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Proteomic evaluation of human umbilical cord tissue exposed to polybrominated diphenyl ethers in an e-waste recycling area

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ABSTRACT

Parental exposure to polybrominated diphenyl ethers (PBDEs) is associated with adverse birth outcomes. This study aims to examine differentially-expressed protein profiles in umbilical cord tissue, derived from mothers exposed to PBDEs, and investigate candidate biomarkers to reveal the underlying molecular mechanisms. Umbilical cord samples were obtained from women residing in an electronic waste (e-waste) recycling area (Guiyu) and reference area (Haojiang) in China. The concentration of PBDEs in umbilical cord tissue was determined by gas chromatography and mass spectrometry (GC/MS). Isobaric tagging for relative and absolute quantification (iTRAQ)-based proteomic technology was conducted to analyze differentially-expressed protein profiles. The total PBDE concentration was approximately five-fold higher in umbilical cords from Guiyu than from Haojiang (median 71.92 ng/g vs. 15.52 ng/g lipid, $P < 0.01$). Neonatal head circumference, body-mass index (BMI) and Apgar1 score were lower in Guiyu and negatively correlated with PBDE concentration ($P < 0.01$). Proteomic analysis showed 697 proteins were differentially expressed in the e-waste-exposed group compared with the reference group. The differentially-expressed proteins were principally involved in antioxidant defense, apoptosis, cell structure and metabolism. Among them, catalase and glutathione S-transferase omega-1, were down-regulated, and cytochrome c was found to be up-regulated, changes which were further verified by enzyme-linked immunosorbent assays. These results suggest that an antioxidant imbalance and cell apoptosis in the umbilical cord following PBDE exposure is associated with neonatal birth outcomes.

1. Introduction

Electronic waste (e-waste) has become a global environmental health problem due to the rapid development of electronic technology (Ogunseitan et al., 2009; Heacock et al., 2016). Guiyu, a town with large-scale e-waste processing in southern China, is one of the largest e-waste sites in the world (Huo et al., 2007; Wu et al., 2010). Informal e-waste dismantling and recycling results in release of various toxic chemicals, including heavy metals, polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs), into the air, water and soil, and has caused severe health problems (Huo et al., 2007; Leung et al., 2007; Wu et al., 2010; Xu et al., 2013, 2014, 2015; Zhang et al., 2014). PBDEs are a group of brominated flame retardants that have been extensively used in various consumer products, such as electronic devices, construction materials, textiles and family furniture (Eskenazi et al., 2013; McDonald, 2002). PBDEs are easily released into the environment during e-waste dismantling, and affect human populations by entering the body via inhalation, ingestion, and dermal contact (Abdallah and Harrad, 2014; Darnerud et al., 2001; Sjodin et al., 2003). Due to their highly lipophilic properties, PBDEs are prone to accumulate in mothers and fetuses, mainly in fatty tissue and breast milk (Ma et al., 2012), umbilical cord blood (Zhao et al., 2016), placenta and fetal membranes (Miller et al., 2009; Solomon and Weiss, 2002; Wu et al., 2010). Since the chemical structure is similar to thyroid hormones and PCBs, PBDEs are inclined to disrupt the thyroid endocrine system (Zheng et al., 2017), and cause altered thyroid hormone homeostasis and neurotoxicity (Chen et al., 2014). Previous studies indicate prenatal exposure to high-levels of PBDEs is related to adverse neonatal birth outcomes and neurodevelopmental effects (Eskenazi et al., 2013; Wu et al., 2010).

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Proteomics is a potent method for detecting global changes in protein expression and identifying potential biomarkers that respond to environmental stressors (Bradley et al., 2002; Martyniuk et al., 2012; Zhang et al., 2012). Studies on the proteomic responses, following PDBE exposure, have been investigated in neonatal mouse brain striatum and hippocampus (Alm et al., 2006), rat cerebellum and hippocampus (Kodavanti et al., 2015), rat neural stem/progenitor cells (Song et al., 2014), *Mytilus galloprovincialis* (Ji et al., 2013) and human umbilical vein endothelial cells (Kawashiro et al., 2009), but have been conducted by conventional proteomics analysis involving two-dimensional electrophoresis (2-DE) combined with tandem mass spectrometry. In our previous study, we performed 2-DE technology to characterize the differential proteomic expression of human placenta and fetal development following e-waste lead and cadmium exposure in utero (Xu et al., 2016a). Isobaric tags for relative and absolute quantification (iTRAQ) proteomics coupled with liquid chromatography-tandem mass spectrometry (LC-MS/MS), which can analyze and quantify up to eight phenotypes with high resolution, has been commonly used to investigate mechanisms of chemical toxicity combined with altered protein expression profiles under chemical contaminant stress (Martyniuk et al., 2012; Ross et al., 2004).

