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Original article

Diallyl trisulfide induces apoptosis and mitotic arrest in AGS human gastric carcinoma cells through reactive oxygen species-mediated activation of AMP-activated protein kinase

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ABSTRACT

Diallyl trisulfide (DATS), one of the principal constituents of garlic oil, is a kind of organosulfur compound with high anti-cancer activity. Although inhibition of cancer cell proliferation by DATS is known to be associated with the induction of apoptosis and cell cycle arrest related to reactive oxygen species (ROS) production, it is still necessary to study the detailed mechanisms. In this study, we investigated the role of ROS on the activation of AMP-activated protein kinase (AMPK) in DATS-induced apoptosis and cell cycle arrest in AGS human gastric carcinoma cells. The results of the present study indicate that DATS inhibited proliferation of AGS cells by promoting apoptosis, and accumulating cellular portion of G2/M phase *via* the induction of cyclin B1 and cyclin-dependent kinase p21(WAF1/CIP1). The phosphorylation of histone H3 was also markedly increased following treatment with DATS, revealing that DATS stimulated a mitotic arrest, not the G2 phase. Furthermore, we found that DATS concurrently induced phosphorylation of AMPK; however, chemical inhibition of AMPK by compound C, an AMPK inhibitor, significantly blocked apoptosis induced by DATS, suggesting that DATS induces cytotoxicity of AGS cells through the AMPK-dependent pathway. Moreover, DATS provoked intracellular ROS generation and the loss of mitochondrial membrane potential, and in particular, when ROS production was blocked by antioxidant N-acety-L-cysteine, both AMPK activation and growth inhibition by DATS were completely abolished. Collectively, these findings suggest that DATS inhibited growth of AGS cells, which was mediated by complex interplay between cellular mechanisms governing redox homeostasis, apoptosis, and cell cycle arrest, through a ROS-dependent activation of AMPK pathway.

1. Introduction

Cell cycle deregulation, which leads to uncontrolled cell proliferation, is one of the most frequent changes that occur during tumorigenesis. Thus, effective strategies to inhibit the abnormal proliferation of cancer cells are the progression of the cell cycle and/or targeting of the machine. Induction of apoptosis, a programed cell death, with cell cycle control has also been evaluated as a key mechanism for the removal of cancer cells [1,2]. Therefore, appropriate treatment methods for overcoming these problems in cancer cells without affecting normal cells are recognized as comprising the most fundamental process of cancer treatment [3,4]. Cancer cells also often exhibit several types of mitochondrial dysfunction, including mitochondrial DNA mutations, changes in energy metabolism, increased reactive oxygen species (ROS) production, and increased mitochondrial membrane potential (MMP), which may be key targets for cancer cell proliferation blocking [5,6]. In particular, since the loss of MMP damages mitochondrial function and induces ROS production, natural products that can control intracellular energy and metabolism have recently become the targets of promising anticancer drugs [6,7].

Diallyl-trisulfide (DATS) is one of the naturally organosulfur compounds derived from the Allium genus, which includes garlic [8,9]. This compound has a strong antioxidant and anti-inflammatory potential, and has proven to be an important candidate cancer preventive with high activity that inhibits the proliferation of cancer cells, and induces apoptosis in many cancers [10,11]. Although inhibition of the proliferation by DATS in some cancer

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cell lines has been reported to be associated with G1 arrest in the cell cycle [12,13], many studies have shown DATS induces G2/M arrest that is associated with apoptosis in multiple cancer types [14–18]. The effects of DATS on cell cycle progression have also been reported to be specific for mitotic arrest, but not G2 phase [14,18–21]. In addition, it has been shown in various previous studies, including the results of our laboratory, that ROS act as key regulators in the induction of apoptosis by DATS [9,16,22–25].

AMP-activated protein kinase (AMPK), a serine/threonine kinase, is a metabolic-sensing protein kinase that plays an essential role in regulating cellular energy homeostasis during cell proliferation [7,26]. AMPK is activated in response to the phosphorylation of the critical amino acid residue Thr172, in response to an increase in the ratio of intracellular AMP to ATP, and phosphorylation by several upstream AMPK kinases [27]. A number of studies have shown that AMPK plays an important role in cell cycle inhibition and induction of pro-apoptotic pathways, often accompanied by increased production of intracellular ROS in many cancer cell types [28,29]. Therefore in recent years, AMPK signaling has been considered as an attractive therapeutic target molecule for cancer treatment. Recently, it has been shown that allicin, a major ingredient of fresh garlic extract, which quickly changes into a series of other sulfur-containing compounds such as DATS, promotes tumor suppressor p53-mediated autophagy in human hepatocellular carcinoma cells, through activation of AMPK signaling [30]. However, the correlation between the ROS generation and AMPK activation in DATS-induced cell cycle arrest and apoptosis has not yet been identified. Therefore, in this study, we performed experiments to investigate the roles of ROS and AMPK in DATS-induced growth inhibition using AGS human gastric adenocarcinoma cells, and found that DATS promotes the AMPK activation through generation of intracellular ROS, and thereby induces mitotic arrest and apoptosis.

