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The coffee diterpene kahweol suppresses the cell proliferation by inducing cyclin D1 proteasomal degradation via ERK1/2, JNK and GKS3β-dependent threonine-286 phosphorylation in human colorectal cancer cells

Gwang Hun Park, Hun Min Song, Jin Boo Jeong*

Department of Medicinal Plant Resources, Andong National University, Andong 36729, Republic of Korea

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ABSTRACT

Kahweol as a coffee-specific diterpene has been reported to exert anti-cancer properties. However, the mechanism responsible for the anti-cancer effects of kahweol is not fully understood. The main aim of this investigation was to determine the effect of kahweol on cell proliferation and the possible mechanisms in human colorectal cancer cells. Kahweol inhibited markedly the proliferation of human colorectal cancer cell lines such as HCT116, SW480. Kahweol decreased cyclin D1 protein level in HCT116 and SW480 cells. Contrast to protein levels, cyclin D1 mRNA level and promoter activity did not be changed by kahweol treatment. MG132 treatment attenuated kahweol-mediated cyclin D1 downregulation and the half-life of cyclin D1 was decreased in kahweol-treated cells. Kahweol increased phosphorylation of cyclin D1 at threonine-286 and a point mutation of threonine-286 to alanine attenuated cyclin D1 degradation by kahweol. Inhibition of ERK1/2 by PD98059, JNK by SP600125 or GSK3β by LiCl suppressed cyclin D1 phosphorylation and downregulation by kahweol. Furthermore, the inhibition of nuclear export by LMB attenuated cyclin D1 degradation by kahweol. In conclusion, kahweol-mediated cyclin D1 degradation may contribute to the inhibition of the proliferation in human colorectal cancer cells.

1. Introduction

In worldwide, colorectal cancer is one of the most common human malignancies with high rate of morality (Siegel et al., 2014). Although the surgery and adjuvant therapy have been regarded as the most effective treatment for human colorectal cancer, the morality rate by colorectal cancer remains high. Thus, the complementary and alternative medicine is considered because of ineffectiveness of these therapeutic approaches and chemoprevention using phytochemicals has received attention as an attractive and promising strategy for human cancer (Wang et al., 2012).

There is growing evidence that coffee consumption in humans can reduce the cancer incidence, including colorectal cancer (Giovannucci, 1998; Sang et al., 2013; Yu et al., 2011). In animal studies using rodents, coffee administration has been reported to decrease the incidence of spontaneous tumors (Stalder et al., 1990; Wurzner et al., 1977). In addition, other studies have reported that coffee exerts protective properties against tumorigenesis by the well-known carcinogens such as nitrosamines, 1,2-dimethylhydrazine and 7,12-dimethylbenz[a]anthracene (Gershbein, 1994; Miller et al., 1988; Nishikawa et al., 1986; Wattenberg, 1983). A thousand of components contained in coffee beans have been identified as being potentially responsible for the cancer chemoprotective effects of coffee. (Cavin et al., 2002).

Among coffee components, kahweol as a diterpene molecule has been regarded as one of the compounds responsible for cancer chemoprevention. Kahweol exerts anti-cancer activity against oral squamous cancer (Chae et al., 2014), breast cancer (Cardenas et al., 2014), pleural mesothelioma (Lee et al., 2012), renal carcinoma (Um et al., 2010) and lung cancer (Kim et al., 2009). In addition, kahweol induces the cytotoxicity through apoptosis and suppression of heat shock protein 70 expression in human colorectal cancer cells (Choi et al., 2015). Although kahweol has been shown to induce cell growth arrest in oral squamous cancer cells (Chae et al., 2014), the underlying mechanism in colorectal cancer cells is not fully

* Corresponding author. Department of Bioresource Sciences, Andong National University, Andong 760749, Republic of Korea.
E-mail address: jjb0403@anu.ac.kr (J.B. Jeong).

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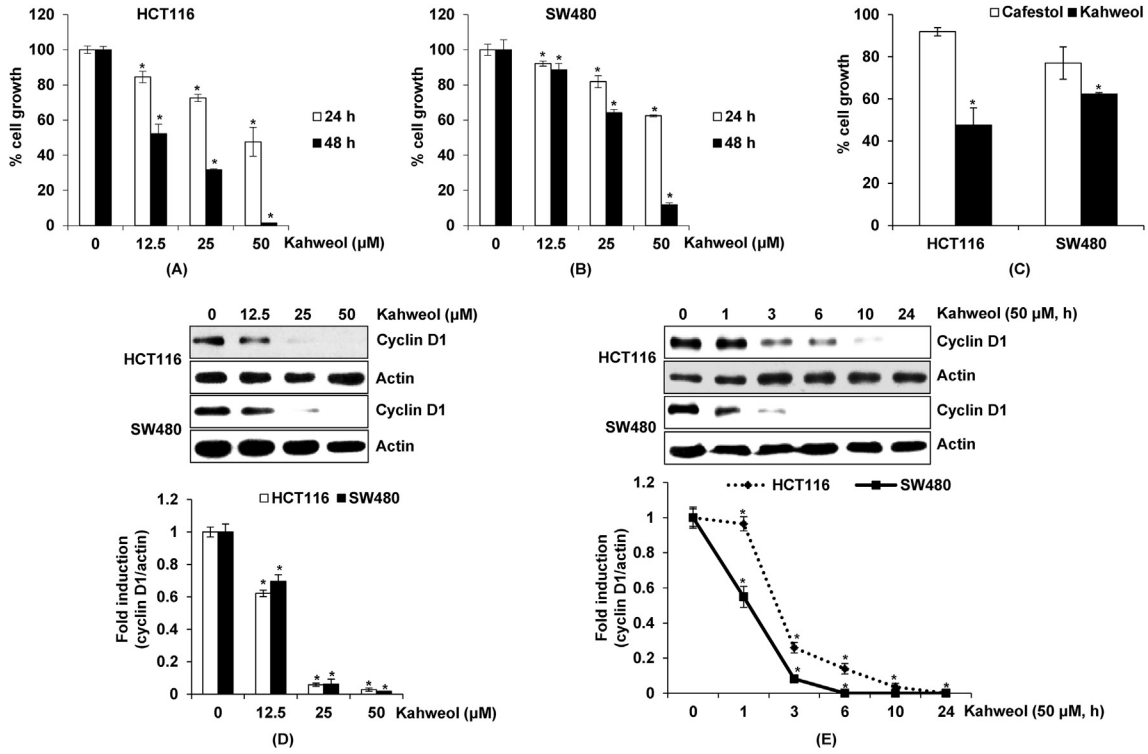

