Mutation Research 773 (2015) 1–8

Contents lists available at ScienceDirect

Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis

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

Benzo[a]pyrene-induced cell cycle progression occurs via ERK-induced Chk1 pathway activation in human lung cancer cells

Bing-Yen Wang[a,b], Sung-Yu Wu[a], Sheau-Chung Tang[a,c,d], Chien-Hung Lai[c,d], Chu-Chyn Ou[e], Ming-Fang Wu[f,g], Yi-Min Hsiao[h,*], Jiunn-Liang Ko[a,f,*]

[a] Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan
[b] Division of Thoracic Surgery, Department of Surgery, Changhua Christian Hospital, Taiwan
[c] Department of Biochemistry, School of Medicine, Tzu Chi University, Hualien, Taiwan
[d] Division of Chinese Medicine, Department of Health, Executive Yuan, Hualien Hospital, Taiwan
[e] School of Nutrition, Chung Shan Medical University, Taichung, Taiwan
[f] Department of Medical Oncology and Chest Medicine, Chung Shan Medical University Hospital, Taiwan
[g] School of Medicine, Chung Shan Medical University, Taichung, Taiwan
[h] Department of Medical Laboratory Science and Biotechnology, Central Taiwan University of Science and Technology, Taichung, Taiwan

ARTICLE INFO

Article history:
Received 24 September 2014
Received in revised form 11 January 2015
Accepted 17 January 2015
Available online 28 January 2015

Keywords:
S phase accumulation
Benzo[a]pyrene
ERK
Chk1
P-p53

ABSTRACT

Benzo[a]pyrene (B[a]P) is a potent lung carcinogen derived from tobacco smoking and environmental contamination. This study aimed to investigate the signal transduction pathway responsible for B[a]P-induced non-small cell lung cancer (NSCLC) development. We exposed the human NSCLC cell lines Calu-1, CL3, H1299, CH27, H23, and H1355 to B[a]P and assessed cell cycle progression using flow cytometry. Expression of cell cycle mediators was measured using Western blot analyses and electrophoretic mobility shift assays (EMSAs). B[a]P exposure dramatically induced S-phase accumulation in H1355 cells. Phospho-p53 (Ser15 and Ser20), phospho-ERK, phospho-p38, and Bax were significantly increased in H1355 cells whereas phospho-Rb was decreased in these cells. In addition, B[a]P induced phosphorylation of checkpoint kinase-1 (Chk1) but not Chk2. EMSA experiments revealed a slower migrating band after c-Myc bound the E-box in response to B[a]P treatment, which was abolished upon the addition of the ERK inhibitor PD98059 in H1355 cells. Phospho-ERK inhibition and dominant negative mutant Chk1 expression reversed B[a]P-induced S phase accumulation and downregulated phospho-Chk1 and phospho-ERK expression. Taken together, these results suggest that activation of ERK and its downstream mediator Chk1 may contribute to B[a]P-induced S phase accumulation in H1355 cells. The results could help in the development of lung cancer treatments that target the Chk1 pathway through ERK.
© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Lung cancer is the most common malignancy worldwide in both men and women, and cigarette smoking is responsible for approximately 90% of lung cancer cases [1]. Benzo[a]pyrene (B[a]P), the most extensively studied polycyclic aromatic hydrocarbon, is a carcinogen found in cigarette smoke and is a likely cause of lung cancer [2,3]. B[a]P binds the aryl hydrocarbon receptor (AHR), which induces transcription of a number of genes involved in B[a]P metabolism including cytochrome P450 enzyme (CYP1A1), which metabolizes B[a]P to form benzo[a]pyrene-diol-epoxide (BPDE). BPDE covalently binds DNA and forms deoxyguanoside-DNA adducts [4] that may result in misreplication and mutagenesis [5]. B[a]P may also exert carcinogenic action when it is metabolized to reactive intermediates that covalently bind DNA and cause a guanine (G)–thymine (T) transversion in the *p53* gene [6].

