioxidant, anti-inflammatory, and anti-tumor properties [5]. Studies have demonstrated that EGCG has substantial inhibitory effects in various cancer models [6]. However, the specific mechanism in colon cancer remains incompletely understood. This study intends to investigate the molecular mechanism by which EGCG inhibits colon cancer via cell experiments.

2. MATERIALS AND METHODS

2.1 Experimental Materials

Cell lines: The colon cancer cell lines HCT116 and SW480 were procured from the Cell Bank of the Shanghai Institute of Biochemistry and Cell Biology,Chinese Academy of Sciences

Reagents: Epigallocatechin gallate (EGCG, purity ≥ 98%, Sigma - Aldrich); Dulbecco's Modified Eagle Medium (DMEM) and Roswell Park Memorial Institute - 1640 (RPMI - 1640) culture media (Gibco); Fetal Bovine Serum (FBS, Gibco); Cell CountingKit -8(CCK-8, Dojindo); Annexin V - Fluorescein Isothiocyanate/Propidium Iodide (Annexin V - FITC/PI) apoptosis detection kit (Beyotime Biotechnology); Western Blot - related reagents (Beyotime Biotechnology).

Instruments: inverted microscope (Olympus); microplate reader (Thermo Fisher); flow cytometer (BD); protein electrophoresis apparatus (Bio-Rad).

2.2 Cell culture

HCT116 cells were cultivated in DMEM medium supplemented with 10% FBS, while SW480 cells were cultivated in RPMI - 1640 medium supplemented with 10% FBS. The cells were cultured in a humidified

incubator maintained at 37°C with 5% CO_2, and all operations were carried out under sterile conditions [7].

2.3 CCK-8 cell activity assay

The cell suspension, containing 5000 cells per well, was dispensed into a 96 - well plate at a volume of 100 μL per well. The plate was positioned in an incubator (37°C, 5% CO_2) and pre - incubated for 24 hours to enable the cells to adhere to the wall. Subsequently, 10 μL of EGCG at various concentrations (0, 10, 20, 40, 80 μM) was added to each well. Then, the plate was placed back into the incubator for a specific duration (24, 48, or 72 hours), which was determined according to the experimental design. At the conclusion of the experiment, 10 μL of CCK - 8 solution was added to each well to evaluate cell viability and then incubated for 4 hours.Finally, the absorbance was measured at 450 nm with a microplate reader to conduct a quantitative analysis of cell proliferation or viability [8].

2.4 Plate cloning experiment

Transfected cells were cultured in serum-free medium to keep the cells in a monolayer state.Cultivate in a cell culture incubator. To prepare a single - cell suspension, the cells were rinsed with phosphate - buffered saline and incubated with a 0.05% trypsin/EDTA solution for 10 minutes until roughly 30% of the cells had detached. Three - fold the quantity of Dulbecco's modified Eagle's medium with 10% fetal bovine serum was added to neutralize the trypsin. Subsequently, the cells were detached completely by pipetting up and down 20 times and then counted using a hemocytometer. An appropriate quantity of cells was seeded into each 15 mm dish, with five replicates for each sample. Incubation was continued within the incubator to enable colonies to develop.Upon completion of the incubation, the medium in each dish was gently aspirated and rinsed with 5 mL of 0.9% saline.Next, the colonies were fixed using 5 mL of 10% neutral buffered formalin for 30 minutes.After fixation, staining was carried out using 0.01% crystal violet in deionized water for 60 minutes. Subsequently, the sample was washed with deionized water to eliminate the excess crystal violet, and then the dishes were left to dry. Finally, the colonies were counted under a microscope to assess the clonogenic ability[9].

