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6-OH-BDE-47 promotes human lung cancer cells epithelial mesenchymal transition *via* the AKT/Snail signal pathway

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ABSTRACT

Hydroxylated polybrominated diphenyl ethers (OH-PBDEs) have been detected in the various human tissues. The OH-PBDEs are suggested to be stronger endocrine-disrupting compounds than PBDEs, therefore the toxicological effects of OH-PBDEs had received lots of attention. However, there is no study about the carcinogenic effect of OH-PBDEs and their estrogen potencies on the tumorigenesis and development of cancer. In the present study, we found that 6-hydroxy-2,2′,4′,4′-tetrabromodiphenyl ether (6-OH-BDE-47), the most abundant OH-PBDE congeners in human serum, promoted the *in vitro* migration of lung cancer A549 and H358 cells by induction of epithelial to mesenchymal transition (EMT). This was confirmed by that 6-OH-BDE-47 significantly down regulated the expression of epithelial markers E-cadherin (E-Cad) and zona occludin-1 (ZO-1) while up regulated the mesenchymal markers vimentin (Vim) and N-cadherin (N-Cad). 6-OH-BDE-47 up regulated the protein while not mRNA levels of Snail, which was the key transcription factor of EMT. Silencing of Snail by use of siRNA attenuated the 6-OH-BDE-47 induced EMT. This suggested that the stabilization of Snail was essential for 6-OH-BDE-47 induced EMT. Further, the treatment of 6-OH-BDE-47 increased the phosphorylation of AKT and ERK in A549 cells. Only PI3K/AKT inhibitor (LY294002), but not ERK inhibitor (PD98059), completely blocked the 6-OH-BDE-47 induced up regulation of Snail and down regulation of E-Cad, suggesting that PI3K/AKT pathway is important for 6-OH-BDE-47-mediated Snail stabilization and EMT in A549 cells. Generally, our results revealed for the first time that 6-OH-BDE-47 promoted the EMT of lung cancer cells *via* AKT/Snail signals. This suggested that more attention should be paid to the effects of OH-PBDEs on tumorigenesis and development of lung cancer.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are flame retardants that have been extensively used in textiles, polymers, rubber, carpets, television sets, computers, and small appliances (Brown et al., 2004). Recent studies revealed that PBDEs have been widely distributed in the environment and bioaccumulated in humans and wildlife (Hites, 2004). It was observed that concentrations of PBDEs have been increasing

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in human tissues such as human milk, serum, and adipose tissue (Chen et al., 2014a; Thomsen et al., 2002). Further, the hydroxylated (OH–) metabolites of PBDEs (OH-PBDEs) have also been detected in human tissues including breast milk, cord blood and serum, and environmental samples (Lacorte and Ikonomou, 2009; Wang et al., 2012). As higher concentrations in the human body are detected, there is increasing concern regarding the toxicity of these compounds.

Previous studies indicated that OH-PBDEs have reproductive toxicity, fetotoxicity, developmental neurotoxicity, and endocrine disrupting effects (Canton et al., 2008; Usenko et al., 2012). They are suggested to be stronger endocrine-disrupting compounds than PBDEs (Dingemans et al., 2008). *In vitro* studies indicated that OH-PBDEs can create a number of effects on cell signaling transduction pathways and cell functions (An et al., 2010; Li et al., 2013). OH-PBDEs increased basal calcium levels and ablated the ability for calcium depolarization in cells, potentially leading to neurodisruption (Dingemans et al., 2008). This might be due to that OH-PBDEs have the similar structures with thyroid hormones and therefore have the most potent in displacing T4 from TTR (Canton et al., 2008). Despite all these studies, there is still a large gap in our knowledge about toxicity of OH-PBDEs and the molecular mechanisms of the toxicity, particularly for the roles of OH-PBDEs on the tumorigenesis and development of cancer.

