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Green tea catechin (–)-epicatechin gallate induces tumour suppressor protein ATF3 via EGR-1 activation

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ARTICLE INFO

Article history:
Received 9 May 2007
Received in revised form 12 July 2007
Accepted 20 July 2007
Available online 30 August 2007

Keywords:
ATF3
ECG
EGCG
EGR-1

ABSTRACT

Epicatechin gallate (ECG) is the third major catechin component in green tea, but it shows strong biological activity in some aspects, including apoptosis, cell growth inhibition, and membrane transport system in various cells. We previously reported that ECG induces activating transcription factor 3 (ATF3), which is involved in pro-apoptosis in HCT-116 cells. In this report, we present a molecular mechanism by which ECG induces ATF3 expression at the transcriptional level. We found that Sp3 contributed to the basal expression of the ATF3 gene, whereas EGR-1 played an important role in ECG-induced ATF3 expression in HCT-116 cells, as assessed by EMSA and co-transfection experiments. These results suggested that EGR-1, a tumour suppressor protein, could substantiate ECG's role of ATF3 expression in human colorectal cancer cells. We also found that pro-oxidant activity of ECG contributed to ECG-induced ATF3 expression.

1. Introduction

Green tea (*Camellia sinensis*) is the most widely consumed beverage in the world, next to water. Tea contains large amounts of flavonoids, and the major flavonoids in green tea are catechins, which include epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC). EGCG is the most abundant catechin in green tea, and has been reported to have biological activities, such as anti-oxidative,[1] pro-oxidative[2] and anti-inflammatory effects[3,4] in a variety of experimental models. Although ECG is the third most abundant constituent amongst the green tea catechins, much attention has been focused on its anti-tumourigenic activity, due to distinguished features of ECG compared to EGCG. For example, it has been reported that ECG has anti-angiogenic,[5] and anti-oxidant[6] activities. We have recently reported that ECG has an anti-tumourigenic effect; it increased the G1-sub population, cleaved poly (ADP-ribose) polymerase (PARP) in HCT-116 cells,[7] and suppressed cyclin D1 and β-catenin pathways in mouse oral SCC7 cancer cells.[8] However, the molecular targets that might be involved in ECG-induced anti-tumourigenesis have not yet been reported.

Activating transcription factor 3 (ATF3) is a member of the ATF/CREB family, and is induced upon exposure of cells to a variety of physiological and pathological stimuli.[9] This response is thought to have cell-defending effects, such as cell cycle arrest and apoptosis.[10,11] On the other hand, ATF3 is also rapidly induced in regenerating liver,[12] or in cells treated with growth-stimulating factors such as serum, epidermal growth factor or fibroblast growth factor.[13] These conflicting results may depend on stimuli or cell types used in the studies. In HCT-116 cells, ATF3 is reported to be increased by nonsteroi-

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0959-8049/$ - see front matter
doi:10.1016/j.ejca.2007.07.020

dal anti-inflammatory drugs (NSAID),[14] conjugated linoleic acid,[15] LY294002,[16] and 3, 3′-diindolylmethane,[17] which are shown to have anti-tumourigenic activity in human colorectal cancer cells. In this study, we focused on the transcriptional regulation of ATF3, affected by green tea catechins. We found that early growth response gene-1 (EGR-1) is involved in ECG-induced ATF3 expression, whereas Sp3 contributed to the basal expression of ATF3 in HCT-116 cells. In addition, ATF3 expression by ECG may result from the oxidative stress generated by ECG in the cell culture media.

2. Materials and methods

2.1. Cell lines, reagents and plasmids

Cell lines were purchased from ATCC (Rockville, MD). HCT-116 and SW480 human colorectal cells were maintained in McCoy's 5A and RPMI medium, respectively, supplemented with 10% foetal bovine serum and gentamicin (10 μg/ml). Catechins (EGCG, ECG, EGC and EC), glutathione (GSH), H_2O_2, and catalase were purchased from Sigma (St Louis, MO). NAG-1 (nonsteroidal anti-inflammatory drug-activated gene-1) antibody was described previously.[18] ATF3, Actin, EGR-1, Sp1 and Sp3 antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The *pATF3* −1850/+34 and *pATF3* −84/+34 luciferase reporter vectors were provided by Dr. S. Kitajima (Tokyo Medical and Dental University, Tokyo, Japan). For the deletion clones of the *ATF3* promoter, *pATF3* −1850/+34 was used and serially deleted using the Erase-a-Base System (Promega, WI). The EGR-1, Ftz and CRE sites between the −514 and +34 region of the *ATF3* promoter were deleted using the QuickChangeII site directed Mutagenesis Kit (Stratagene, TX). To delete these sites, the following primers were used: del EGR-1 F, 5′-GCTGGTGTGTGTCTCAGTGAGGGAACGCGC-3′; del EGR-1 R, 5′-GCCAGCCCAGGCGCGTTCCCTCACTGAGAC-3′; del Ftz F, 5′-GTTCGGCCGGTTCTCCCGGGTAGCATTACG-3′; del Ftz R, 5′-CCCAGGCTGACGTAATGCTACCCGGGAGAA-3′; del CRE F, 5′-CGGGAAGCTATTAATAGCATGCCTGGGACT-3′; and del CRE R, 5′-CCGTGTTGCCAGTCCCAGGCATGCTATTAA-3′. *EGR-1* (pcDNA3.1-EGR-1/NEO) and Sp3 (pCMV4-Sp3flu) expression vectors were previously described[19,20].