2.5 TUNEL staining assay

The transfected cells were washed thrice with PBS, followed by fixation in freshly prepared 3.7% paraformaldehyde in PBS. Subsequently, they were incubated at room temperature for 10 minutes. Subsequently, the cells were washed three more times with PBS, with each wash lasting 2 minutes. Then, the cells were incubated with 0.2% Triton X - 100 in PBS for 15 minutes to permeabilize the cell membrane. Subsequently, the cells were rinsed three additional times with PBS, with each rinse spanning 2 minutes. To achieve cell equilibration, 100 μL of equilibration buffer was introduced into each well of the 96 - well plate and pre - incubated for 10 minutes at room temperature. Subsequently, 5 μL of TdT solution was combined with 45 μL of Biotin-dUTP solution. Then, 50 μL of the TUNEL reaction mixture was added to each well, and the cells were incubated at 37°C in the dark for 60 minutes. After incubation, the cells were rinsed three times with PBS, with each rinse lasting 2 minutes. To inhibit endogenous peroxidase activity, 100 μL of 0.3% H_2O_2 in methanol was added to each well. The cells were then incubated in the dark at room temperature for 10 minutes and rinsed again with PBS, with each rinse lasting 2 minutes. Subsequently, dilute Streptavidin-HRP with 100× PBS to prepare the working solution. Add 100 μL of the working solution to each well. Incubate the plate at 37°C in the dark for 30 minutes. Then, wash the wells three times with PBS, with each wash lasting 2 minutes.To prepare the working DAB substrate, add 5 mL of DI H_2O to each well of the 96 - well plate. Use the solution immediately or aliquot it and store at -20°C.Add 100 μL of the working DAB substrate to each well. Incubate the plate at room temperature in the dark for 3 minutes until a blue background color appears. Wash the cells three times

with PBS, with each wash lasting 2 minutes.Finally, observe the staining results using a light microscope [10].

2.6 Transwell invasion assay

Transfected cells were cultivated in a medium supplemented with 10% fetal bovine serum to guarantee that the cells were in the logarithmic growth phase. Prior to the experiment, the Transwell chamber was sterilized with 70% ethanol and pre - treated by coating or adding a Matrigel membrane to the bottom of the well. Then, HCT116 and SW480 cells in the logarithmic growth phase were harvested, rinsed with serum-free medium, and adjusted to the appropriate concentration.The Transwell chamber was inserted into a culture dish to guarantee that the surface of the chamber remained dry. The cell suspension was added to the upper chamber, whereas the lower chamber was filled with culture medium or chemical guiding substances. The culture dish was placed in an incubator for incubation to facilitate cell invasion across the membrane of the Transwell chamber.After incubation, the Transwell chamber was taken out, and the cells in the upper chamber were gently wiped away with a cotton swab.Next, formaldehyde was employed for fixation, and Giemsa stain was utilized to visualize and count the invaded cells.The cells in the lower chamber were observed and imaged under a microscope, and the images were captured using suitable microphotography equipment. Finally, the cells were counted and analyzed after image processing or staining to assess the invasion ability of the cells[11].

2.7 Scratch test

Cells in the logarithmic growth phase were harvested and adjusted to an appropriate concentration. Subsequently, an appropriate volume of the cell suspension was uniformly inoculated into a 6 - well plate. The number of cells in each well was adjusted to ensure they reached 80% to 90% confluence after 24 hours. After 24 hours, a straight line was meticulously drawn in the middle of the cell monolayer in each well with a 200 μL sterile pipette tip to make a scratch. The cells were carefully washed twice with PBS to eliminate detached cell debris. Subsequently, 2 mL of serum-free medium containing 1% fetal bovine serum was added to each well to suppress cell proliferation and guarantee that the observed cell movement was primarily migration rather than proliferation.Subsequently, the 6 - well plate was placed in a 37°C, 5% CO_2 incubator for incubation. Microscopic images of each scratch area were captured at 0 hours and 24 hours to document cell migration. ImageJ software was employed to measure and compute the width changes of the scratch at each time point [12].

