Biochemical and Biophysical Research Communications 473 (2016) 586–592

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Bakuchiol sensitizes cancer cells to TRAIL through ROS- and JNK-mediated upregulation of death receptors and downregulation of survival proteins

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ARTICLE INFO

Article history:
Received 17 March 2016
Accepted 27 March 2016
Available online 28 March 2016

Keywords:
Bakuchiol
TRAIL
Death receptor
JNK
ROS

ABSTRACT

We investigated whether bakuchiol, an analog of resveratrol enhances the apoptosis ability of tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) in cancer cells. Bakuchiol enhanced expression of cell death receptor (DR) in TRAIL-sensitive and -resistant colon cancer cells in a dose-dependent manner. A combination of bakuchiol with TRAIL significantly inhibited cell growth of TRAIL sensitive HCT116 and TRAIL resistant HT-29 cells. The expression of TRAIL receptors; DR4 and DR5 was significantly increased by treatment of bakuchiol, however, the expression of survival proteins (e.g., cFLIP, survivin, XIAP and Bcl2) was suppressed. Moreover, the expression of apoptosis related proteins such as cleaved caspase-3, -8, -9 and PARP was increased by combination treatment of bakuchiol and TRAIL. Depletion of DR4 or DR5 by small interfering RNA significantly reversed the cell growth inhibitory effects of bakuchiol in HCT116 and HT-29 cells. Pretreatment with the c-Jun N-terminal kinase (JNK) inhibitor SP600125 and the reactive oxygen species (ROS) scavenger N-acetylcysteine reduced the bakuchiol induced cell growth inhibitory effects. The collective results suggest that bakuchiol facilitates TRAIL-induced apoptosis in colon cancer cells through up-regulation of the TRAIL receptors; DR4 and DR5 via ROS/JNK pathway signals.

1. Introduction

Tumor necrosis factor (TNF)-α-related apoptosis-inducing ligand (TRAIL), a member of the TNF ligand superfamily, is considered a potential agent for cancer therapeutics due to its ability to induce apoptosis selectively in a variety of cancer cells without toxicity in normal human cells [1,2]. Although many cancer cells express functional TRAIL receptors; death receptor 4 (DR4) and DR5, resistance to TRAIL is common because decreased level or mutation of DR4 and DR5 or the loss of distal signaling cascades [3,4]. For these reasons TRAIL alone may not be sufficient to treat many malignant tumors. Sensitization of cancer cells to TRAIL can be restored by treatment with subtoxic concentration of chemotherapeutic drugs through upregulation of TRAIL receptors, DR4 and DR5 [5,6]. Several recent studies have suggested that the DR4 and DR5 TRAIL receptors are up-regulated by different mechanisms such as MAPKs including extracellular signal-regulated kinases (ERK)1/2, p38 MAPK and c-Jun NH2-terminal kinase (JNK), reactive oxygen species (ROS) and C/EBP homologous transcription factor (CHOP) [7–9].

Bakuchiol, a prenylated phenolic monoterpene isolated from the seeds of *Psoralea corylifolia* L. (Leguminosae) and commonly used in traditional Chinese and Indian folkloric medicine as a kidney-tonifying agent for alleviating asthma, diarrhea and osteoporosis osteoporosis [10–12]. Bakuchiol also induces caspase-3-dependent apoptosis through the activation of JNK (c-jun N-terminal kinase), followed by Bax translocation into the mitochondria in rat liver myofibroblasts [13]. Recent study has demonstrated that bakuchiol has anti-tumor effect in lung cancer cells through S phase arrest, caspase 9/3 activation, p53 and Bax up-regulation and Bcl-2 down-regulation by reactive oxygen species-related apoptosis [12]. Especially, bakuchiol is an analog of resveratrol (3,5,4-trihydroxy-

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http://dx.doi.org/10.1016/j.bbrc.2016.03.127
0006-291X/

trans-stilbene) that is a phytoalexin found in grapes, berries, and peanuts, and is one of the most promising agents for cancer prevention [14–16]. Moreover, resveratrol enhances antitumor activity of TRAIL in prostate cancer xenografts through activation of FOXO transcription factor [17], resveratrol sensitized melanomas to TRAIL through modulation of antiapoptotic gene expression [18], resveratrol-mediated sensitization to TRAIL-induced apoptosis depends on death receptor and mitochondrial signaling [19] and resveratrol is a potent sensitizer of tumor cells for tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis through p53-independent induction of p21 and p21-mediated cell cycle arrest associated with survivin [20]. We hypothesized that the bakuchiol that is an analog of resveratrol can enhance the sensitivity of cancer cells to TRAIL. In this study, we demonstrated that the bakuchiol enhances antitumor activity of TRAIL in colon cancer cells.

2. Materials and methods

2.1. Materials

Soluble Recombinant human Apo2L/TRAIL was purchased from Peprotech (Rocky Hill, NJ).

