MASTER'S THESIS

Metabolic changes as potential biomarkers for assessing the mode of benzo[a]pyrene-induced cell death in human hepatoma ($HepG_2$) cells

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Metabolic Changes as Potential Biomarkers for Assessing the Mode of Benzo[a]pyrene-induced Cell Death in Human Hepatoma ($HepG_2$) Cells

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Abstract

The major objective of this study is to evaluate the feasibility of incorporating metabolic changes as biomarkers for studying the mechanism of actions of environmental toxicants in human liver cells. Cellular metabolism is the pathway through which substances including nutrients as well as environmental compounds could be handled upon entering cells. While there have been extensive studies on cellular metabolism and nutrition, the metabolic changes upon exposure to environmental toxicants have not been carefully addressed. In the present study, we investigated the actions of Benzo[a]pyrene (BaP) on cellular metabolism in HepG2 cells with the purpose of correlating cellular metabolism with toxicant-induced cell death. In the first study, metabolic changes in the HepG2 cells upon exposure to BaP were determined. In a second study, we evaluated the cellular functional changes-induced cell death in the same model. Finally, the signaling pathways that regulate and correlate with the mode of cell death induced by BaP were studied. Besides offering an insight on the metabolic regulation of cell death, the potential application of cellular metabolic intermediates as biomarkers of cell death will be discussed.

BaP is a ubiquitous environmental pollutant formed during the combustion of fossil fuels, grilling, barbecuing and smoking of food. Recent studies have demonstrated that, other than inducing carcinogenesis in target tissues, high level of BaP could cause cell death. However, the metabolic changes during BaP exposure and the signaling pathways that associated with the progression of cell death is not known. In Chapter 2 of this thesis, attempt was made to examine the changes of oxidative metabolism of glutathione and subsequent cellular events induced by BaP in the $HepG_2$ cells. It was found that exposure to BaP could result in an increase in cellular glutathione level in a dose- and time-dependent manner. This was correlated to an increase in the activities of both γ-glutamylcysteine synthase (γ-GCS) activity and the phase II detoxification enzyme, glutathione S-transferase. Reactive oxygen species (ROS) were maintained at basal level. These results indicate that BaP induced a strong self-defense mechanism, attenuating oxidative stress during the metabolism of BaP and enhancing its detoxification.

Chapter 3 shows the cellular and biochemical pathways in BaP-induced cell death. It was observed that a decreased rate of cell proliferation and later massive cell death after 48 to 72 hrs incubation, but interestingly cells died not through apoptosis. Instead, cell death occurred through a necrotic pathway as demonstrated by histological and biochemical features. Oxidative stress was not observed during the cell death and lowering of GSH level as well as other apoptosis inhibitors did not rescue cells from death. BaP induced DNA damage in HepG2 cells, which in turn activated poly(ADP-ribose)polymerase-1 (PARP-1), a nuclear enzyme responsible for repairing DNA damage. Once activated, PARP-1 catalyzes the formation of ADP-ribose polymers on acceptor proteins at the expense of NAD^+.

Incubation of cells with high extracellular concentration of NAD^+ (5 mM) after BaP treatment could elevate intracellular NAD^+ level and block cell death. Inhibitor of PARP-1 suppresses both overactivation of PARP-1 activity and NAD^+ depletion. These results elucidated a sequence of events linking cellular metabolism to the progression of cell death induced by this organic toxicant.

The signaling pathway through which BaP causes cell death was studied in Chapter 4. BaP induced accumulation and activation of p53, which occurred as early as 12 hr after exposure. Activation of p53 was evidenced by its phosphorylation at serine 15 (Ser15) and acetylation at lysine 382 (Lys382). Chemical inhibition and siRNA-mediated knockdown of p53 expression both suppressed its phosphorylation as well as cell death. BaP also activated p38 MAPK and ERK, but not JNK, at 6 hr after exposure. Both pharmacological inhibition and siRNA knockdown of p38 and ERK suppressed phosphorylation of p53 at Ser15, but the accumulation of p53 was only moderately reduced. Acetylation of p53 at Lys 382 was not affected by these inhibitors, suggesting that acetylation stabilizes p53 in response to DNA damage. Furthermore, inhibition of DNA-PK, ATM, and ATR also suppressed p53 phosphorylation, implying an involvement of multiple pathways of p53 activation. In summary, the current study demonstrated that both MAPK and p53 activation are required for BaP-induced necrotic cell death. The results also provide a novel model for studying the regulation between p53 and p38 MAPK in the progression of cellular necrosis.

In the present study, we investigated a series of events leading from DNA damage to cell death and identified a novel pathway that mediates BaP-induced cell death. The research outlines the sequence of biochemical and molecular signaling events which could lead to cell death. The key players of these events may constitute potential biomarkers for assessing toxicity of this and other environmental toxicants. The role cellular metabolism plays in assessing toxicants-induced cell death is also discussed (Chapter 5). In future studies, it is proposed that research should be conducted to investigate whether the events observed are specific for the HepG2 cells by conducting the same study in hepatocytes of non-tumor origin.

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