Biochemical Pharmacology 91 (2014) 457–473

Contents lists available at ScienceDirect

Biochemical Pharmacology

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

c-Jun NH_2-terminal kinase-induced proteasomal degradation of c-$FLIP_{L/S}$ and Bcl_2 sensitize prostate cancer cells to Fas- and mitochondria-mediated apoptosis by tetrandrine

Pankaj Chaudhary, Jamboor K. Vishwanatha *

Department of Molecular and Medical Genetics, Texas Center for Health Disparities and Institute for Cancer Research, University of North Texas Health Science Center, Fort Worth, TX 76107, USA

ARTICLE INFO

Article history:
Received 4 August 2014
Accepted 13 August 2014
Available online 30 August 2014

Keywords:
Tetrandrine
Prostate cancer
Apoptosis
Reactive oxygen species
Ubiquitination

ABSTRACT

Tetrandrine, a constituent of Chinese herb *Stephania tetrandra*, causes cell death in prostate cancer, but the molecular mechanisms leading to apoptosis is not known. Here we demonstrated that tetrandrine selectively inhibits the growth of prostate cancer PC3 and DU145 cells compared to normal prostate epithelial PWR-1E cells. Tetrandrine-induced cell death in prostate cancer cells is caused by reactive oxygen species (ROS)-mediated activation of c-Jun NH_2-terminal kinase (JNK1/2). JNK1/2-mediated proteasomal degradation of c-$FLIP_{L/S}$ and Bcl_2 proteins are key events in the sensitization of prostate cancer cells to Fas- and mitochondria-mediated apoptosis by tetrandrine. Tetrandrine-induced JNK1/2 activation caused the translocation of Bax to mitochondria by disrupting its association with Bcl_2 which was accompanied by collapse of mitochondrial membrane potential (MMP), cytosolic release of cytochrome *c* and Smac, and apoptotic cell death. Additionally, tetrandrine-induced JNK1/2 activation increased the phosphorylation of Bcl_2 at Ser70 and facilitated its degradation via the ubiquitin-mediated proteasomal pathway. In parallel, tetrandrine-mediated ROS generation also caused the induction of ligand-independent Fas-mediated apoptosis by activating procaspase-8 and Bid cleavage. Inhibition of procaspase-8 activation attenuated the cleavage of Bid, loss of MMP and caspase-3 activation suggest that tetrandrine-induced Fas-mediated apoptosis is associated with the mitochondrial pathway. Furthermore, most of the signaling effects of tetrandrine on apoptosis were significantly attenuated in the presence of antioxidant *N*-acetyl-L-cysteine, thereby confirming the involvement of ROS in these events. In conclusion, the results of the present study indicate that tetrandrine-induced apoptosis in prostate cancer cells is initiated by ROS generation and that both intrinsic and extrinsic pathway contributes to cell death.

© 2014 Elsevier Inc. All rights reserved.

1. Introduction

Prostate cancer is one of the most prevalent malignancies affecting men of all ages worldwide and is the second leading cause of cancer-related death in American men [1]. Prostate cancer development is initially androgen dependent, so the basic therapeutic strategy has been the deprivation of androgen [2]. Although most patients initially respond to androgen deprivation therapy by showing low prostate-specific antigen values, they invariably relapse with a more aggressive form of prostate cancer termed androgen-independent or castration-resistant prostate cancer (CRPC) [3,4]. Yet, the molecular mechanisms that promote the development of CRPC are not fully understood. Prostate tumors that recur after androgen deprivation therapy have been shown to have amplified androgen receptor

Abbreviations: ASK1, apoptosis signal-regulating kinase 1; Bax, Bcl_2-associated X protein; Bcl_2, B-cell lymphoma 2; Bid, BH3 interacting-domain death agonist; CRPC, castration-resistant prostate cancer; DAPI, 4′,6-diamidino-2-phenylindole; DHE, dihydroethidium; DISC, death-inducing signaling complex; DMSO, dimethylsulfoxide; FADD, Fas-associated protein with death domain; FasL, Fas ligand; FITC, fluorescein isothiocyanate; H2DCF-DA, 2′,7′-dichlorodihydrofluorescein diacetate; IP, immunoprecipitation; JNK, c-Jun NH_2-terminal kinase; MAPK, mitogen-activated protein kinases; MMP, mitochondrial membrane potential; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide; NAC, *N*-acetyl-L-cysteine; *PARP*, poly (ADP-ribose) polymerase; PI, propidium iodide; ROS, reactive oxygen species; S.E., standard error of the mean; Smac, second mitochondria-derived activator of caspases; z-DEVD-FMK, Z-Asp-Glu-Val-Asp fluoromethylketone; z-IETD-FMK, Z-Ile-Glu(O-ME)-Thr-Asp(O-Me) fluoromethylketone.