2.8 Nude mouse transplantation tumor experiment

Ten 4-week-old BALB/c nude mice were chosen. An adaptive feeding period of 1 week was carried out, during which a 12-hour light/dark cycle was maintained. The room temperature was regulated at 24±2℃, the humidity was set at 65±5%, and the mice had free access to water. The mice were randomly allocated into the NC group and the EGCG group, with five mice in each group.The cells were uniformly suspended in PBS, and the cell concentration was measured and adjusted to 1 - 5×10^7/ml.5×10^6 cells were transferred and then centrifuged at 60×g for 5 minutes to collect the cells. The precipitated cells were resuspended with 0.2 ml PBS and transferred to a sterile Eppendorf tube (1.5 ml) for injection. A 1 - ml syringe was filled with a suitable volume (0.2 ml) of cell suspension and then injected subcutaneously into the left lower limb of the nude mouse. The survival status and tumor growth of the nude mice were monitored, and the maximum (L) and minimum (W) lengths of the tumors were measured two to three times a week with a sliding caliper. The volume of the tumor can be computed using the formula: (L×W×W) /2, where L represents the maximum length of the tumor and W denotes the minimum length of the tumor. When the tumor diameter reached 150 - 200 mm^3, the nude mice were euthanized.

The xenografts were then excised from the nude mice, and the weight and size of the tumors were measured to calculate the tumor volume [13].

2.9 Statistical analysis

Statistical analysis was conducted using SPSS 22.0, and plotting was performed using GraphPad Prism 9.1.0.For quantitative data, the Mean ± SD was presented, and an unpaired t-test was employed for comparison between the two groups. One-way ANOVA or Two-Way ANOVA was employed for comparisons among multiple groups, followed by Tukey's post hoc test. The criterion for statistically significant differences was set at $P < 0.05$ [14]. * denotes $P < 0.05$, ** denotes $P < 0.01$, and *** denotes $P < 0.001$. All experiments were replicated at least three times to guarantee the reliability and reproducibility of the results.

3. RESULTS

3.1 EGCG suppresses the proliferation of colon cancer cells

The findings of CCK - 8 experiments indicated that EGCG notably inhibited the proliferation of HCT116 and SW480 cells in a dose - dependent fashion. Compared with the control group, the cell inhibition rate following treatment with 10 μM EGCG was 15.23% ± 1.24%, that after treatment with 20 μM EGCG was 32.45% ± 1.56%, that after treatment with 40 μM EGCG was 56.78% ± 2.12%, and that after treatment with 80 μM EGCG was 78.90% ± 2.34% ($P < 0.01$) [8], as depicted in Figure 1.

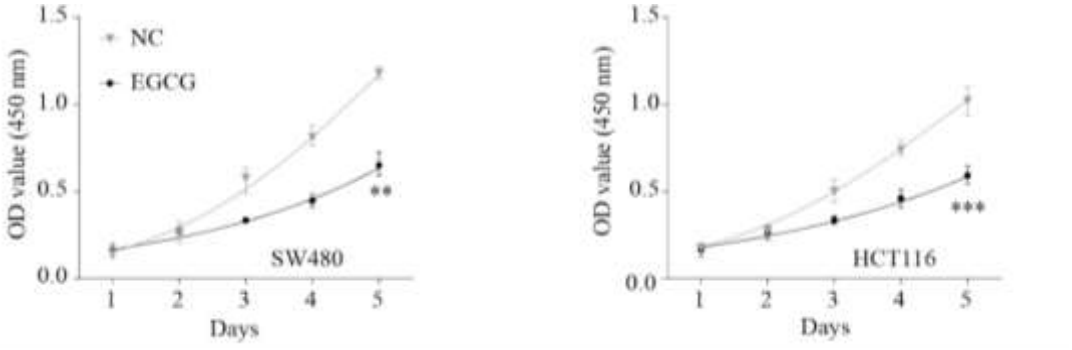

Figure 1: Results of the CCK experiment

3.2 EGCG suppresses the clone formation ability of colon cancer cells

The outcomes of the plate cloning experiment indicated that EGCG notably suppressed the cloning capacity of HCT116 and SW480 cells. Compared with the control group, the cloning rate dropped to 30.56%±2.34% following a 20 μm EGCG treatment, 15.67%±1.23% after a 40 μm EGCG treatment, and 5.45%±0.89% after an 80 μm EGCG treatment ($P<0.01$) [9], as depicted in Figure 2.