Several studies have focused on the association between B[a]P-induced apoptosis and its effects on various cellular signaling pathways, including members of the mitogen activated protein kinases (MAPKs) such as extracellular signal-regulated kinase (ERK), c-jun N-terminal kinase (JNK), and p38 MAPK [5]. Activation of MAPK signaling leads to diverse cellular responses, including cell proliferation, differentiation, and apoptosis [7,8]. Other findings have revealed that B[a]P rapidly activates p38 MAPK and c-Jun N-terminal kinase (JNK) in lung cells [9–11].

* Corresponding authors at: Institute of Medicine, Chung-Shan Medical University, 110, Section 1, Chien-Kuo North Road, Taichung 40203, Taiwan.
Tel.: +886 4 24730022x11694; fax: +886 4 24751101 (Jiunn-Liang Ko); Department of Medical Laboratory Science and Biotechnology, Central Taiwan University of Science and Technology, Taichung, Taiwan (Yi-Min Hsiao).
E-mail addresses: ymhsiao@ctust.edu.tw (Y.-M. Hsiao), jlko@csmu.edu.tw (J.-L. Ko).

http://dx.doi.org/10.1016/j.mrfmmm.2015.01.009
0027-5107/© 2015 Elsevier B.V. All rights reserved.

Research in mouse hepatoma cell lines found that B[a]P induces apoptosis by causing p53 accumulation and phosphorylation and downregulating the Bcl-2 proteins Bcl-xl, Bad, and Bid [9]. Furthermore, reactive metabolites of B[a]P induce Bax translocation to the mitochondria as well as p53 accumulation and translocation to the nucleus.

Genetic analyses indicate that checkpoint kinase 1 (Chk1) is involved in the G2-M damage checkpoint and is activated in response to DNA damage. Chk1 prevents cells from entering mitosis by inhibiting the cell cycle machinery [12]. Ataxia telangiectasia and Rad3-related (ATR) kinase, ataxia telangiectasia mutated (ATM) kinase, and DNA-dependent protein kinase (DNA-PK) are key mediators of the cellular response to DNA damage. DNA double-strand breaks (DSBs) and single-stranded DNA have been shown to activate ATM-Chk2 and ATR-Chk1, respectively [13]. In addition, the ATR-Chk1 pathway is the primary direct effector of DNA damage and replication checkpoints and is essential for survival of many cell types. A previous study found rapid and persistent activation of ERK1/2 in response to B[a]P exposure in lung cancer cell lines. B[a]P increases expression of the ligands for epidermal growth factor receptor (EGFR) and increases the proliferative potential of lung adenocarcinoma cells via EGFR signaling [14]. It is well known that Chk1 activation requires ongoing replication. It wonders that ERK inhibition simply inhibits DNA synthesis or precludes the checkpoint from being activated as a secondary consequence of S-phase arrest.

The purpose of this study was to elucidate the effects of B[a]P on cell cycle progression in lung cancer cell lines. In this study, we found that B[a]P induced phosphorylation of ERK1/2 via phosphorylation and activation of Chk1, resulting in S phase accumulation of human H1355 lung cancer cells.

2. Materials and methods

2.1. Chemicals

B[a]P was dissolved in dimethylsulfoxide (stock solution 2 mM) and diluted in the cell media for each experimental cycle. RPMI-1640 was obtained from GIBCO Invitrogen (Carlsbad, CA). dNTPs were purchased from Amersham Pharmacia Biotech (Piscataway, NJ). Pro Taq polymerase was obtained from Protech (Taiwan).

2.2. Cell culture

H1355, H23, and CH27 cells were cultured in RPMI-1640. H1299 cells were cultured in Dulbecco's modified Eagle's media (DMEM). CL-3 and Calu-1 were cultured in F12 and McCoy's 5A media, respectively. Cells were maintained at 37 °C in a 5% CO_2-humidified atmosphere in the appropriate media containing 10% fetal bovine serum (FBS; Life Technologies, Inc., Rockville, MD), 2 mM L-glutamine, 0.2–0.37% sodium bicarbonate, 100 units/ml penicillin, and 100 μg/ml streptomycin (Life Technologies, Inc.). Cell lines were purchased from the American Type Culture Collection with the exception of CH27 cells, which were kindly provided by Dr. Hsu, S-L. (Taichung Veterans General Hospital, Taiwan).

