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Impairment of cell cycle progression by sterigmatocystin in human pulmonary cells *in vitro*

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ABSTRACT

Sterigmatocystin (ST) is a carcinogenic mycotoxin that is commonly found in human food, animal feed and in the indoor environment. Although the correlation between ST exposure and lung cancer has been widely reported in many studies, the cytotoxicity of ST on human pulmonary cells is not yet fully understood. In the current study, we found that ST could induce DNA double-strand breaks in a human immortalized bronchial epithelial cell line (BEAS-2B cells) and a human lung cancer cell line (A549 cells). In addition, the effects of ST on cell cycle arrest were complex and dependent on the tested ST concentration and cell type. Low concentrations of ST arrested cells in the G_2/M phase in BEAS-2B cells and in the S phase in A549 cells, while at high concentration both cells lines were arrested in S and G_2/M phases. Furthermore, we observed that the modulation of cyclins and CDK expression showed concomitant changes with cell cycle arrest upon ST exposure in BEAS-2B and A549 cells. In conclusion, ST induced DNA damage and affected key proteins involved in cell cycle regulation to trigger genomic instability, which may be a potential mechanism underlying the developmental basis of lung carcinogenesis.

1. Introduction

Sterigmatocystin (ST) is a carcinogenic mycotoxin produced as a secondary metabolite by the *Aspergillus*, *Penicillium* and *Bipolaris* species (Schroeder and Kelton, 1975) and it has been recognized as a 2B carcinogen (potential human carcinogen) by the International Agency for Research on Cancer (IARC, 1976). As a contaminant, ST is commonly detected in human food, animal feed and in the indoor environment, such as carpets and building materials (Engelhart et al., 2002; Nielsen et al., 1999). Several *in vivo* studies have shown that ST may induce lung adenocarcinoma in mice (Fujii et al., 1976; Huang et al., 2004; Xing et al., 2007). In addition, our previous *in vitro* studies have demonstrated that ST could induce malignant transformations in human fetal lung tissue (Cao et al., 2000). Taken together, these studies suggested that ST is a potent lung carcinogen. Thus, further information on the biological effects of ST in human pulmonary cells is required to understand its potential carcinogenicity in the mammalian species.

It has been demonstrated that ST can cause DNA damage in several cell lines, including A549 cells, a human esophageal epithelial cell line (Het-1A), and Chinese hamster ovary cells (CHO-K1) and transformed rat fibroblasts (AWRF) *in vitro* (Jaksic et al., 2012; Stetina and Votava, 1986). Cells respond to DNA damage by undergoing cell cycle arrest to facilitate DNA repair (Adamson et al., 2005; Bork et al., 2010). Carcinogenesis results from an imbalance in cell cycle regulation (Chen et al., 2010). Accumulated evidence demonstrates that cell cycle arrest is the most common effect of several mycotoxins in animal tissue *in vivo* or in human cells *in vitro*. Studies have shown that citrinin causes a significant enhancement in cell cycle arrest at the G_0/G_1 phase and G_2/M phase in mouse skin (Kumar et al., 2011). In addition, deoxynivalenol induced G_2/M phase arrest in human intestinal epithelium cells (Yang et al., 2008). Xie et al. found that ST could induce G_2/M phase arrest in mouse embryo fibroblasts (MEFs) (Xie et al., 2000). Thus, we sought to determine whether ST induced DNA damage and interfered with cell cycle progression in human pulmonary cells.

Progression through the cell cycle in mammalian cells is regulated by the cyclin-dependent kinase (CDK) family of serine/threonine kinases and their regulatory partners cyclins (Perez-Roger et al., 2000). The deregulated expression of CDK or cyclins cause a loss in cell cycle control and thus enhances carcinogenesis (Okamoto et al., 1994; Pucci and Giordano, 1999). CDK4, Cyclin D1, CDK2 and Cyclin E are important regulators of G_1-S phase progression and have been associated with the development of many types of cancer, such as tumors in the lung, cervix, breast, and bladder (Alao, 2007; Foulkes et al., 2004; Kanai et al., 1998;

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Yoshinouchi et al., 2000). Recently, we found that ochratoxin A (OTA) could induce human gastric epithelium cells (GES-1) arrested in G_2/M phase that was associated with down-regulation of G_2/M regulatory proteins including CDK1 and Cyclin B1 (Cui et al., 2010). Moreover, GES-1 cells and Het-1A cells exposed to ST have been reported to demonstrate an increase in the number of cells in the G_2/M phase (Wang et al., 2012; Xing et al., 2011), which is mediated by CDK1, Cyclin B1 and Cyclin A. In eukaryotic cells, Cyclin A, Cyclin B1 and CDK1 play a critical role in regulating cell cycle progression. Expression of CyclinA becomes detectable near the G_1/S transition, peaks in the S phase, and interacts with both CDK2 and CDK1 to ensure completion of DNA replication before entering into mitosis (King et al., 1994). The progression from G_2 phase to mitosis is mainly regulated by the CyclinB1–CDK1 complex (Smits and Medema, 2001). CyclinB1 accumulates in the S and G_2 phases to form the inactive mitosis-promoting factor (MPF) consisting of CyclinB1 and CDK1. Dephosphorylation of CDK1 at Tyr15 is required for the MPF activation, and the activated MPF complex initiate mitosis (Morita et al., 2001). Abnormal expression and/or phosphorylation of these factors have been reported in many human cancers and cell lines suggesting their importance in carcinogenesis (Cahill et al., 1998; Gemma et al., 2000; Ohshima et al., 2000; Scolnick and Halazonetis, 2000). Until recently, the effect of ST on the regulation of cyclins and CDKs in human pulmonary cells was not sufficiently clear.

