Carcinogenesis of benzo[a]pyrene and its relation to cell sycle regulation

Proceedings: International Symposium of the Kanazawa University 21st-Century COE Program Vol. 2 (2004)
Hayakawa, K., Kizu, R., Kamata, N. (eds.)

ISSN 1348-3048

Carcinogenesis of Benzo[*a*]pyrene and Its Relation to Cell Cycle Regulation

Ryoichi KIZU and Kazuichi HAYAKAWA
Department of Biological Sciences, Kanazawa University, Kanazawa, Ishikawa 920-1192, JAPAN

Abstract - Relationship between benzo[*a*]pyrene (BaP) -induced cell death and cell cycle regulation was investigated in human cervix carcinoma HeLa cells. The cell cycle of HeLa cells treated with BaP was arrested at G_2 phase by checkpoint mechanism but the high mobility group 1 (HMG1) protein stimulated the progression of cell cycle from G_2 to M phase. HMG1 protein has been known to bind to the BaP-adduct sites of DNA. The masking of BaP-adduct sites of DNA by HMG1 protein would be responsible in part to carcinogenicity of BaP.

I. Introduction

Polycyclic aromatic hydrocarbons (PAHs), typified by the benzo[*a*]pyrene (BaP), are toxic and ubiquitous environmental pollutants. They are generally formed and emitted into the environment as a result of incomplete combustion of fossil fuels, wood and other organic materials and from industrial processes. Humans and animals are exposed to PAHs from environmental (air, water), dietary and occupational sources, and also from cigarette smoke. A number of PAHs have been shown to be carcinogenic in human and laboratory animals and the principal concern regarding exposure to PAHs has been cancer risk.

Many PAHs including BaP undergo metabolic activation to metabolites which are capable of mutating oncogenes and/or tumor suppressor genes to effect carcinogenic transformation of mammalian cells by forming PAH metabolite-DNA adducts [1-3]. Cells have evolved mechanisms to prevent mutagenic misreplication of damaged DNA, called "checkpoint". The major checkpoints are G_1- and G_2-checkpoints. The checkpoints can arrest the progress of cells with damaged DNA through the cell cycle, prior to entry into critical phases such as DNA replication (G_1 arrest) and cell division (G_2 arrest). A crucial requirement for PAH-induced carcinogenesis is that mutations in genomic DNA are caused and fixed by misreplication of damaged genes. Such pathways, however, are poorly understood.

In this paper, I present a possible molecular mechanism for BaP-induced mutations and a significance of role of high mobility group-1 (HMG1) proteins in disruption of G_2-checkpoint which leads to carcinogenicity of BaP.

II. Experimental

Cell human cervix carcinoma HeLa. *Chemicals* BaP, nocodazole (Noco; microtubule-interfering agent, M phase blocker), butyrolactone I (BL; cyclin B/cdc2 kinase inhibitor, G_2 phase blocker). *Cell treatment* HeLa cells were treated with 3 μM BaP alone or in combination with 0.3 μM Noco or 20 μM BL for 24, 48, 72 or 96 h. *Estimation of BaP-DNA adduct* HPLC analysis of BaP-tetrol released from DNA of BaP-treated cells by acid hydrolysis. *Analysis of cell cycle distribution* flow cytometry coupled with propidium iodide staining. *Plasmid* pcDNA3.1-HMG1 expressing human HMG1 protein.

III. Results and Discussion

BaP-DNA adduct, cell survival and cell cycle distribution of HeLa cells treated with BaP alone

First, I measured the amount of BaP-DNA adduct, cell survival and cell cycle distribution of HeLa cells treated with BaP alone. Amount of BaP-DNA adduct was evaluated by analyzing BaP-tetrol released from DNA because the principal carcinogenic metabolite of BaP is *anti*-7,8-dihydoroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene and its DNA adduct is hydrolyzed to yield BaP-tetrol. As shown in Fig. 1 (circle), amount of BaP-DNA adduct increased in time-dependent manner up to 72 h. It is noteworthy that alive cell fraction significantly declined after 48 h as shown Fig. 2 (circle) while cell cycle was arrested at G_2/M phase up to 48 h as shown in Figs. 3-A and 3-B, suggesting that dead cells are primarily originated from cells arrested at G_2/M phase.