The umbilical cord is not only responsible for the transport of nutrients and oxygen between fetus and mother, but also an ideal source of stem cells in tissue engineering and regenerative medicine (Chen et al., 2013). Xenobiotics are prone to accumulate in umbilical cord tissue during blood transportation and can cause varying degrees of damage to the structure and tissue of the umbilical cord. For instance, lead acetate has a toxic effect on the self-renewal, multipotent differentiation potential and hematopoiesis-promoting function of umbilical mesenchymal stem cells, and PBDEs cause oxidative stress in human umbilical vein endothelial cells (Charnley and Putzrath, 2001; Kawashiro et al., 2009; Sun et al., 2012; Zeng et al., 2014). However, the underlying mechanism of its effects remains unclear. Until now, no studies have investigated the effects of in utero PBDE exposure on protein expression profiles in the umbilical cord, and the correlation between umbilical PBDE exposure and fetal growth. Given the critical role of the umbilical cord in fetal growth and tissue medicine, we sought to examine the differentially-expressed protein profiles in umbilical cords from mothers exposed to PBDEs, and screen for biomarkers of its effects. In the current study, we applied an iTRAQ-based proteomic approach to investigate altered protein expression profiles of human umbilical cord tissue exposed to PBDE. This is the first study to examine the relationship between health risks of the neonate and PBDE levels in umbilical cord tissue from an e-waste recycling area. We focus on 3 differentially-expressed proteins, catalase (CAT), glutathione S-transferase omega-1 (GSTO1) and cytochrome c (Cyt c) in umbilical cord. These proteins involve antioxidant defense and cell apoptosis, which may affect fetal development and growth (Kawashiro et al., 2009). Our results may provide new insight for toxicity health risks of prenatal PBDE exposure on fetal development and growth by the novel proteomics.

2. Materials and methods

2.1. Study population

A total of 300 healthy pregnant women were recruited from a local hospital in both Guiyu and Haojiang between March and August 2012. Prior to enrollment, all participants answered a detailed questionnaire involving information covering maternal age, maternal weight and height, prenatal maternal smoking and drinking, education, father smoking, pregnancy complications, residential history and distance from an e-waste recycling site. Neonatal birth outcomes, including birth weight, body length and head circumference, were measured by medical professionals after delivery, and information was obtained from hospital records. The study protocol was approved by the Human Ethics Committee of Shantou University Medical College. Umbilical cord tissue samples were collected immediately following delivery by medical professionals. All tissue samples were taken from the neonatal side of the umbilical cord. After rinsing with cold sterile phosphate-buffered saline, samples were immediately frozen in liquid nitrogen, and then stored at − 80 °C until analysis.

2.2. Determination of PBDEs in umbilical cord tissue

Chemical analysis of PBDEs in umbilical cord tissue was with slight modifications of the procedure described in previous studies (Kawashiro et al., 2008; Xu et al., 2015). A PBDE standard mixture solution containing BDE-17, -28, -47, -66, -71, -85 -99, -100, -138, -153, -154, -183, -190, and -209, and individual standards BDE-15 and BDE-77, were purchased from AccuStandard (New Haven, CT, USA). BDE-15 and BDE-77 were used as internal and recovery standards, respectively. A 500 mg umbilical cord tissue sample was first extracted three times by addition of 10 mL of a 2:1 acetone/hexane solution and homogenizing for 1 min using a Pro200 homogenizer (Pro Scientific, USA). The supernatant was transferred and filtered through pre-cleaned (hexane) glass wool and anhydrous sodium sulfate. After concentrating to approximately 2 mL with a water bath, 20% of the extract was used for lipid gravimetric determination. The rest was filtered through a florisil glass column to remove lipids (lipid-adjusted PBDE concentration was used in the data analysis), then eluted with hexane for cleanup. The extract was concentrated to 1 mL under nitrogen for further cleanup with a multilayer silica gel column (1 g anhydrous sodium sulfate, 1 g of neutral silica, 1 g of basic silica, 3 g of acidic silica, 1 g of neutral silica, 1 g anhydrous sodium sulfate). The extracts were eluted with 50 mL dichloromethane, and then evaporated under a nitrogen stream until dryness, and resolubilized in 50 μL hexane. BDE-15 was used as the internal injection standard for analysis by gas chromatography/mass spectrometry (GC/MS). Samples were ultimately analyzed with an Agilent 7890A-5975C GC/MS (Agilent Technologies, America) with a negative chemical ion source.

For every ten samples, a solvent blank and a procedural blank were processed to make sure that samples were not contaminated. Instrumental accuracy was determined 7 times by constantly quantifying the minimum concentration of standard solution for the calibration curve. Relative standard deviation (RSD%) (tri-deca-BDE) was within 0.40%–4.00%. The limits of detection (LOD) (signal/noise of 3) was defined as three times the standard deviation (SD) of the method blanks analyzed in parallel with the study samples in 10 μL hexane (in the absence of detectable blanks) and ranged from 0.011 to 0.034 ng/mL for tri- to nona-BDE, and 0.070 ng/mL for BDE-209. The ten-point calibration curves showed excellent linearity ($r^2 > 0.999$). For recovery, a spiked sample with surrogate was determined for each batch. The average recoveries for surrogate standard ranged from 81%–95%.

2.3. Protein preparation, digestion, and labeling with iTRAQ reagents

Twenty-four umbilical cord tissues (twelve from the e-waste-exposed group and twelve from the reference group) were separated into three replicates for proteomic analysis, each replicate included eight samples (four samples/group). All samples with no birth complications and no birth defects, and pregnant women who smoked were excluded. From each of the 24 individual umbilical tissues, 100 mg was homogenized with lysis buffer (8 M urea, 40 mM Tris-HCl with 1 mM PMSF, 2 mM EDTA and 10 mM dithiothreitol, pH 8.5) and two magnetic beads (diameter 5 mm) were used to extract in a 1.5 mL centrifuge tube. The mixtures were placed into a Tissue Lyser for 2 min at 50 Hz torelease proteins. After centrifugation at 25,000*g* and 4 °C for 20 min, the supernatant was transferred to a new tube, reduced with 10 mM dithiothreitol at 56 °C for 1 h and alkylated with 55 mM iodoacetamide in the dark at room temperature for 45 min. Following centrifugation at 32,000*g* and 4 °C for 20 min, the supernatant was removed and