Lung cancer, which killed an estimation of 1.4 million people annually, is one of the leading causes of cancer deaths worldwide (Reck et al., 2013), which is still increasing both in prevalence and mortality worldwide. At the same time, lung cancer is also the most difficult cancers to treat due to the limited treatment options for advanced lung cancer. Metastasis is suggested to be the main reason for the mortality caused by lung cancer. Clinical studies indicated that more than 70% of NSCLC patients show metastases to the regional lymph nodes or to distant sites at the initial presentation (Reck et al., 2013). Previous studies indicated that estrogen signals played an important role in the tumorigenesis and development of lung cancer (Siegfried and Stabile, 2014). Further, high levels of polyaromatic hydrocarbons (PAHs) in air correlated with increased levels of DNA adducts in peripheral lymphocytes, and with an increased incidence of lung cancer (Hemminki and Veidebaum, 1999). However, the carcinogenic effects of OH-PBDEs and their estrogen potency on the tumorigenesis and development of lung cancer had not been investigated.

In this study, the effects of 6-hydroxy-2,2′,4′,4′-tetrabromodiphenyl ether (6-OH-BDE-47), which has been identified as the most abundant hydroxylated PBDE congeners in human serum (Zota et al., 2011), on the migration of lung cancer cells were investigated. We found that 6-OH-BDE-47 can promote the epithelial to mesenchymal transition (EMT) of lung cancer cells *via* the AKT/Snail signal pathway. To the best of our knowledge, this is the first study to illustrate effects of 6-OH-BDE-47 on migration of lung cancer cells.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals were of reagent grade or better and were purchased from Sigma–Aldrich (Deisenhofen, Germany). BDE-47 and 6-OH-BDE-47 purchased from AccuStandard (New Haven, CT) were dissolved in dimethyl sulfoxide (DMSO) to prepare a 10 mM stock solution, and stored at −20 °C. Primary antibodies against E-cadherin (E-Cad), zona occludin-1 (ZO-1), N-cadherin (N-Cad), fibronectin (FN), vimentin (Vim), GAPDH, and horseradish peroxidase-conjugated secondary antibody were purchased from Cell Signaling Technology (Beverly, MA, USA). PrimeScript® RT reagent kit and SYBR® Premix Ex Taq™ were products of TaKaRa. E.Z.N.A® HP Total RNA kit was bought from Omega Bio-Tek (Doraville, USA). The final concentration of DMSO in the cell cultures was less than 0.5%.

2.2. Cell line and cell culture

Human lung cancer cell line A549 and H358 were obtained from the American Type Culture Collection. The cells were cultured in RPMI 1640 with 10% fetal bovine serum, 100 U/mL penicillin and 100 g/mL streptomycin at 37 °C in a humidified 5% CO_2 atmosphere. Twenty-four hours before the experiments, the medium was removed and replaced with RPMI 1640 without phenol red supplemented with 5% dextran-coated, charcoal treated FBS (5% DC-FBS) to exclude estrogenic effects caused by the medium.

2.3. Wound healing assay

For the *in vitro* wound healing assay, confluent monolayers of A549 cells were scratched using sterile pipette tips. Then the cells were cultured in serum-free media for the indicated times. The migration distance of the cells into the scratched area was measured in five randomly chosen fields. A Zeiss LSM 510 microscope was used to obtain images. Scale bars were generated and inserted by LSM software.

2.4. In vitro migration and invasion assay

Cell migration and invasion assays were conducted as described (Qian et al., 2005) with a slight modification. Briefly, cells were serum-starved overnight. The transwells were coated with Matrigel Matrix (BD Biosciences) at 20 mg/mL used for invasion assay, and uncoated filters were used for migration assay. The top chamber of transwell was loaded with 0.2 mL of 4×10^5 cells/mL in serum-free media and the bottom chamber was loaded with 0.6 mL of DMEM medium containing 0.2% FBS. Cells were treated with 6-OH-BDE-47 in the transwells at 37 °C in a 5% CO_2 atmosphere for the indicated times. Migrated or invaded cells were fixed, stained, and counted using phase-contrast microscopy. Each migration and invasion assay was repeated in three independent experiments.

2.5. Real-time PCR detection of mRNA

The mRNA expression was determined by real-time PCR. Total RNA was isolated from cells and tumor tissues using the Trizol reagent (Takara, Japan) according to the manufacturer's instructions. Complementary DNA (cDNA) corresponding to 0.8 μg of total RNA was used per reaction (20 μL) in a real-time