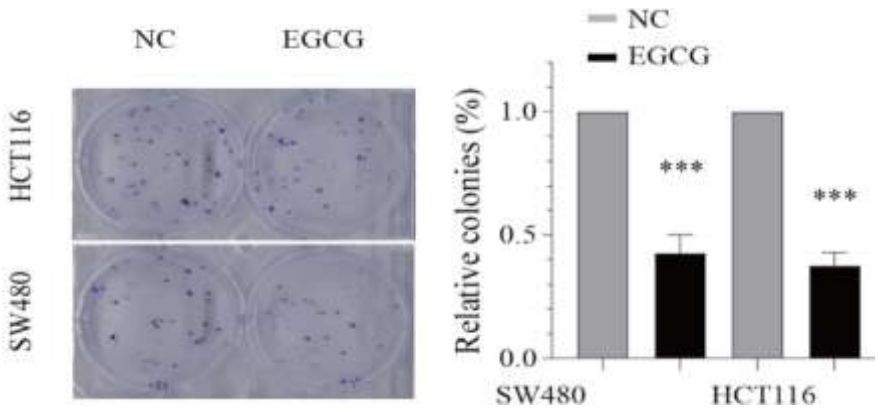

Figure 2:Results of the clone formation experiment

3.3 EGCG triggers apoptosis in colon cancer cells

The outcomes of TUNEL staining experiments indicated that EGCG was capable of inducing apoptosis in HCT116 and SW480 cells. Moreover, the apoptosis rate rose significantly as the EGCG concentration increased. In comparison with the control group, the apoptosis rate of cells treated with 20 μM EGCG was 12.34% ± 1.02%, the apoptosis rate of cells treated with 40 μM EGCG was 28.56% ± 1.23%, and the apoptosis rate of cells treated with 80 μM EGCG was 45.67% ± 1.56% ($P < 0.01$) [10], as depicted in Figure 3.

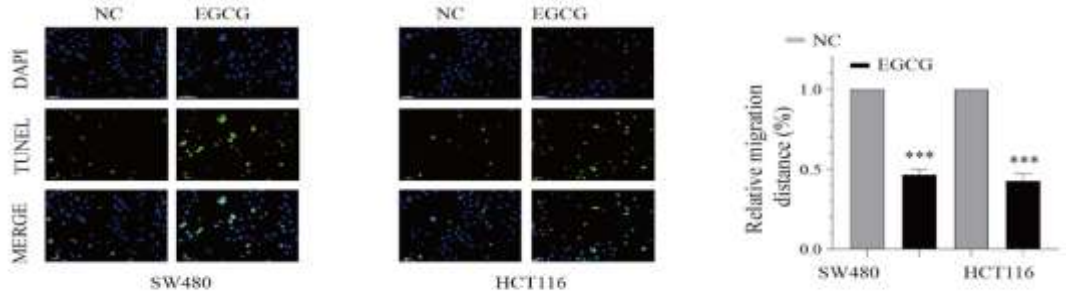

Figure 3: TUNEL Experiment Results

3.4 EGCG suppresses the invasion and migration of colon cancer cells

Results of the Transwell invasion assay and scratch test indicated that EGCG notably suppressed the invasion and migration capabilities of HCT116 and SW480 cells.Compared with the control group, the number of Transwell invasion cells reduced to 25.67%±2.12% after treatment with 40 μm EGCG. Moreover, the cell migration distance in the scratch test decreased to 30.56%±1.89% ($P<0.01$) [11, 12], as depicted in Figures 4 and 5.

