DOCTORAL THESIS

Mass spectrometry-based metabolomics to unveil the polybrominated diphenyl ether-47 induced alteration in breast carcinoma

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ABSTRACT

Polybrominated diphenyl ethers (PBDEs) are commonly used to prevent the development of fire in various factory products. Due to the adverse effects on human health and bio-accumulation capacity, PBDEs are considered as one kind of persistent organic pollutants. 2,2',4,4'-Tetrabromodiphenyl ether (BDE-47) is one of the most frequently detected PBDE congeners in humans. Although numerous studies have shown the close connection between BDE-47 and human health, few reports were related to breast carcinoma. *In vivo* study of the association between BDE-47 and breast cancer was also scarce. In this study, both *in vitro* and *in vivo* experiments were conducted to explore the influence of BDE-47 to breast cancer.

Firstly, we performed the *in vitro* study by exposing different concentrations of BDE-47 (5, 10 μM) to MCF-7 breast cancer cells. Nontargeted metabolomics analysis was conducted by using ultra-high performance liquid chromatography coupled with mass spectrometry (UHPLC-MS). Results showed that the toxicity to MCF-7 cells gradually increased when the concentration of BDE-47 exceeded 1 μM in the medium. Pyrimidine metabolism, purine metabolism and pentose phosphate pathway (PPP) were the most influenced metabolic pathways, and the metabolites in the three metabolic pathways were significantly downregulated. Moreover, the increase of reactive oxygen species was detected by using the 2',7'-dichlorodihydrofluorescein diacetate staining assay. Results suggested that the BDE-47 induced oxidative stress by downregulating the NADPH generation in PPP. The pyrimidine metabolism and purine metabolism might be downregulated by the

downregulation of mRNA transcripts. Therefore, BDE-47 could induce oxidative stress in breast cancer cells by inhibiting PPP and disordering the metabolism of the entire cell subsequently.

Secondly, we constructed a breast cancer nude mouse model, performed *in vivo* exposure of BDE-47 to the mice, and conducted mass spectrometry-based metabolomics and lipidomics analysis to investigate the metabolic changes in mice. Results showed that the tumor sizes were positively associated with the dosage of BDE-47. Metabolomics and lipidomics profiling analysis indicated that BDE-47 induced significant alterations of metabolic pathways in livers, including glutathione metabolism, ascorbate and aldarate metabolism, and lipids metabolism, etc. The upregulations of phosphatidylcholines and phosphatidylethanolamines suggested the membrane remodeling, and the downregulations of Lyso-phosphatidylcholines and Lyso-phosphatidylethanolamines might be associated with the tumor growth. Targeted metabolomics analysis revealed that BDE-47 inhibited fatty acid β-oxidation (FAO) and induced incomplete FAO. The inhibition of FAO and downregulation of PPARγ would contribute to inflammation, which could promote tumor growth. In addition, BDE-47 elevated the expression of the cytokines TNFRSF12A, TNF-α, IL-1β and IL-6, and lowered the cytokines SOCS3 and the nuclear receptor PPARα. The changes of cytokines and receptor may contribute to the tumor growth of mice.

Based on the findings from breast cancer cells and nude mouse assays, we noticed that fatty acid metabolism was influenced by BDE-47 exposure. To have a

comprehensive understanding of the impact, we performed targeted metabolomics analysis of fatty acids. Short-chain fatty acids (SCFAs) and hydroxylated short-chain fatty acids (OH-SCFAs) are crucial intermediates related to a variety of diseases, such as bowel disease, cardiovascular disease, renal disease and cancer. We developed a global profiling method to screen SCFAs and OH-SCFAs by tagging these analytes with d_0/d_6-N, N-dimethyl-6,7-dihydro-5H-pyrrolo[3,4-d] pyrimidine-2-amine (d_0/d_6-DHPP) and UHPLC-MS/MS in parallel reaction monitoring (PRM) mode. The derivatization procedure was simple and rapid. The targeted compounds could be derivatized within three minutes under mild condition and analyzed without the need of further purification. The derivatization significantly improved the chromatographic performance and mass spectrometry response. The d_6-DHPP tagged standards were used as internal standards, which remarkably reduced the matrix effects. The use of high resolution PRM mode made it possible to identify unknown SCFA and OH-SCFA species. The developed method was successfully applied to the analysis of mouse feces, serum, and liver tissue samples harvested from the breast cancer nude mice that had been exposed to BDE-47. By using the developed method, 40 analytes (10 SCFAs and 30 OH-SCFAs) were characterized. Semi-quantitative analysis indicated that the exposure of BDE-47 to the mice altered the SCFA and OH-SCFA metabolism, especially in the high dose group.

In addition, medium- and long-chain fatty acids (MLFAs) are essential energy sources in cells and possess vital biological functions. Characteristics of MLFAs in

biosamples can contribute to the understanding of biological process and the discovery of potential biomarkers for relevant diseases. However, there are obstacles of the MLFAs determination because of the poor ionization efficiency in mass spectrometry and structural similarity. Herein, a derivatization strategy was developed by labeling with d_0-DHPP and detecting with UHPLC-MS/MS in multiple reaction monitoring (MRM) mode. The parallel isotope labeled internal standards were generated by tagging d_6-DHPP to MLFAs. The simple and rapid derivatization procedure and mild reaction conditions greatly reduced the potential of MLFA degradation. With the methodology, the chromatography performance was greatly improved, and the mass spectrum response was enhanced up to 1, 600 folds. Finally, the developed derivatization method was applied to serum samples to analyze the alteration of MLFAs induced by BDE-47 exposure in breast cancer nude mice. The semi-quantitative results demonstrated that the BDE-47 exposure significantly influenced the MLFA metabolism.

Together, mass spectrometry-based targeted and nontargeted metabolomics of *in vitro* and *in vivo* studies suggested that BDE-47 impacted multiple metabolic pathways and was positively associated with breast tumor growth in mice. This study might further our understanding of the health risks of BDE-47 to breast cancer.

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