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The coffee diterpene kahweol sensitizes TRAIL-induced apoptosis in renal carcinoma Caki cells through down-regulation of Bcl-2 and c-FLIP

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ABSTRACT

Kahweol, a coffee-specific diterpene, found in the beans of *Coffea arabica*, has potent anti-carcinogenic, anti-tumor, and anti-inflammatory properties. TRAIL is a potential anti-cancer compound that induces apoptosis in a wide variety of cancer cells, but not in most normal human cell types. In the present study, we show that kahweol sensitizes human renal cancer cells, but not normal human mesangial cells, to TRAIL-mediated apoptosis. Moreover, treatment with a combination of kahweol and TRAIL induces significant apoptosis in various cancer cell types, thus presenting an attractive novel strategy for cancer treatment. Our experiments show that treatment with a combination of kahweol and TRAIL-induced apoptosis, and stimulated of DEVDase activity, DNA fragmentation, and cleavage of PARP, which was prevented by pretreatment with z-VAD, indicative of cell death via a caspase-dependent pathway. Kahweol-induced down-regulation of Bcl-2 and ectopic expression of Bcl-2 led to attenuation of kahweol plus TRAIL-mediated apoptosis, indicative of Bcl-2 involvement in the apoptotic process. In addition, the c-FLIP and caspase signal pathways seem to play a crucial role in apoptosis triggered by the combination of kahweol and TRAIL in Caki cells. Our results collectively demonstrate that down-regulation of Bcl-2 and c-FLIP contributes to the sensitizing effect of kahweol on TRAIL-mediated apoptosis in cancer cells.
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1. Introduction

Renal cancer carcinoma (RCC) represents approximately 2–3% of general malignancies and 80–85% of kidney tumors in adults [1,2]. RCC is resistant to both chemotherapy and radiotherapy [1,2]. Therefore, development of novel therapeutic strategies for treatment of malignant RCC is required. Recent studies have focused on tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) as a potential anti-cancer drug because the compound induces apoptosis in a wide variety of transformed and cancer cells but not in most normal human cell types [3,4]. However, some cancer cell types are resistant to the apoptotic effects of TRAIL [5,6]. TRAIL-resistant cancer cells can be sensitized by chemotherapeutic drugs *in vitro*, indicating that combination therapy is a possible treatment option. Therefore, to develop effective TRAIL-based cancer therapy, clarification of the molecular mechanisms of TRAIL resistance, and identification of sensitizers capable of overcoming TRAIL resistance are essential.

Kahweol is a diterpene molecule found in considerable quantities in coffee beans. The compound exhibits a wide variety of biological activities, including anti-carcinogenic, anti-tumor, and anti-inflammatory properties [7–10]. The anti-carcinogenic properties of kahweol may in part be due to the induction of phase II detoxifying, down-regulation of signal transducer and activator of transcription 3 (STAT3) [7,11]. We previously reported that kahweol induces apoptosis in human leukemia cells [12]. However, the precise mechanism underlying kahweol-induced sensitization of TRAIL is not fully understood. In the present study, we examined the capacity of kahweol to enhance TRAIL-mediated apoptosis, and explored the biochemical mechanisms underlying this process.

We investigated whether kahweol affected TRAIL-induced apoptosis in human renal cancer cells. Kahweol treatment selectively rendered human renal cancer cells, but not normal cells, more sensitive to the cytotoxic activity of TRAIL, suggesting that combined treatment with kahweol and TRAIL could be a safe and

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effective anti-cancer therapy. Moreover, Bcl-2 down-regulation induced by kahweol seemed to be involved in the mechanism of sensitization to TRAIL-induced apoptosis.

2. Materials and methods

2.1. Cells and materials

Caki, MDA231, SK-Hep1, and HT29 cells were obtained from the American Type Culture Collection (ATCC: Rockville, MD). Mouse kidney cells of the TMCK-1 line were the kind gift of Dr. T.J. Lee (Yeungnam University, Korea). The culture medium used in all experiments was Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 20 mM HEPES buffer, and 100 μg/ml gentamicin. Primary human mesangial cells (Cryo NHMC) and the corresponding growth medium (CC-3146 MsGM) were acquired from Lonza Walkersville Inc. (Walkersville, MD). Kahweol acetate (Fig. 1) was purchased from LKT Laboratories, Inc. (W St. Paul, MN). A 50 mM solution of kahweol was initially prepared in DMSO, stored as small aliquots at −20 °C, and thawed and diluted in cell culture medium, as required. Recombinant human TRAIL/Apo2 ligand (the nontagged 19 kDa protein, amino acid residues 114–281) was purchased from KOMA Biotech Inc. (Seoul, Korea). Anti-Bcl-2, anti-Bax, anti-Bcl-xL, anti-c-IAP1, anti-XIAP, anti-procaspase-3, anti-PARP, anti-FADD, anti-DR5, anti-DR4, anti-Mcl-1, anti-c-FLIP, anti-NF-κB p65, anti-Ref and anti-ERK antibodies were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Kahweol, Asp-Glu-Val-Asp-chromophore-p-nitroanilide (DEVD-pNA) and benzyloxy carbonyl-Val-Ala-Asp-fluoromethyl ketone (z-VAD-fmk) were purchased from Biomol (Plymouth Meeting, PA).