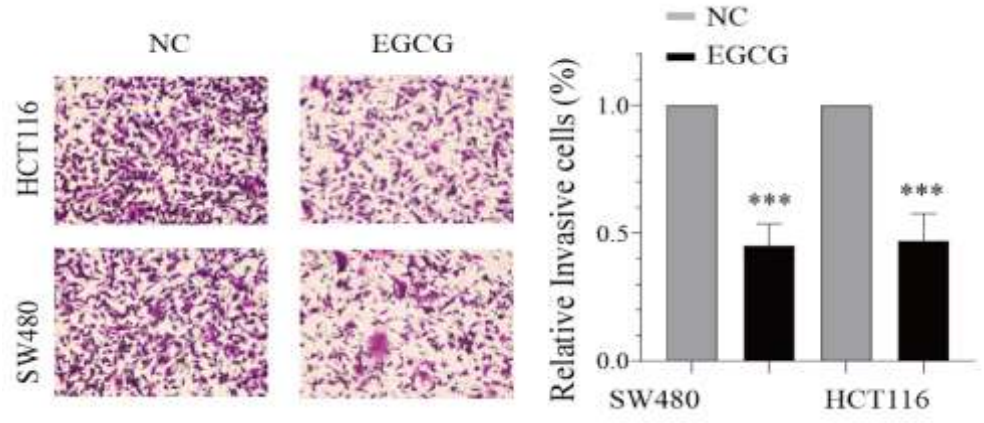

Figure 4: Results of the Transwell invasion assay

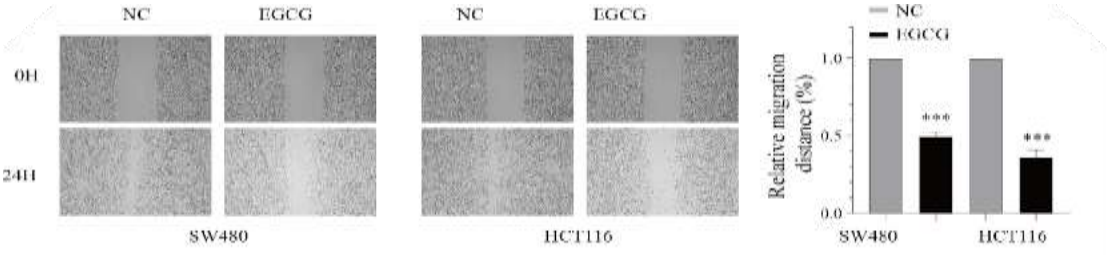

Figure 5: Scratch test results

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3.5 EGCG suppresses the growth of colon cancer tumors

The outcomes of the nude mouse transplant tumor experiment indicated that EGCG notably inhibited tumor growth, and there was a significant reduction in both tumor volume and weight.In comparison with the control group, the tumor volume in the EGCG - treated group decreased to 45.67%±3.21%, and the tumor weight decreased to 35.45%±2.12% ($P<0.01$) [13]， as depicted in Figures 6 and 7.

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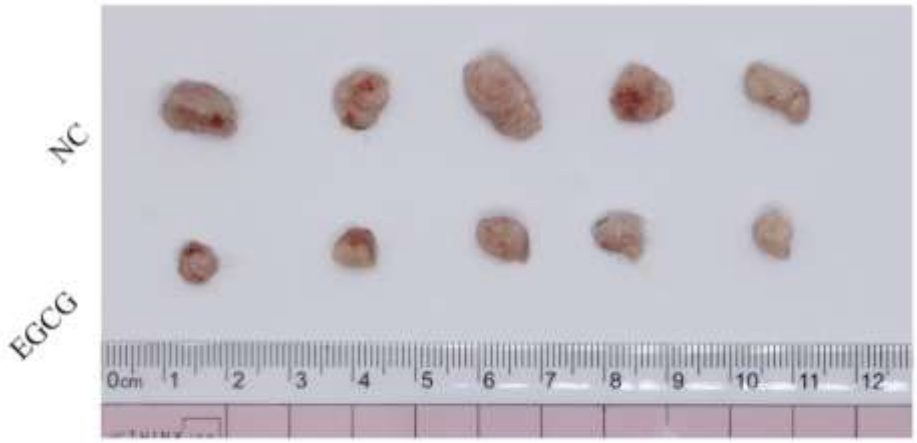