2.3. shRNA VSV-G pseudo-lentivirus production and infection

Lentiviral infection of H1355 cells led to stable integration and expression of short hairpin RNA (shRNA) targeting the MAPK1(ERK) (NM_138957) mRNA sequences. Individual clones were identified by their unique TRC number: shLuc TRCN0000072246 for vector control, shMAPK1 (#50) TRCN0000010050 (target sequence: TATCCATTCAGCTAACGTTCT) and shMAPK1 (#95) TRCN0000342295 (target sequence: TATCCATTCAGCTAACGTTCT) targeted to MAPK1.

2.4. Western blot analyses

Cells were plated in multi-well systems at a density of 5×10^5 cells/ml. After seeding for 18 h, cells were treated with or without B[a]P for the indicated times. Cells were lysed in RIPA buffer containing a protease inhibitor cocktail (Roche, Basel, Switzerland) and protein concentrations were assayed using a Bio-Rad Protein Assay Kit (Bio-Rad, Hercules, California, USA). Equal amounts of total protein were loaded into 15% SDS-PAGE gels and electrophoresed. Membranes was blocked with 5% non-fat milk (for nonphosphorylated antibodies) or 5% BSA (for phosphorylated antibodies) in Tris–buffered saline containing 0.1% Tween-20 (TBST). Membranes were incubated overnight with the following primary antibodies: p53 (DAKO, DO-7), phospho-p53 (Cell Signaling, Danvers, MA, USA, #9919), p21 Waf1/CIP1 (Cell

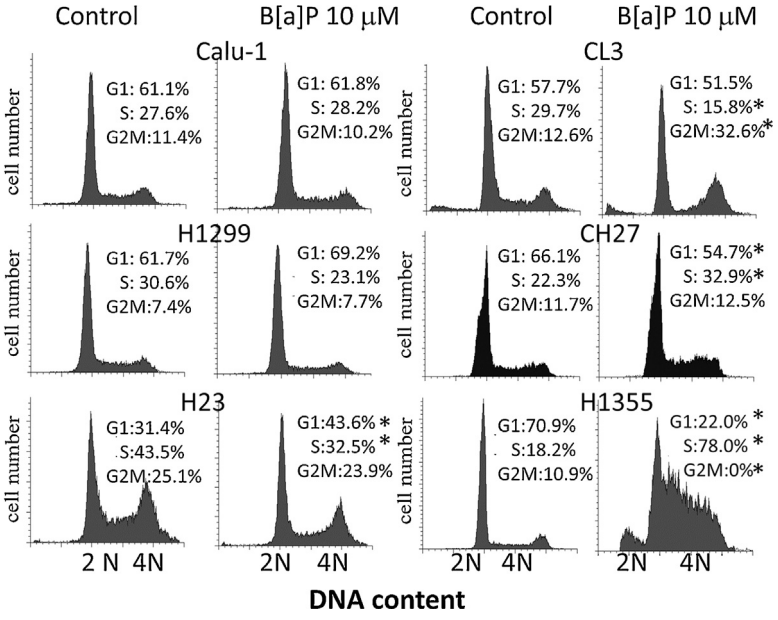

Fig. 1. Cell cycle distribution in lung cancer cells. Calu-1, H1299, H23, H226, CL3, and H1355 cells (5×10^5 cells/60-mm dish) were treated with 10 μM B[a]P for 48 h. Cells were evaluated by flow cytometry and data were acquired using Cellquest. Acquisitions were analyzed and quantified using ModFit LT 3.0. Data are shown as the percentage of nuclei in the sample and are representative of one experiment ($n = 3$). *$P < 0.05$ compared with untreated cells.

Download English Version:

https://daneshyari.com/en/article/8455725

Download Persian Version:

https://daneshyari.com/article/8455725

Daneshyari.com