Mutation Research 755 (2013) 24–29

Contents lists available at SciVerse ScienceDirect

Mutation Research/Genetic Toxicology and Environmental Mutagenesis

journal homepage: www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Opposite effects of JNK and p38 MAPK signaling pathways on furazolidone-stimulated S phase cell cycle arrest of human hepatoblastoma cell line

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ARTICLE INFO

Article history:
Received 10 October 2012
Received in revised form 30 March 2013
Accepted 27 April 2013
Available online 6 May 2013

Keywords:
JNK
p38 MAPK
S phase cell cycle arrest
Furazolidone
Cell growth suppression
HepG2 cells

ABSTRACT

Furazolidone (FZD), a synthetic nitrofuran with a broad spectrum of antimicrobial actions, is known to induce genotoxicity and potential carcinogenicity in several types of cells, but little is known about its p38 mitogen-activation protein kinase (p38 MAPK) and c-Jun N-terminal protein kinase (JNK) pathways in human hepatoblastoma cell line (HepG2). Given the previously described essential roles of p38 MAPK and JNK pathways in HepG2 cells, we undertook the present study to investigate the roles of p38 MAPK and JNK pathways in cell cycle arrest of HepG2 cells stimulated with FZD. Here we reported that FZD could obviously induce S phase cell cycle arrest, suppress cell growth, increase the activity of phosphorylated p38 (p-p38), and decrease the activity of phosphorylated JNK (p-JNK) in HepG2 cells. Simultaneously, inhibition of p38 MAPK pathway could significantly reduce FZD-stimulated S phase cell cycle arrest, active cell growth, decrease the activity of p-p38, and increase the activity of p-JNK. To the opposite, inhibition of JNK pathway could significantly increase FZD-stimulated S phase cell cycle arrest, suppress cell growth, decrease the activity of p-JNK, and increase the activity of p-p38. These results demonstrate that JNK and p38 MAPK pathways have opposite roles in FZD-stimulated S phase cell cycle arrest of HepG2 cells. FZD induces S phase cell cycle arrest and suppresses cell proliferation of HepG2 cells via activating the pathway from p38 to p-p38 and inhibiting the pathway from JNK to p-JNK.

1. Introduction

Furazolidone (FZD) (3-(5-nitrofurfurylideneamino)-2-oxazolidinone) is a synthetic nitrofuran with a broad spectrum of antimicrobial actions. The drug nowadays is widely used in the treatment of certain bacterial and protozoal infections in human [1]. Until now, FZD has been shown to induce genotoxicity and potential carcinogenicity in several types of human cells in vitro [2]. In our previous studies, we found that FZD could obviously induce S phase cell cycle arrest and inhibit cell growth in human hepatoblastoma cell line (HepG2), probably through reactive oxygen species (ROS)-induced oxidative DNA damage [1,2]. Although FZD has toxic and carcinogenic effects in human liver cells, the mechanisms of its adverse effects on abnormal progression of the cell cycle remain to be investigated in a transformed human liver cells [1].

JNK and p38 MAPK constitute together with extracellular signal-regulated kinases (ERKs) to make up the family of mitogen-activated protein kinases (MAPKs) [3,4]. The mitogen-activated protein kinases (MAPKs) family is one of the signal pathways which has been implicated with oxidative stress induced cell death cascade [5,6]. Among the MAPKs family, activation of p38 MAPK usually causes cell cycle arrest and cell growth arrest, whereas activation of JNK is commonly linked to promoting cell apoptosis and cell death, thus JNK and p38 MAPK are also called as stress activated protein kinase (SAPK) [7,8]. For mammalian cells, researchers have reported that accumulation of H_2O_2 can activate JNK/p38 MAPK pathways [9].

