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DIMINISHED EGCG-MEDIATED APOPTOSIS IN THE DIFFERENTIATED HL-60 CELLS

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Summary

The major polyphenol of green tea, (-)-epigallocatechin-3-gallate (EGCG) has been shown to induce apoptosis in HL-60 cells and many cancer cell lines. Some anti-tumor compounds induce apoptosis and lead to side effects in normal cells, so the protection of them is one of the most critical issues in cancer therapy. Here we show that the apoptosis induction by EGCG differs between non-differentiated and differentiated HL-60 cells. HL-60 cells, a human promyelocytic leukemia cell line, can differentiate to a phenotype resembling to normal cells. HL-60 cells were maintained in RPMI 1640 medium supplemented with 10 % fetal bovine serum and 0.5 nM phorbol-12-myristate-13-acetate (PMA) for 1 day, 1 μM all-trans-retinoic acid (ATRA) for 3 days or 100 nM 1,25-dyhydroxyvitamin D3 (VD3) for 3 days. The higher survival rate of PMA-, ATRA- and VD3-differentiated cells, as determined by the Trypan blue dye exclusion assay, indicated higher EGCG tolerance of these cells than non-differentiated HL-60 cells. We found that the gene expression of Bcl-2A1, which inhibits apoptosis, was increased in all differentiated HL-60 cells. These results suggest that EGCG induces apoptosis more effectively in cancer cells than in normal cells.

Keywords

EGCG, apoptosis, HL-60 cells

Introduction

The major polyphenol of green tea, (-)-epigallocatechin-3-gallate (EGCG) has been shown to induce apoptosis in HL-60 cells and many cancer cell lines. Some anti-tumor compounds induce apoptosis and lead to side effects in normal cells, so the protection of them is one of the most critical issues in cancer therapy. EGCG has been shown to induce preferentially in cancerous cells over normal counter cells [1]. However, differences in sensitivity for EGCG-mediated apoptosis between non-differentiated and differentiated HL-60 cells have not been reported. In the present study, we examined if differentiation of these cells to a phenotype resembling to normal cells causes the difference in sensitivity for EGCG-mediated apoptosis.

Materials and methods

Human promyelocytic leukemia HL-60 cells were obtained from Riken Gene Bank (Ibaraki, Japan) and maintained in 10% fetal bovine serum in RPMI 1640 medium containing 50 U/ml penicillin, 50 μg/ml streptomycin, and 2.5 μg/ml amphotericin B at 37°C under a 5% CO_2 atmosphere. Phorbol-12-myristate-13-acetate (PMA) and

all-trans-retinoic acid (ATRA) were obtained from Wako Pure Chemicals Co., (Osaka, Japan). 1,25-dihydroxyvitamin D_3 (VD3) was kindly provided by Dr. R. Nozawa (The university of Shizuoka). To induce differentiation, HL-60 cells were treated with 0.5 nM PMA for 1 day, 1 μM ATRA for 3 days or 100 nM VD3 for 3 days. The survival rate was determined by the Trypan blue dye exclusion assay. Detection of apoptotic bodies and examination of DNA ladder formation were carried out as described previously [2]. For the reverse transcription-polymerase chain reaction (RT-PCR) and quantitative (qPCR), total RNA was extracted from 1×10^6 cells and examined using the Thermal Cycler Dice Real Time System (TaKaRa Bio.) according to the manufacturer's instructions. Primers used were:

BCL2-F: TCGCCCTGTGGATGACTGAG
BCL2-R: CAGAGTCTTCAGAGACAGCCAGGA
BCL2A1-F: CATCAAGAAACTTCTACGACAGCA
BCL2A1-R: AGTCATCCAGCCAGATTTAGGTTC
67LR-F: GCCATTGAAAACCCTGCTG
67LR-R: GCTGCCTGGATCTGGTTAGTG

Results and discussion

Incubation with EGCG in HL-60 cells resulted in appearance of apoptotic bodies (Fig. 1) and a formation of DNA ladder, the characteristic features of apoptosis. Apoptosis-induction was further supported by the finding that a non-specific caspase inhibitor Z-Asp-CH_2-DCB prevented this formation. Previously, it has already been reported that tea polyphenols induced apoptosis in these cells [3].

When PMA-, ATRA- and VD3-differentiated HL-60 cells were incubated with EGCG, the cell survival rate of them became much higher than that of the non-differentiated cells. The results of RT-PCR and qPCR indicated that the gene expression of Bcl-2A1, which inhibits apoptosis [4], was increased in all differentiated HL-60 cells. The Bcl-2 mRNA level was decreased by PMA and ATRA treatments.

These results suggest that EGCG induces apoptosis more effectively in cancer cells than in normal cells, and that it may be useful as a chemopreventive agent.

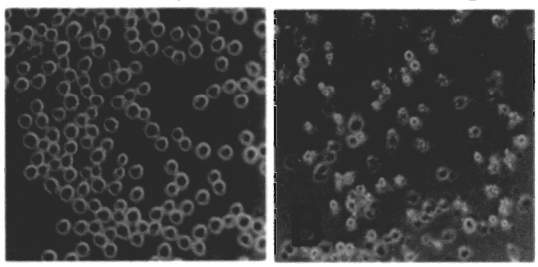

Fig. 1 Detection of apoptotic bodies in the case of HL-60 cells incubated with EGCG at 50 μM for 4 hr. A, untreated cells; B, EGCG-treated cells

References

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