MECHANISM OF APOPTOSIS INDUCTION SELECTIVE FOR CANCER CELLS BY EGCG

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Summary

Many researches have reported anti-cancer activity of (-)-epigallocatechin-3-gallate (EGCG) and its cancer cell specificity. However, there have been few reports on the mechanism. In our previous study, we have disclosed that Fas protein is the first acting target in apoptosis induction by EGCG. In this study, we investigated the interaction between EGCG and TRAIL-R3 (DcR1), TRAIL-R4 (DcR2), DcR3, and TRAIL-R5 (OPG), which are decoy receptor family proteins, to see whether there is a relation to cancer cell-specific activity of EGCG. EGCG inhibited cell growth and induced DNA fragmentation in transformed WI-38VA cells, but did not affect normal cells WI-38 up to 200 μM. RT-PCR showed that these decoy receptors were strongly expressed in normal cells, whereas there were a little differences in mRNA expression of death receptors. The results of the binding assay between EGCG and TNF receptor family proteins using an EGCG affinity column showed that EGCG bound to DcR3 and OPG. These results suggest that lower expression of decoy receptors in transformed cells may explain their higher sensitivity to apoptosis-induction by EGCG as compared to normal cells.

Key words

EGCG, WI-38, WI-38VA, DcR3, OPG

Introduction

It has been reported cancerous cells are more sensitive to (EGCG)-induced apoptosis than normal counterparts. However, the reason why EGCG induces apoptosis in cancerous cells selectively is not known. We have disclosed that Fas protein is the first acting target in apoptosis-induction by EGCG. In this study, we examined a possibility that the selective apoptosis-inducing activity of EGCG may be caused by the interaction between EGCG and cell surface receptor proteins, using WI-38 and WI-38VA.

Material and Methods

Both types of cells were treated with EGCG for 17 hr, DNA were extracted and analyzed for DNA fragmentation. From both types of cells total RNA was extracted, and gene expression levels were compared by RT-PCR using target primers (TNFR, Fas, DcR1, DcR2, DcR3, OPG). Total proteins were extracted and expression of OPG and DcR3 was analyzed by Western blotting. The supernatant from WI-38 cells cultured for 48 hr was analyzed by affinity chromatography using an EGCG-Sepharose column, and by Western blotting using antibodies against OPG and DcR3.

Results and Discussion

Firstly, although EGCG at least up to 200 μM did not affect WI-38, EGCG suppressed proliferation of WI-38VA and induced DNA fragmentation in WI-38VA even at 50 μM (Fig. 1). Secondly, RT-PCR showed that decoy receptor (DcR2, DcR3 and OPG) mRNAs were expressed more in WI-38 cells, while expressions of death receptors were at similar levels (Fig. 2, Fig. 3). Thirdly, Western blotting analysis showed that higher protein levels of DcR3, OPG, and TNF-R2 in WI-38 cells than in WI-38VA cells (Fig. 4). Finally, EGCG affinity chromatography and Western blotting analysis demonstrated the binding interaction of EGCG with OPG and DcR3 (Fig. 5). These results suggest that lower expression of decoy receptors in transformed cells may explain their higher sensitivity to apoptosis induction by EGCG as compared to normal cells.

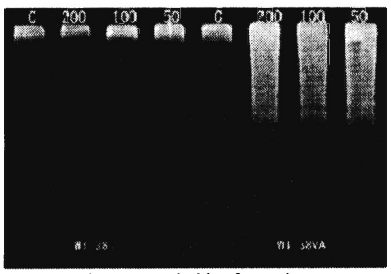

Fig.1. DNA ladder formation

Fragmentation of DNA from WI-38 and WI-38VA cells treated with EGCG at concentration indicated (μM) for 17 hr.

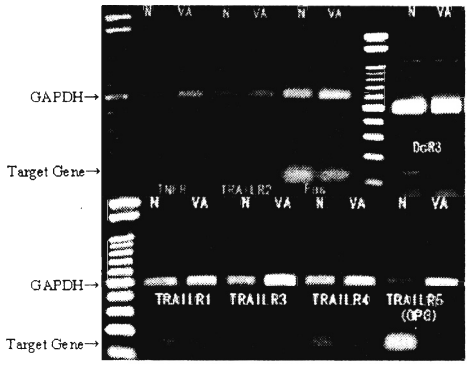

Fig. 2. Expression of TNF receptor family mRNA
N: WI-38 VA: WI-38VA

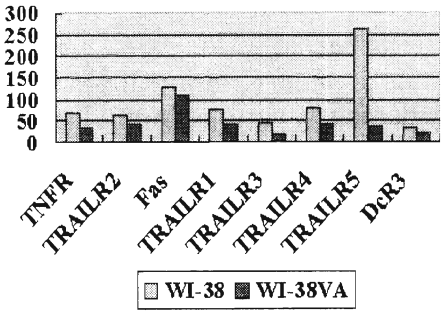

Fig. 3. Expression level of TNF receptor family mRNA

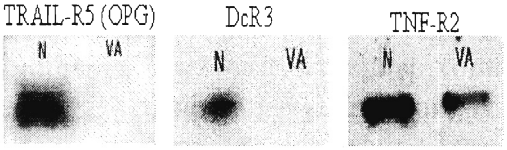

Fig. 4. Protein production levels (Western blotting)

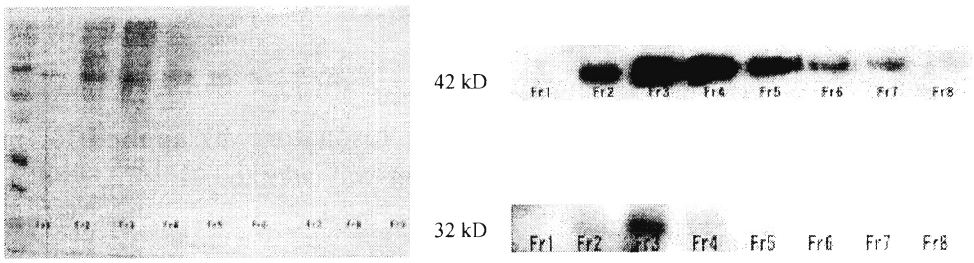

Fig. 5. Coomassie Brilliant Blue straining (left) and Western blotting of fractions bound to and eluted with 4M urea / 1M NaCl from an EGCG affinity column (right)