While BaP treatment caused G_2/M arrest, cells at G_1 phase were observed up to 96 h. To address whether cells at G_1 phase are ones arrested at G_1 phase or ones that come from M phase, HeLa cells were treated with BaP in combination with 0.3 μM Noco. Co-treatment with Noco, a M phase blocker, eliminated G_1 phase cells, indicating that G_1 phase cells observed on BaP single treatment are ones came from M phase.

Effect of BL on the BAP-DNA adduct, cell survival and cell cycle distribution

The above results suggest that while cell cycle is arrested at G_2/M phase in most of cells treated with BaP, cell cycle progress from G_2 phase to M phase and then G_1 phase in some cells with DNA damage. Then, HeLa cells were treated with BaP in combination with BL, a cyclin B/cdc2 kinase inhibitor / G_2 blocker. As can be seen in Fig. 3-C, it was confirmed that BL effectively blocked the cell cycle at G_2/M phase. Although the amount of BaP-DNA adduct are almost same as that of cells treated with BaP alone as shown in Fig. 1 (triangle), alive cell fraction significantly increased as shown in Fig. 2 (triangle). These results support the hypothesis that dead cells observed with BaP single treatment are primarily originated from cells arrested at G_2/M phase.

Effect of overexpression of HMG1 protein on the BaP-DNA adduct, cell survival and cell cycle distribution

HMG1 protein is a nuclear non-histone protein and plays an important role in transcription. HMG1 protein has been known to bind to naturally occurring and chemicals-induced bending sites in DNA [4]. It is also reported that DNA-bending (approximately 23°) is induced at BaP-DNA adduct sites [4]. If HMG1 protein binds to BaP-DNA adduct sites and keep them from checkpoint system, cell cycle would progresses even in cells with damaged DNA. Next, we evaluated the effects of overexpression of HMG1 protein on BaP-DNA adduct, cell survival and cell cycle distribution in HeLa cells transiently transfected with pcDNA3.1-HMG1 plasmid vector expressing human HMG1 protein. While overexpression of HMG1 protein did not affect the formation of BaP-DNA adduct (Fig. 1, square symbol), it significantly decreased the alive cell fraction (Fig. 2, square symbol). As can be seen in Fig. 3-D, HMG1 over expression increased the fractions of G_1 and sub-G_1 (dead cell) phase cells. HMG1 overexpression stimulates progression of cell cycle from G_2/M to G_1 and cell death.

All these results suggest that cell cycle of the cells treated with BaP is arrested at G_2 phase by checkpoint mechanism but the progression of cell cycle from G_2 to M phase occur in some cells by masking of BaP-DNA adduct sites with HMG1. The HMG1 protein-mediated "stealth" effect would be responsible in part to carcinogenicity of BaP.

References

[1] Hemminki, K. DNA adducts, mutations and cancer. *Carcinogenesis*, **14**: 2007-2012 (1993).
[2] Dipple, A. DNA adducts of chemical carcinogens. *Carcinogenesis*, **16**: 437-441 (1995).
[3] Loechler, E.L. How are potent bulky carcinogens able to induce such a diverse array of mutations? *Mol. Carcinog.*, **13**: 213-219 (1995).
[4] Webb, M., and Thomas, J.O. Structure-specific binding of two tandem HMG box of HMG1 to four-way junction DNA is mediated by the A domain. *J. Mol. Biol.*, **294**: 373-387 (1999).
[5] Xu, R., Mao, B., Amin, S., and Geacintov, N.E. Bending and circularization of site-specific and stereoisomeric carcinogen-DNA adducts. *Biochemistry*, **37**: 769-778 (1998).