2.2. Reverse transcription-polymerase chain reaction (RT)-PCR

Total RNA was extracted using TRIZOL reagent (Invitrogen, Carlsbad, CA), and 5 μg of total RNA was reverse transcribed using an iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA). One μl of synthesised cDNA was added to a 25 μl PCR reaction mixture with human *ATF3* gene-specific primers (F: 5′-GTTTGAGGATTTTGCTAACCTGAC-3′ and R: 5′-AGCTGCAATTCTTATTTCTTTCTCGT-3′) and human glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene-specific primers (F: 5′-TCAACGGATTTGGTCGTATT-3′ and R: 5′-CTGTGGTCATGAGTCCTTCC-3′). The thermal cycling conditions were as follows: initial denaturation at 94 °C for 2 min, followed by 30 and 25 cycles (ATF3 and GAPDH, respectively) of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1

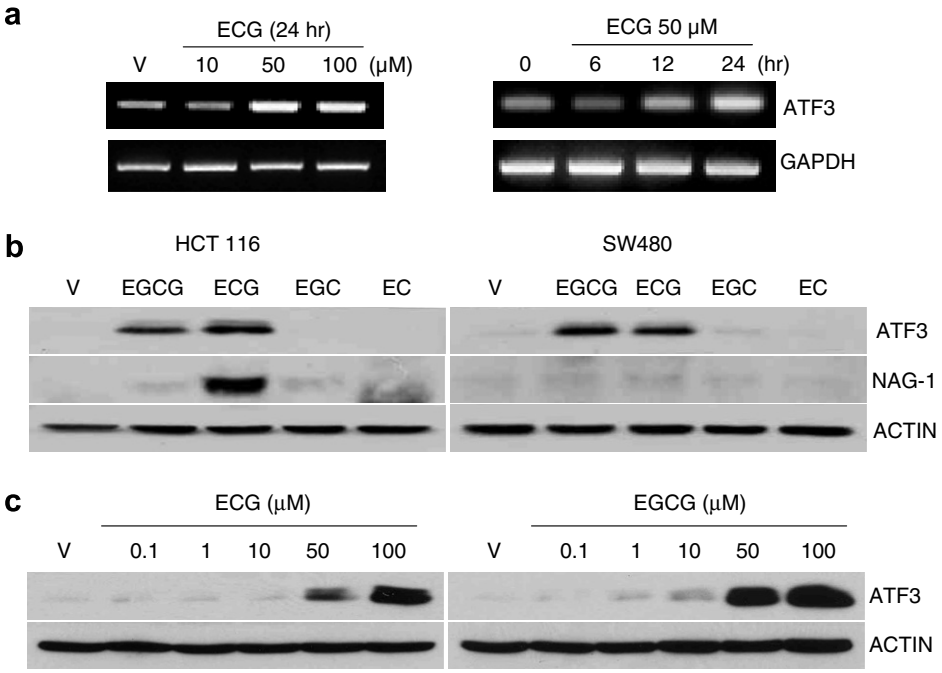

Fig. 1 – ECG induces ATF3 expression in colorectal cancer cells. (a) HCT-116 cells were treated with the indicated concentration of ECG for 24 h. RT-PCR was performed using ATF3- and GAPDH-specific primers as described in the Materials and Methods section. (b) HCT-116 and SW480 cells were treated with 50 μM of EGCG, ECG, EGC and EC, respectively for 24 h. Thirty μg of total cell lysates were loaded, and Western analysis was performed using ATF3 (1:500), NAG-1 (1:1,000) and Actin (1:500) antibodies. (c) HCT-116 cells were treated with 0, 0.1, 1, 10, 50, and 100 μM of ECG and EGCG for 24 h. Thirty μg of total cell lysates were loaded, and Western analysis was performed using ATF3 and Actin antibodies.