Fig. 1. Effect of kahweol on cell growth and cyclin D1 expression in human colorectal cancer cells. (A, B) HCT116 or SW480 cells (1×10^4/well) were plated overnight and then treated with kahweol at the indicated concentrations for 24 h or 48 h (C) HCT116 or SW480 cells (1×10^4/well) were treated with 50 μM of kahweol or 50 μM of cafestol for 24 h. Cell proliferation was measured using MTT assay as described in Materials and methods. *$P < 0.05$ compared to cell without treatment. (D) HCT116 and SW480 cells (2×10^6/well) were plated overnight and then treated with kahweol at the indicated concentrations for 24 h (E) HCT116 and SW480 cells (2×10^6/well) were plated overnight and then treated with 50 μM of kahweol for the indicated times. Cell lysates were subjected to SDS-PAGE and the Western blot was performed using antibody against cyclin D1. Actin was used as internal control for Western blot analysis. Each bar shows the mean ± S.D. of three independent experiments, performed in triplicate.

understood.

Cyclin D1 as an important regulator of the G1-to-S phase transition has been observed to be overexpressed in various cancer types such as lymphoid, breast, esophageal, lung and bladder tumors (Diehl, 2002; Landis et al., 2006; Li et al., 2006; Sherr, 1996). In human colorectal cancer, cyclin D1 has been reported to be overexpressed in 68.3% of cancer case, which indicates that deregulation of cyclin D1 is associated with colon tumorigenesis (Bahnassy et al., 2004; Holland et al., 2001). Therefore, it has been accepted that the control of cyclin D1 level may provide a promising chemopreventive and therapeutic way for human colorectal cancer.

In this study, we assessed the effect of kahweol on cell growth and elucidated the molecular mechanism for kahweol-mediated cyclin D1 downregulation in human colorectal cancer cells.

2. Materials and methods

2.1. Chemical reagents

Cell culture media, Dulbecco's Modified Eagle medium (DMEM)/F-12 1:1 Modified medium (DMEM/F-12) was purchased from Lonza (Walkersville, MD, USA). PD98059, SB203580, SP600125, LiCl, BAY11-7082, MG132, cycloheximide (CHX), leptomycin B (LMB), 3-(4,5-dimethylthizaol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and kahweol (≥95%) were purchased from Sigma Aldrich (St. Louis, MO, USA). Antibodies against cyclin D1, phospho-cyclin D1 (Thr286), phospho-ERK1/2, total-ERK1/2, phospho-JNK, total-JNK, phospho-GSK3β, total-GSK3β, HA-tag and β-actin were purchased from Cell Signaling (Bervely, MA, USA). All chemicals were purchased from Fisher Scientific, unless otherwise specified.

2.2. Cell culture and treatment

Human colon cancer cell lines, HCT116 and SW480 were purchased from Korean Cell Line Bank (Seoul, Korea) and grown in DMEM/F-12 supplemented with 10% fatal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin. The cells were maintained at 37 °C under a humidified atmosphere of 5% CO_2. Kahweol was dissolved in dimethyl sulfoxide (DMSO) and treated to cells. DMSO was used as a vehicle and the final DMSO concentration did not exceed 0.1% (v/v).

2.3. Cell proliferation assay

Cell growth was measured using MTT assay system. Briefly, cells (1×10^4/well) were grown in 96-well plate overnight. The cells were treated with 0, 12.5, 25 and 50 μM of kahweol for 24 and 48 h. Then, the cells were incubated with 50 μl of MTT solution (1 mg/ml) for an additional 2 h. The resulting crystals were dissolved in DMSO. The formation of formazan was measured by reading absorbance at a wavelength of 570 nm.

2.4. Reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA was prepared using a RNeasy Mini Kit (Qiagen, Valencia, CA, USA) and total RNA (1 μg) was reverse-transcribed using a Verso cDNA Kit (Thermo Scientific, Pittsburgh, PA, USA) according to the manufacturer's protocol for cDNA synthesis. PCR was carried out using PCR Master Mix Kit (Promega, Madison, WI, USA) with human primers for cyclin D1 and GAPDH as followed: cyclin D1: forward 5′-aactacctggaccgcttcct-3′ and reverse 5′-