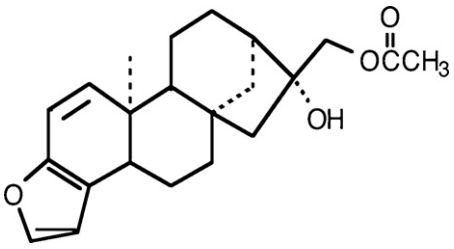

Fig. 1. Chemical structure of kahweol.

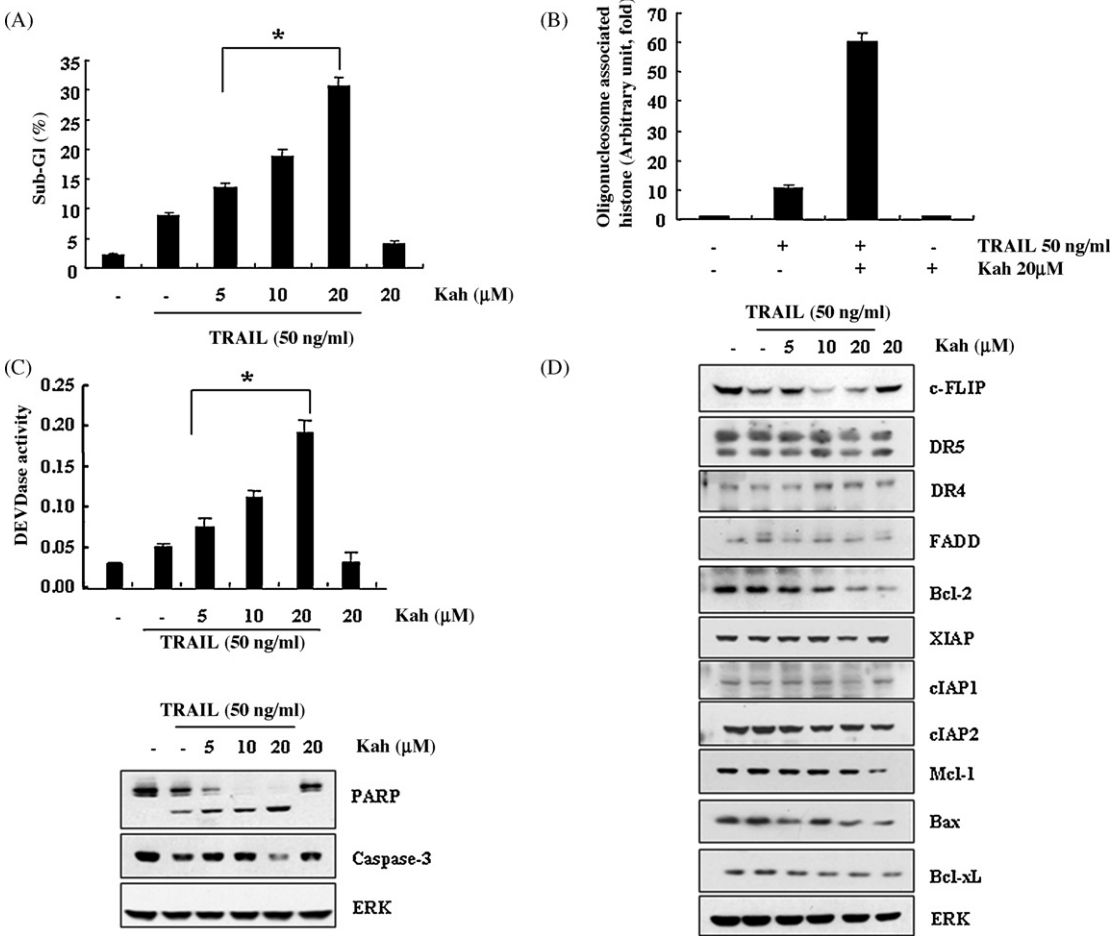

Fig. 2. Kahweol sensitizes cancer cells to TRAIL-induced apoptosis. (A) Caki cells were treated for 24 h with TRAIL (50 ng/ml), either in the absence or the presence of kahweol (5, 10 and 20 μM). After 24 h, apoptosis was analyzed as the proportion of cells in the sub-G1 fraction, as assessed by FACS. Data are presented as means obtained from three independent experiments and bars represent standard deviations. The statistical test was Student's *t*-test for unpaired values. $^*p < 0.05$ vs. control cells. (B) Fragmentation of genomic DNA in Caki cells treated for 24 h with the indicated concentrations of kahweol and TRAIL. DNA fragmentation in Caki cells was determined using a DNA fragmentation detection kit. Data are presented as means ± SD ($n = 3$). (C) Activation of caspases during kahweol-sensitized TRAIL-induced apoptosis. Cells were treated with the indicated concentrations of kahweol and TRAIL. DEVDase activity was determined as described in Section 2. Data are presented as mean values from three independent experiments, and bars represent standard deviations. The statistical test was Student's *t*-test for unpaired values. $^*p < 0.05$ vs. control cells (Top). Equal amounts of cell lysates (40 μg) were subjected to electrophoresis and analyzed by Western blotting for PARP, procaspase-3, and ERK (as control normalization) (Bottom). (D) Equal amounts of cell lysates (40 μg) were subjected to electrophoresis and analyzed by Western blotting for c-FLIP, DR5, DR4, FADD, Bcl-2, XIAP, cIAP1, cIAP2, Mcl-1, Bax, Bcl-xL, and ERK for normalization.

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