JNK and p38 MAPK, two distinct members of the mitogen-activated protein (MAP) kinase family, regulate genes expression in response to various extracellular stimuli, yet their physiological functions are not completely understood [10–12]. The JNK and p38 MAPK signal transduction pathways play important roles in regulation of cell cycle and cell proliferation in mammalian cells in a manner inextricable from other signal transduction system by sharing substrate and cross-cascade interaction [13,14]. Activated p38 MAPK pathway can cause serious cell cycle arrest and inhibit cell growth in somatic cells [15–18]. Recently it was reported that JNK and p38 MAPK involved in various vertebrate cells growth processes such as adipocytes, cardiomyocytes, erythroblasts,

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http://dx.doi.org/10.1016/j.mrgentox.2013.04.015

myoblasts and neurons [19,20]. It seems that MAP kinase signaling pathway represents a potential target for therapeutic intervention [21,22]. Therefore, a better understanding of the relationship between MAP kinase signal transduction system and the regulation of cell cycle progress is essential for the rational design of novel pharmacotherapeutic approaches.

The cell cycle is divided into mainly four phases, including G_0/G_1, S and G_2/M phases [23]. On stimuli of growth or other stress factors, cells exit from the G_0 phase, a quiescent state, and enter into the G_1 phase, an active state, and then progress into S phase in which DNA synthesis occurs. Following the G_1/S transition is the G_2/M transition, in which mitotic events occur [24]. Loss of normal cell cycle control, especially S phase cell cycle arrest has been believed to play an important role in the pathogenesis of most cancers [25]. During the S phase cell cycle arrest, some related signaling pathways are activated and thereby mediating the cell death cascade reactions and inhibiting cell proliferation [2,22].

Although the major roles of FZD in the human liver cells relate to its inhibitory effects on monamine oxidase, little is known regarding the effects of JNK and p38 MAPK pathways on FZD-stimulated cell cycle arrest of human liver cells in vitro. This prompted us to focus on the intracellular signaling pathways in this study. In our previous studies, we found that FZD could induce S phase cell cycle arrest and inhibit cell growth in HepG2 cells, probably through ROS-induced oxidative DNA damage [1,2]. Together with the above studies, we suspect MAPK pathways might have an effect on regulating cell cycle arrest and cell growth suppression induced by FZD via activating or inhibiting JNK and p38 MAPK.

2. Materials and methods

2.1. Reagents

Furazolidone (3-(5-nitrofurfurylideneamino)-2-oxazolidinone) (CAS no. 67-45-8) was purchased from Sigma Chemical Co. (St. Louis, MO). SB203580 and SP600125 were from Calbiochem (San Diego, CA). Antibodies against p-p38, p-JNK, total p38, total JNK were from Santa Cruz Biotechnology (Santa Cruz, CA). secondary anti-mouse IgG and anti-rabbit IgG were from Cell Signaling (Beverly, MA). Dulbecco's MEM cell culture medium (DMEM) and fetal calf serum (FCS) were obtained from GIBCO (Invitrogen, Carlsbad, CA). Diethylpyrocarbonate (DEPC), leupeptin, aprotinin, pepstatin, and phenylmethyl sulfonyl fluoride (PMSF) were purchased from Sigma (St. Louis, MO).

2.2. Cell culture

This study was carried out using an established human hepatoblastoma cell line (HepG2) provided by Department of Pharmacology and Toxicology, College of Veterinary Medicine, China Agricultural University (Beijing, China). This is a standard cell line with a stable karyotype and has been widely used in mutagenicity research. The cells were grown and maintained in DMEM medium supplemented with 10% fetal bovine serum, 100 IU/mL penicillin and 100 μg/mL streptomycin and the culture were incubated at 37 °C in a 5% CO_2 incubator at 95% humidity.

2.3. Cell proliferation assay

Cells were suspended at a final concentration of 10^6 cells/mL in 96-well plate in triplicate. Reagents were added to each well at various combinations and incubated for 24 h at 37 °C in 5% CO_2. MTT (3-(4,5)-dimethylthiahiazo(-z-y1)-3,5-diphenytetrazol-iumromide) assays were performed according to the manufacturer's instructions (Roche, Switzerland).

2.4. Cell cycle analysis

After treatments, cells ($1–2 \times 10^6$) were washed twice with PBS and harvested with 0.05% trypsin in 0.15% Na_2EDTA. Cells were then centrifuged, washed in PBS, fixed with ice-cold 70% ethanol, and stored overnight at 4 °C. Fixed cells were washed in PBS, and were incubated with propidium (125 μg/mL) and RNase A (100 μg/mL) for 60 min at 4 °C. Data acquisition was performed using an argon laser fluorescence activated cell analyzer (FACscan, BD Bioscience, San Jose, CA).

