Chemosphere 213 (2018) 559–567

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Genome-wide identification of the interactions between key genes and pathways provide new insights into the toxicity of bisphenol F and S during early development in zebrafish

Feng Yang [a, b, 1], Wenhui Qiu [a, b, *, 1], Rongzhen Li [a, b], Jiaqi Hu [a, b], Shusheng Luo [a, b], Ting Zhang [a, b], Xin He [a, b], Chunmiao Zheng [a, b, **]

[a] Guangdong Provincial Key Laboratory of Soil and Groundwater Pollution Control, School of Environmental Science and Engineering, Southern University of Science and Technology, Shenzhen 518055, China
[b] State Environmental Protection Key Laboratory of Integrated Surface Water-Groundwater Pollution Control, School of Environmental Science and Engineering, Southern University of Science and Technology, Shenzhen 518055, China

HIGHLIGHTS

- Three overlapping key pathways related to BPF and BPS exposure were identified.
- The hub genes affected by BPF and BPS exposure were *mst1ra*, *pik3cb*, and *prkcdb*.
- BPF and BPS may potentially be carcinogenic.

ARTICLE INFO

Article history:
Received 20 July 2018
Received in revised form
19 September 2018
Accepted 20 September 2018
Available online 21 September 2018

Handling Editor: David Volz

Keywords:
Bisphenol F
Bisphenol S
KEGG
GSEA
Hub genes
Carcinogenicity

ABSTRACT

Bisphenol F (BPF) and bisphenol S (BPS) have been widely used as alternatives to bisphenol A (BPA). With their increasing use, BPF and BPS have also been released into the environment; thus, their potential risks to aquatic organisms and humans are drawing attention. The objective of this study was to identify the interactions between key pathways and hub genes in zebrafish following BPF and BPS exposure, and to evaluate the potential risks to human health. We identified three key pathways using KEGG over-representation test and Gene Set Enrichment Analysis (GSEA): 'Necroptosis,' 'Adipocytokine signaling pathway,' and 'C-type lectin receptor signaling pathway.' Moreover, three hub genes (*mst1ra*, *prkcdb*, and *pik3cb*) and detailed interactions among the pathways were examined by the analyses of PPI network, subcellular location, and shortest-pathway. Surprisingly, all three pathways were strongly associated with a potential risk of cancer, as reported previously. In addition, the results of KOBAS shown in 'Pathways in Cancer' and 'Cancers' belong to the top 10 terms in pathway enrichment analyses using genes related to BPF or BPS in human, as was found using GenCLiP. Moreover, the Kaplan-Meier survival analysis was performed using homologenes (MST1R, PIK3CB and PRKCD) of hub genes in human to evaluate whether exposure to bisphenols may adversely affect breast cancer. Taken together, these studies demonstrate the potential carcinogenicity of BPF and BPS. To our knowledge, this is the first study on three overlapping key pathways and three hub genes to investigate BPF and BPS exposure-related mechanisms and subsequent interactions in zebrafish.

1. Introduction

Bisphenol A (BPA) has been used for the manufacturing of plastics, shopping receipts, food packaging, and other products, and is one of chemicals manufactured in high volumes globally, with a more than five million tons output in 2008 (Bailin et al., 2008). Owing to their large amounts and multiple industrial applications,

* Corresponding author. School of Environmental Science, Engineering and Shenzhen Key Laboratory of Soil and Groundwater Pollution Control, Southern University of Science and Technology, Shenzhen, Guangdong, 518055, China.
** Corresponding author. School of Environmental Science, Engineering and Shenzhen Key Laboratory of Soil and Groundwater Pollution Control, Southern University of Science and Technology, Shenzhen, Guangdong 518055, China.
E-mail addresses: qiuwh@sustc.edu.cn (W. Qiu), zhengcm@sustc.edu.cn (C. Zheng).
[1] These authors contributed equally.

https://doi.org/10.1016/j.chemosphere.2018.09.133
0045-6535/

BPA has been widely distributed in the environment, and has raised considerable public health concerns as it was broadly observed at low nanomolar levels in a variety of human samples such as urine, serum, and breast milk (Zhang et al., 2013). Furthermore, in the past few years, various studies have revealed numerous adverse effects of environmental BPA exposure on humans and animals, including ontogenetic and endocrine disruptions, and interference with neural networks, as well as with cardiovascular, metabolic, and immune systems (Peretz et al., 2014; Rochester and Bolden, 2015). As a result, several regulatory agencies have recently banned the use of BPA in various products for infants (Rochester and Bolden, 2015). Manufacturers thus have been searching for alternatives to produces BPA-free products.