2.1.1. Extraction of bakuchiol from P. corylifolia

Dried seeds of *P. corylifolia* L were extracted with boiling distilled water for 3 h and the extract concentrated, and partitioned with n-butanol. The butanol fraction was dissolved with methanol, and partitioned with CH2CL2 to give a CH2CL2 fraction. The bioactive CH2CL2 fraction was separated by sephadex LH-20 column chromatography to obtain 3 fractions (A–C). The fraction B was chromatographed on a Sephadex LH-20 column with CH2Cl2: MeOH (40:1) to give 3 fractions (B1–B3). Fraction B1 was separated by silica gel column chromatography (n-hexane: EtOAc (4:1)) to yield (S)-bakuchiol. All extracts were quantitatively analyzed by HPLC system and a UV detector. The detector wavelength was set at 245 nm.

2.2. Cell culture and regents

HCT116 (human colorectal carcinoma) and HT-29 (human colorectal adenocarcinoma) cancer cells were obtained from the American Type Culture Collection (Manassas, VA). HCT116 and HT-29 cancer cells were grown at 37 °C in 5% CO_2 humidified air in

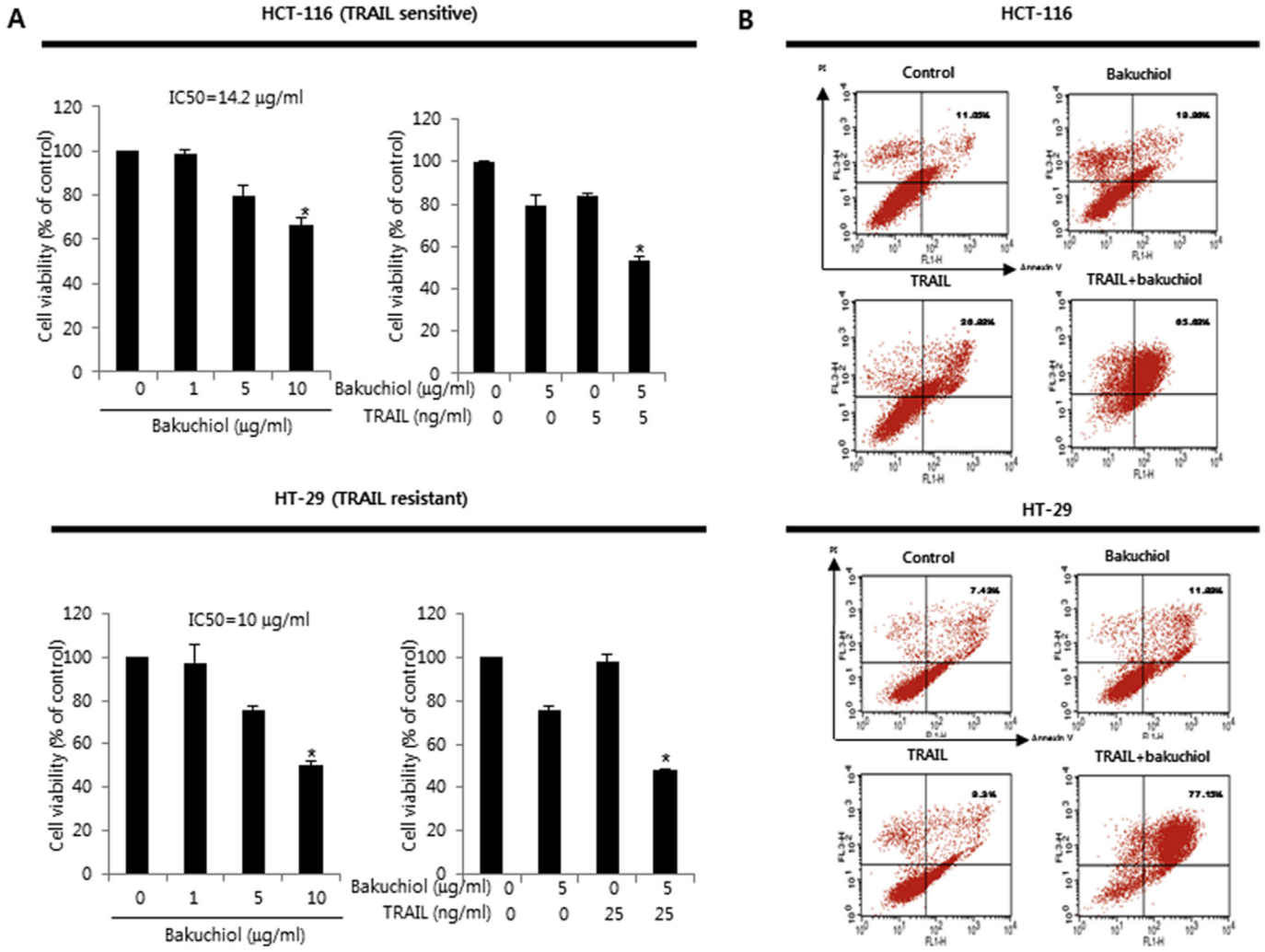

Fig. 1. Bakuchiol enhanced TRAIL-induced cytotoxicity and apoptosis in colon cancer cells. A, Cell viability was determined by direct counting viable cells after pretreated with bakuchiol (5 μg/ml) for 24 h and exposed to TRAIL in HCT116 and HT-29 cells as described in materials and methods. B, Apoptosis was analyzed by Annexin V/FITC assay. Data means ± SD expressed as percentage of control value, which is set to 100%. At least three independent experiments were carried out in triplicate. *, $p < 0.05$, significantly different from control cells.