2.5. Western blot analysis

Cultured cells were scraped, centrifuged, resuspended, and lysed in a lysis buffer containing 50 mM Tris–HCl (pH7.4), 2% SDS, 150 mM NaCl, 1 mM EDTA, 50 mM NaF, 0.5 mM Na_3VO_4, and 1 μg/mL each of protein inhibitors leupeptin, aprotinin, pepstatin, and phenylm-ethyl sulfonyl fluoride (PMSF). Equal amount of cellular protein was loaded into each well of polyacrylamide-SDS gel. After electrophoresis, proteins were transferred to nitrocellulose membranes (Mini-Protean and Trans-Blot systems, Bio-Rad Laboratories, Hercules, CA). The membranes were blocked with milk and were incubated with specific primary and secondary antibodies. Proteins were detected by enhanced chemiluminescence and band image was recorded by exposure to Kodak film. Band intensity was quantified by thin layer scanner (AlphaImager TM2200, Alpha Innotech, CA) relative to the band intensity of β-actin, which was used as an internal control.

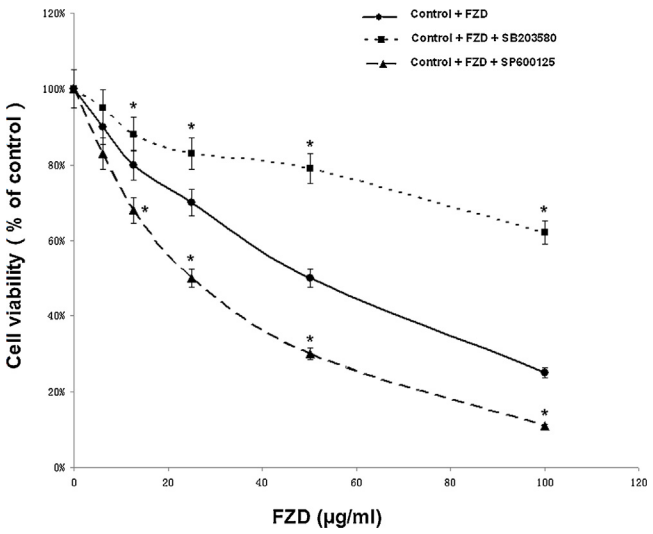

Fig. 1. Involvement of p38 MAPK and JNK in cell viability of HepG2 cells induced by FZD after 24 h incubation. Values were expressed as mean ± SD of three independent experiments (*$P < 0.05$, compared with control + FZD).

2.6. Statistical analysis

All statistical analysis were performed using SPSS for windows (SPSS, Inc., Chicago, IL). The data were analyzed by the one-way analysis of variance (ANOVA) followed by post hoc Dunnett's *t*-test. $P < 0.05$ was considered statistically significant. All experiments were repeated at least three times.

3. Results

3.1. The effect of JNK/p38 MAPK on FZD-stimulated cell growth suppression of HepG2 cells

To test the role of JNK and p38 MAPK in FZD-stimulated cell growth suppression, the HepG2 cells were exposed to 0–100 μg/mL FZD for 24 h and cell growth was monitored by MTT [1,2]. Result of MTT assay showed a dose decrease in cell viability of HepG2 cells at all tested concentrations of FZD (0–100 μg/mL). Our studies showed that IC_{50} values in HepG2 cells for 24 h approximately was 50 μg/mL [2]. For examination of the role of p38 MAPK in FZD-stimulated cell growth suppression, the cells were pretreated with SB203580 (a specific p38 MAPK inhibitor) before exposure of cells to FZD. The results showed that pretreatment of the cells with SB203580 (20 μM) resulted in an inhibitory effect of FZD-stimulated cell growth suppression, significantly increased the percentage of cell viability at all tested concentrations of FZD (Fig. 1). On the other hand, the cells were pretreated with SP600125 (a specific JNK inhibitor) before exposure of cells to FZD. The results showed that pretreatment of the cells with SP600125 (20 μM) resulted in an activated effect of FZD-induced cell growth suppression, significantly decreased the percentage of cell viability at all tested concentrations of FZD (Fig. 1). These results indicate that JNK and p38 MAPK have opposite roles in FZD-induced cell proliferation suppression of HepG2 cells.