

Epigallocatechin-3-gallate induced anticancer efficiency on Caco2 cells through the inhibition of cell proliferation following apoptosis

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Epigallocatechin-3-gallate (EGCG) has been a major active polyphenolic compounds in natural foods. It is well known that the polyphenols have strong antioxidant effects and have the effect of converting harmful substances such as active oxygen into harmless substances. Therefore, they were expected to help prevent lifestyle-related diseases. However, although in recent years it has begun to be reported that polyphenols have significant therapeutic and chemotherapy-preventing potential for various types of cancer, the molecular mechanism involved in EGCG induced cell death in Caco2 cells was still not revealed. Therefore, in this study, we evaluated the mechanism behind EGCG- induced cell death in Caco2 cells.

Caco2 cells were cultured in EMEM supplemented with 10% FBS and 1% non-essential amino acids in a humidified incubator at 37°C with 5% CO₂. After 24 h incubation, Caco2 cells were treated with different concentrations of EGCG for 48 h. Trypan blue and LDH assays were used for cytotoxicity. Glutathione reductase and GSH were measured for the oxidative stress status by EGCG. The changes of DNA fragmentations in the cells were analyzed by agarose electrophoresis. mTOR, SIRT1, Akt, p53, NFκB, FOXO, and XIAP were measured by Western blotting. Cleaved caspase-3, p51, and Bax were also determined to prove activation of intrinsic apoptosis pathway by the polyphenols. Statistical analyses were performed by one-way ANOVA followed by a multiple comparison test. $p < 0.05$ was defined as statistically significance.

A significant decreased cell viability ($p < 0.05$) and increased LDH activity in the culture medium ($p < 0.01$) were observed in the Caco-2 cells exposed to 100 μM EGCG ($n = 3$). In addition, DNA fragmentation and intracellular GSH level reduction (84.6 ± 4.33 to 56.3 ± 2.84 nmol/mg of protein: mean \pm SE, $n = 4$) provided evidence of apoptotic types of cell death in Caco2. To analyze cell death mechanism, we found involvement of SIRT1. In the present study we found a negatively regulated activation of SIRT1 ($p < 0.05$) allows p53 to be activated to arrest cell cycle and inactivate NF-κB, FOXO and Akt ($p < 0.05$) to hindered cell cycle regulation and subsequent cell proliferation suppression. On the other hand, downregulation of XIAP ($p < 0.05$) and increased cleaved caspase-3, p51 and Bax ($p < 0.05$) induced cell death in Caco2 cells via apoptosis.

We aimed to unveil the anticancer effect of EGCG against Caco2 cells and their underlying mechanisms. For the first time we revealed, negatively regulated SIRT1 induce apoptosis in Caco2 cells with treatment of EGCG. Apoptosis is regulated by different transcriptional factors, which mainly hindered Caco2 cells to progress cell proliferation and subsequently apoptosis. On the other hand, downregulated XIAP also take crucial role to initiate apoptosis. Therefore, our results showed potentiality of EGCG for colon cancer therapeutic strategy. However, further *in vivo* and clinical studies will be needed to reveal the detailed mechanism.

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References

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