* Corresponding author at: Department of Molecular and Medical Genetics, University of North Texas Health Science Center, 3500 Camp Bowie Boulevard, Fort Worth, TX 76107, USA. Tel.: +1 817 735 0422; fax: +1 817 735 2118.
E-mail address: Jamboor.Vishwanatha@unthsc.edu (J.K. Vishwanatha).

http://dx.doi.org/10.1016/j.bcp.2014.08.014
0006-2952/© 2014 Elsevier Inc. All rights reserved.

gene, resulting in increased androgen receptor expression and hypersensitivity to low levels of circulating androgens [5–7]. Certain growth factors such as epidermal growth factor and insulin-like growth factor-I have been shown to activate androgen receptor in the absence of androgen [8]. Overexpression of antiapoptotic proteins such as Bcl_2 and c-FLIP are seen in CRPC, which have been implicated in promoting tumor survival and reducing sensitivity to chemotherapy [9,10]. Currently there is no effective therapy for CRPC and the median survival after the development of CRPC is 20–24 months [11,12]. The CRPC is an invariably lethal condition, which frequently metastasize and is associated with a significant morbidity and mortality. Therefore, identification of agents that will selectively kill or sensitize castration-resistant tumor cells with no additional toxicity to normal tissues would have significant impact on existing therapies.

Tetrandrine, a benzylisoquinoline alkaloid, is a calcium channel blocker isolated from the Chinese herb *Stephania tetrandra* S. Moore [13–15]. Tetrandrine is used in traditional Chinese medicine as an antirheumatic, anti-inflammatory, and antihypertensive agent for the past several years [16–19]. Tetrandrine has been used as an antifibrotic drug to treat the lesions of silicosis in China since the 1960s. Tetrandrine has been shown to be a potent inhibitor of P-glycoprotein drug efflux [20–22]. Compared to verapamil, etoposide and cytarabine, tetrandrine was more effective in reversing drug resistance to daunorubicin, vinblastine and doxorubicin in leukemia cells [21,22]. Tetrandrine exerts cytotoxic effect by inhibiting cell proliferation and inducing apoptosis in various cancer cells including breast cancer, lung cancer, hepatoma, glioma, leukemia and colon cancer [23–29]. In addition, tetrandrine modulates many cellular signaling events, including cell cycle arrest, mitogen-activated protein kinase activation, NF-κB signaling, Wnt/β-catenin signaling, and the transforming growth factor-β signaling pathway [24,27,28,30–32]. Recent studies have indicated that tetrandrine used alone can exhibit significant anti-cancer activity against cancer cells by inhibiting pathways involved in cell proliferation, migration and angiogenesis [26,28]. Despite its potential as an anti-cancer agent, the effects of tetrandrine on prostate cancer have not been studied. In the present study, we elucidate the mechanism through which tetrandrine induces proapoptotic effect in androgen-independent prostate cancer PC3 and DU145 cells. The results of these studies show that tetrandrine-induced apoptosis in prostate cancer cells is dependent on reactive oxygen species (ROS) generation and that contributes to cell death. Furthermore, we demonstrate for the first time that ROS-mediated activation of JNK1/2 leads to ubiquitin-mediated proteasomal degradation of $c\text{-}FLIP_{L/S}$ and Bcl_2, and sensitize prostate cancer cells to Fas- and mitochondria-mediated apoptosis by tetrandrine.

2. Materials and methods

2.1. Cell lines and culture conditions

Human prostate carcinoma cell lines, PC3 and DU145, and the normal epithelial prostate cell line, PWR-1E, were obtained from the American Type Culture Collection (Rockville, MD). The prostate cancer cell lines were cultured in RPMI-1640 (Hyclone, Logan, UT) supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA), 50 mg/ml penicillin and 50 mg/ml streptomycin (Invitrogen, Carlsbad, CA), and maintained in an incubator with a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. The PWR-1E cells were cultured in keratinocyte growth medium supplemented with 5 ng/ml human recombinant epidermal growth factor and 0.05 mg/ml bovine pituitary extract (Invitrogen, Carlsbad, CA) and maintained in an incubator under the conditions described above.