Our previous immunohistochemical study confirmed that ST induced lung adenocarcinoma in NIH mice induced by alveolar type II pneumocytes (Shen et al., 2005). To further explore the toxicity and putative carcinogenic effect of ST on human lung cells, we selected a human immortalized bronchial epithelial cell line (BEAS-2B), which has been previously used as an *in vitro* model to study human lung toxicity (Reddel et al., 1988) as well as A549 cells, a human lung cancer cell line, with properties of alveolar type II pneumocytes. We evaluated the effect of ST on DNA damage and cell cycle progression using flow cytometric analysis and western blotting analyses.

Table 1
The tail DNA %, tail length, olive tail moment in BEAS-2B and A549 cells after ST treatment for 24 h.

Groups	Tail DNA %	Tail length	Olive tail moment
BEAS-2B cells			
Solvent control	1.83 ± 0.16	7.13 ± 1.29	0.49 ± 0.04
24 μM ST	35.4 ± 3.4*	144.63 ± 9.72*	35.97 ± 1.56*
A549 cells			
Solvent control	2.11 ± 0.16	8.49 ± 0.75	1.01 ± 0.19
24 μM ST	25.18 ± 2.87*	126.38 ± 4.59*	22.44 ± 2.71*

Data represent the means ± SD.
* $p < 0.05$, compared with solvent control group.

2. Materials and methods

2.1. Chemicals and reagents

Highly purified ST (>99% purity, benzene-free) was purchased from ENZO Life Sciences, Inc. (Farmingdale, NY, USA). ST was diluted in dimethyl sulfoxide (DMSO) (Sigma–Aldrich, MO, USA). The primary antibodies used for Western blotting analyses included rabbit anti-human CDK1, Cyclin B1, Cyclin E1, Cyclin D1, CDK4 and phospho-γ-H2AX (Ser-139) monoclonal antibodies (Epitomics, CA, USA), rabbit anti-human phospho-Cdc2 (Tyr-15) monoclonal antibody (Cell Signaling Technology, MA, USA), and rabbit anti-human Cyclin A and CDK2 polyclonal antibodies (Anbo, CA, USA).

2.2. Cell culture and treatment

BEAS-2B cells and A549 cells were purchased from the American Type Culture Collection (ATCC, Manassas VA). BEAS-2B and A549 cells were cultured in DMEM/F-12 and RPMI, respectively, supplemented with 100 U/ml penicillin, 100 U/ml streptomycin, and 10% fetal bovine serum (FBS) in 5% CO_2/95% air. The cells were then treated with solvent (DMSO, final solvent concentration 0.1%) alone or with different concentrations of ST (6, 12 and 24 μM) for 24 h.

2.3. Alkaline comet assay

Cells were scraped, counted, and diluted to 2×10^5 cells/ml in PBS. The fully microscope slides were covered with 100 μl of 0.75% normal melting point agarose. The agarose was allowed to solidify in 4 °C for 5 min. Cells were then

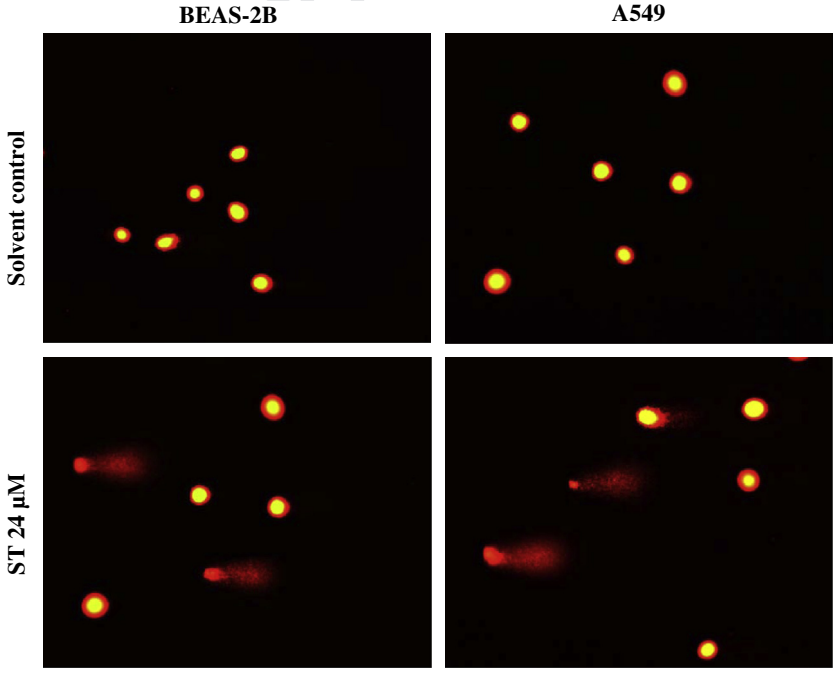

Fig. 1. ST causes DNA damage in BEAS-2B and A549 cells *in vitro*. BEAS-2B and A549 cells were treated with the vehicle alone or induced with 24 μM ST. Comet assay was performed as described in materials and methods. The results are representative of 3 independent experiments.

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