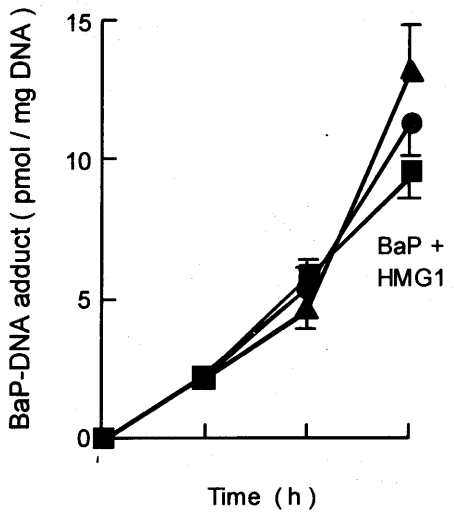

Fig. 1. BaP-DNA Adduct Levels in HeLa Cells Treated with BaP

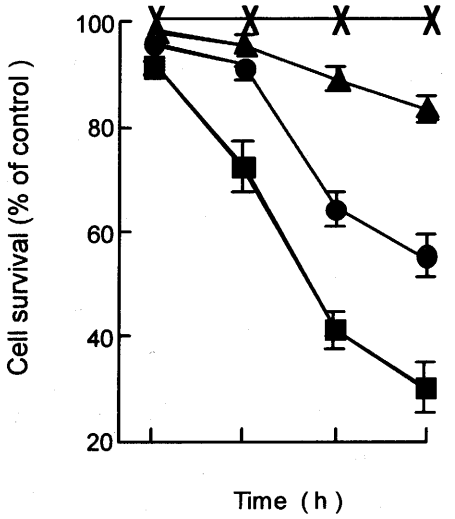

Fig. 2. Cell Survival of HeLa Cells Treated with BaP

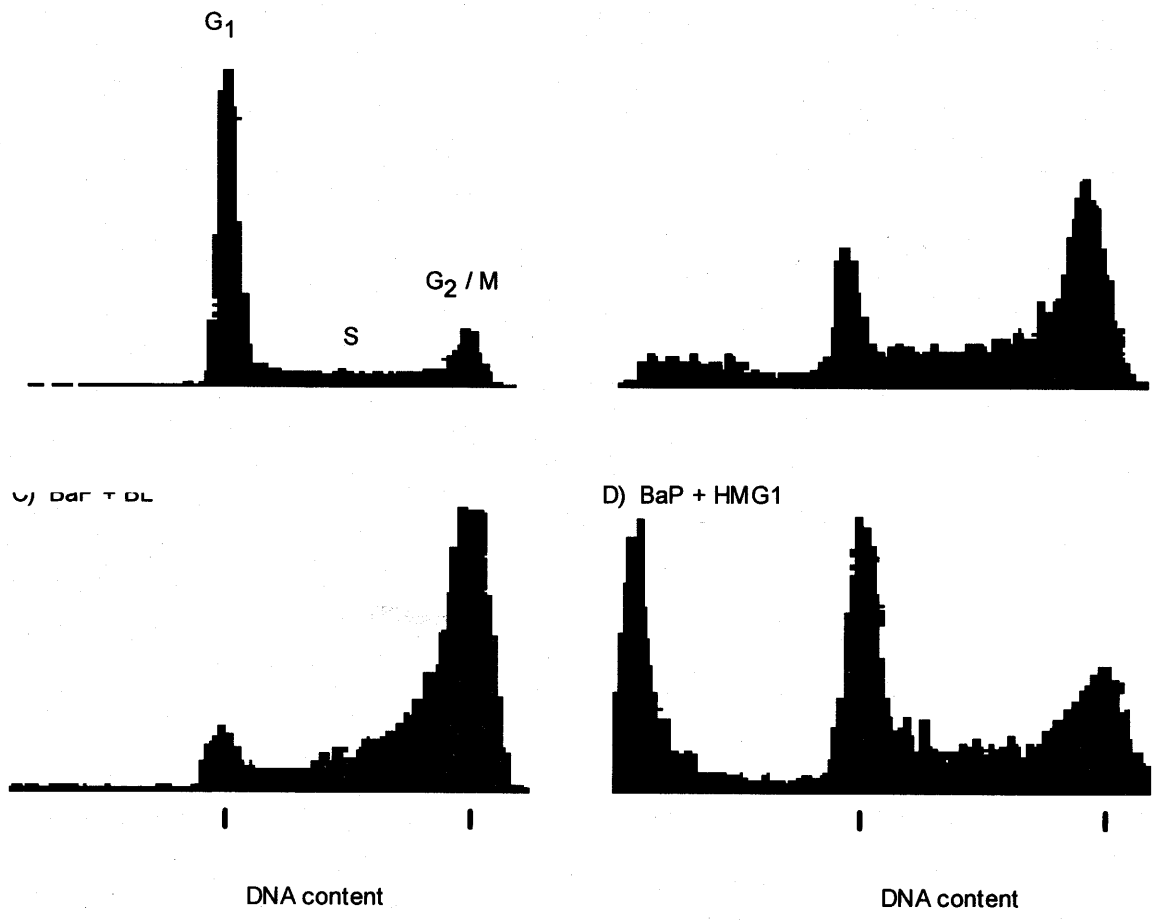

Fig. 3. Cell Cycle Distribution of HeLa Cells Treated with BaP