2.2. Materials

Tetrandrine was purchased from Enzo Life Sciences (Farmingdale, NY). The cell fractionation kit was purchased from MitoScience Inc. (Eugene, OR), protein A/G-agarose from Santa Cruz Biotechnology (Santa Cruz, CA), and MG132 and z-DEVD-FMK from Cayman Chemical (Ann Arbor, MI). Antibodies against Bax, Bcl_2, Apaf-1, cytochrome *c*, c-Jun, Fas, FADD, FasL, $c\text{-}FLIP_{L/S}$ and GAPDH were obtained from Santa Cruz Biotechnology (Santa Cruz, CA), whereas ubiquitin, PARP, Bid, p-JNK Thr183/Tyr185, JNK1/2, p-Bcl_2 Ser70, p-ASK1 Thr845, ASK1, p-SEK1 Thr261, SEK1, p-c-Jun Ser63, Smac and caspase-3, -8 and -9 were from cell signaling technology (Danvers, MA). The cell culture medium RPMI-1640 and fetal bovine serum were from GIBCO (Invitrogen, Carlsbad, CA). All other reagents and chemicals were purchased from Sigma–Aldrich (St. Louis, MO).

2.3. Cytotoxicity assay

Proliferation of the PWR-1E, PC-3 or DU145 cells in the presence of tetrandrine was measured by the MTT assay [33]. Briefly, 2×10^4 cells in an aliquot of 190 μl of full serum medium were seeded in 96-well flat bottomed plates for 24 h to allow attachment to the culture plates. After confirming cell attachment, 10 μl of PBS containing various amounts of tetrandrine (final concentration 0–80 μM) were added in each well and incubated for different time intervals. After 12, 24 or 48 h of incubation at 37 °C, 10 μl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium Bromide (MTT) solution (5 mg/ml in PBS) was added to each well, and the plate was further incubated for 4 h at 37 °C. The plate was then centrifuged at $2000 \times g$ for 10 min and the medium was aspirated from each well. Dimethylsulfoxide (100 μl) was added to each well and the formazan dye crystals formed in cells were dissolved by shaking the plates at room temperature for 1 h. The absorbance of formazan at 562 nm was measured using a plate reader (Synergy 2, BioTek Instruments, Inc.).

2.4. Preparation of cell extracts and western blot analysis

After treatment, cells were collected, washed with cold PBS and then incubated in 150 μl of radioimmunoprecipitation assay (RIPA) lysis buffer (50 mM Tris–HCl, pH 7.5; 150 mM sodium chloride; 0.5% sodium deoxycholate; 1% Nonidet P-40; 0.1% sodium dodecyl sulfate; 1 mg/ml aprotinin; 1 mg/ml leupeptin; 1 mM Sodium orthovanadate; 1 mM phenylmethanesulfonyl fluoride) at 4 °C for 30 min. After sonication on ice, cell debris was removed by centrifugation at $12{,}000 \times g$ for 10 min at 4 °C. Protein concentrations were determined by Pierce BCA protein assay kit (Thermo Scientific, Rockford, IL). Cell extracts were separated on 4–20% Bis-Tris Nu-PAGE gel (Invitrogen Corporation, CA) using MES buffer and transferred onto nitrocellulose membrane (Bio-Rad). Membranes were blocked with 5% fat-free milk in Tris-buffered saline containing 0.05% Tween 20 (TBST) at room temperature for 60 min, and incubated overnight at 4 °C with the appropriate primary antibody in 5% milk in TBST. After three washings with TBST, the membrane was incubated with the appropriate secondary antibody (Promega, WI) at room temperature for 2 h. After washing again with TBST, the membranes were developed using ECL plus (Amersham Pharmacia Biotech, IL), and the image was captured using alpha-imager Fluoretech HD2. Isolation of mitochondrial and cytoplasm enriched fractions was by the MitoSciences cell fractionation kit as per the manufacturer's instructions (MitoSciences Inc., Eugene, OR).

2.5. Immunoprecipitation

After treatment, normalized amounts of cell lysate (400 μg of proteins) were incubated with the appropriate primary antibodies

Download English Version:

https://daneshyari.com/en/article/2512174

Download Persian Version:

https://daneshyari.com/article/2512174

Daneshyari.com