2. Materials and methods

2.1. Reagents and antibodies

DATS (allyl trisulfide, Di-2-propenyl trisulfide) was purchased from LKT Laboratories (St Paul, MN, USA). RPMI-1640 medium, fetal bovine serum (FBS), while other tissue culture reagents were obtained from WelGENE Inc. (Daegu, Republic of Korea). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), propidium iodide (PI), 4′,6-diamidino-2-phenylindole (DAPI), *N*-acetyl-L-cysteine (NAC), 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylimidacarbocyanine iodide (JC-1), 5-fluorouracil (5-FU) and sulforaphane were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). 5,6-carboxy-2′7′-dichlorofluorescin diacetate (DCF-DA) and annexin V-fluorescein isothiocyanate (FITC) apoptosis detection kit were purchased from Molecular Probes Inc. (Eugene, OR, USA) and BD Bioscience (San Jose, CA, USA), respectively. Primary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and Cell Signaling Technology

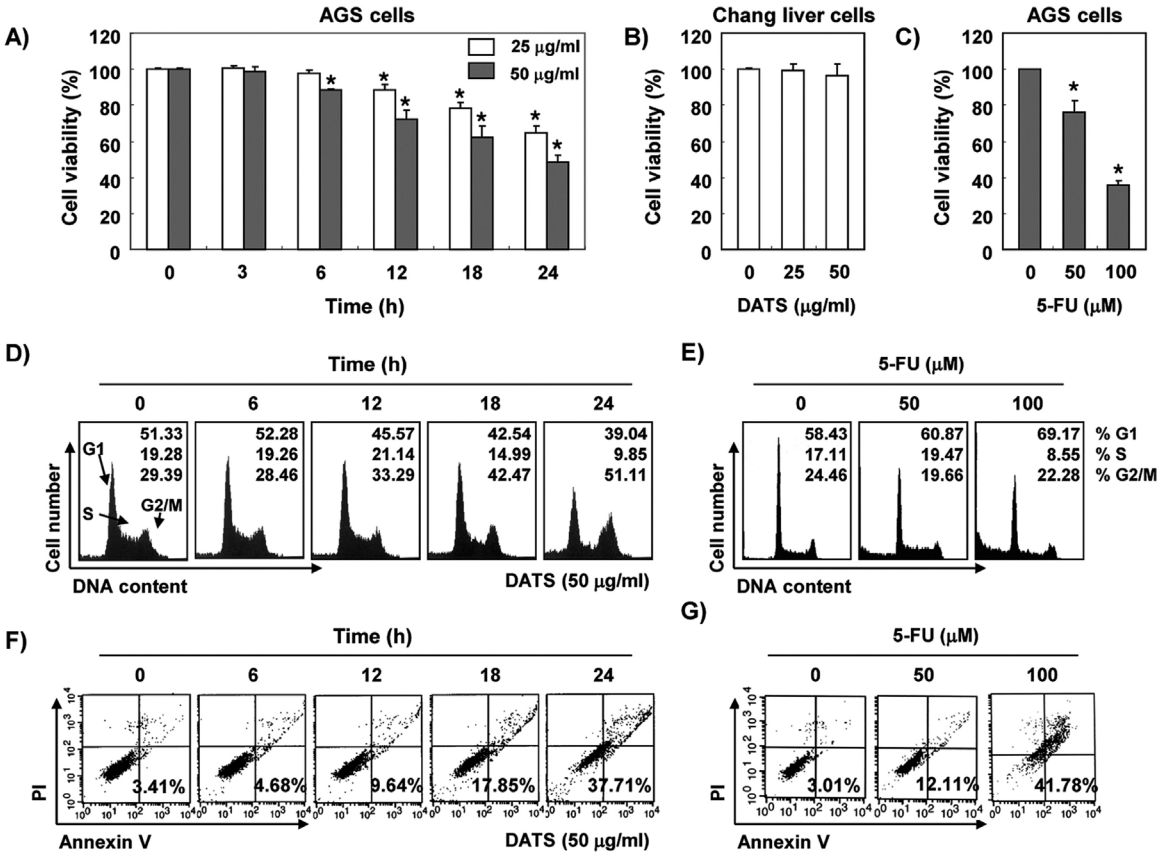

Fig. 1. Effects of DATS on the cell viability and cell cycle progression in AGS cells. (A–C) AGS (A and C) and Chang liver (B) cells were treated with the indicated concentrations of DATS or 5-FU as a positive control for the indicated times (A) or 24 h (B and C). Cell viability was determined by MTT assay. The data are the mean ± SD of three separate experiments (*, $p < 0.05$ vs. untreated control). (D and E) After treatment with 50 μM DATS for the indicated times (D) or 50 and 100 μM of 5-FU (E), the cells were stained with PI, and analyzed by flow cytometry. (F and G) The cells grown under the same conditions as (D and E) were collected and stained with annexin-V and PI, and the percentages of apoptotic cells were then analyzed using flow cytometric analysis. Each point represents the mean of two independent experiments.