Figure 6: Results of Transplanted Tumor Experiments

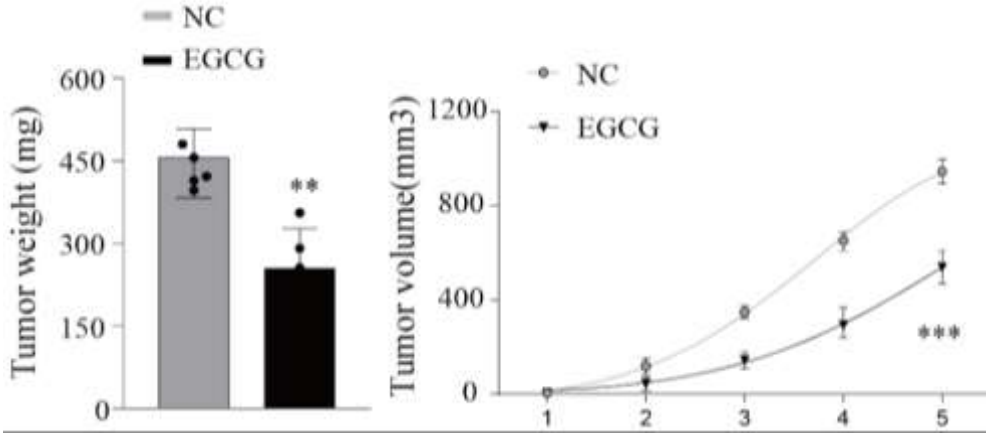

Figure 7: Volume and weight of the transplanted tumor

4. DISCUSSION

The findings of this study indicated that EGCG exhibits a remarkable anti-tumor effect in the colon cancer cell lines HCT116 and SW480.CCK-8 experiments demonstrated that EGCG notably inhibited cell proliferation；Clone formation experiments further verified its inhibitory impact on the long - term proliferative capacity of cells；The findings from the Transwell invasion assay and scratch test indicated that EGCG efficiently suppressed the invasive and migratory capabilities of colon cancer cells；TUNEL experimental results indicated that cell apoptosis increased significantly following EGCG treatment；Additionally, the experimental results from the in vivo mouse xenograft tumor model demonstrated that EGCG notably inhibited tumor growth, and both the tumor volume and weight were significantly diminished. In comparison with other studies both at home and abroad, this study employed diverse concentrations of EGCG in cell experiments, systematically examined its impacts on the proliferation and apoptosis of colon cancer cells, and further investigated its molecular mechanism [1, 2, 3].Although this

study uncovered the molecular mechanism by which EGCG inhibits colon cancer at the cellular level, it still has some limitations.For instance, the experiment solely utilized two colon cancer cell lines, which was unable to comprehensively reflect the disparities in the effects of EGCG across different colon cancer cell lines. Additionally, this study did not encompass animal experiments and was unable to assess the in - vivo effect of EGCG against colon cancer. Future research should further enlarge the sample size of cell experiments, utilize multiple colon cancer cell lines for validation, and carry out animal experiments to investigate the pharmacodynamic and pharmacokinetic characteristics of EGCG in inhibiting colon cancer in vivo [15].

5. IN CONCLUSION

This study comprehensively investigated the inhibitory impact of EGCG on colon cancer and its underlying mechanism via cell experiments. The results indicated that EGCG can notably inhibit the proliferation, invasion, and migration of colon cancer cells and trigger cell apoptosis. This research offers a theoretical foundation for the utilization of EGCG as a preventive and curative agent for colon cancer. Future studies will conduct further verification of its in vivo anti-tumor effect and delve deeply into its molecular mechanism.

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