Bisphenol F (BPF) and bisphenol S (BPS) have replaced BPA in many industrial applications containing polycarbonates and epoxy resins. The structure and physicochemical properties (LogKow and pKa) of BPF and BPS were similar to BPA (Niu et al., 2017) as shown in supporting information (Table S1). As a consequence, BPF and BPS have become emerging environmental contaminants, and are present ubiquitously in the environment owing to their increasing use. BPF and BPS have been detected in surface water, sediment, sewage effluent, and indoor dust, generally at lower concentrations than BPA, but approximately in the same order of magnitude (Song et al., 2014; Yang et al., 2014). For instance, BPS detected in surface waters of Adyar River in India have been found to range from ND to 7200 ng/L and BPF concentrations as high as 2850 ng/L were found in the Tamagawa River in Japan (Yamazaki et al., 2015). Moreover, BPF and BPS have been found in many daily products, such as personal care products (e.g., body wash, hair care products, makeup, lotions, and toothpaste), paper products (e.g., currency, flyers, tickets, mailing envelopes, and airplane boarding passes), and food (e.g., dairy products, meat and meat products, vegetables, canned foods, and cereals) (Liao and Kannan, 2013). In recent studies, BPF and BPS have even been detected in human breast milk (Niu et al., 2017). Human exposure to these substitute chemicals thus warrants further research to assess potential health risks.

Accumulating evidence suggests that BPF and BPS exhibit similar levels of toxicity to organisms as BPA (Rochester and Bolden, 2015). Previous studies report that hormonal activities (estrogenic, antiestrogenic, androgenic, and antiandrogenic) were significantly altered *in vitro* and *in vivo* after BPF and BPS exposure, in a similar mode of action (MOA) as BPA (Rajasarkka et al., 2014; Rosenmai et al., 2014). Thus, BPF and BPS have been categorized as xenoestrogens, indicating their potential to mimic the effects of endogenous estrogen and, thus, to disturb the reproductive system (Rochester and Bolden, 2015). Endocrine disruptors are associated with increased incidences of breast, prostate, and testis cancer, as well as diabetes, obesity, and decreased fertility (Pupo et al., 2012). What's more, BPF and BPS showed effects such as oxidative stress (Zhang et al., 2016b; Qiu et al., 2018b), DNA damage (Lee et al., 2013), cellular apoptotic and survival signaling (Salvesen and Walsh, 2014), which were involved in progress of cancer (Lambert et al., 2017). Furthermore, various *in vivo* and *in vitro* studies have reported that low-dose BPA exposure can result in mammary neoplastic lesions (Wang et al., 2017). Hence, BPF and BPS, similar potency to BPA, which was suggested to have potential carcinogenic risk.

Zebrafish (*Danio rerio*) show high genetic homology to humans, with gene conservation rates of approximately 70%, and are commonly used in toxicology studies (MacRae and Peterson, 2015). Many functionally essential genes and molecular compounds found in humans (e.g. those involved in developmental processes and toxicological responses) can also be found in zebrafish. Thus, it is well worth exploring the potential toxicity of BPF and BPS in zebrafish. However, recent studies on BPF and BPS are almost exclusively focused on endocrine toxicity and cytotoxicity (Rochester and Bolden, 2015), but other toxicity and its action mechanism remains limited. This is due to the lack of comprehensive studies on the key molecular and adverse outcome pathways of BPF and BPS. High-throughput transcriptome technology (RNA-Seq) provides insight into expression profiles of many hundreds or thousands of genes. Furthermore, pathway analysis technologies allow mapping of gene expression data as pathway maps, based on their respective functional annotation and known molecular interactions (Kanehisa et al., 2016). Thus, the present study aimed to observe the core pathways and genes affected by BPF and BPS exposure in zebrafish using RNA-Seq.

2. Materials and methods

2.1. Chemicals

BPF (CAS Number 620-92-8, 98%) and BPS (CAS Number 80-09-1, 98%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The compounds were dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich) to obtain stock solutions of 10 g/L each. Fresh stock solution was prepared on a weekly basis and stored at 4 °C. All other chemicals were of analytical grade and were also purchased from Sigma-Aldrich.

2.2. Zebrafish maintenance

Wild-type adult zebrafish were kept under a light-dark cycle of 14:10 h, and under laboratory conditions at 28 ± 0.5 °C. Adult male and female zebrafish were allowed to breed, according to the standard breeding protocols. Embryos were then collected within 1 h after spawning and were examined under a stereomicroscope to remove unfertilized eggs.

2.3. Experimental design

Zebrafish embryo toxicity tests were performed as described previously (Wu et al., 2011). Briefly, zebrafish embryos (4 h post fertilization) were randomly distributed in Petridishes (100 embryos per dish) immediately before chemical exposure (50 mL). The embryos were exposed to BPF and BPS at concentrations of 100 μg/L in E3 medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$, 0.3 mM $MgSO_4$), for 120 h and at 28 ± 0.5 °C. A control group was incubated with 0.005% DMSO in the embryo medium in parallel. All treatment solutions were replaced completely every 24 h. Concentrations of control, BPF, and BPS in the exposure solutions after 24 h treatment were measured using LC-MS/MS (Agilent 1260 Infinity, Santa Clara, CA, USA) and the detail methods were present in the SI and no significant changes were detected between initial concentrations and observed values after 24 h (Table S2). Zebrafish larvae of the control and experimental groups were collected after 120 h of exposure, and subsequently immersed in liquid nitrogen. The larvae were then stored at −80 °C until RNA-Seq and quantitative PCR (qPCR) analyses.

2.4. RNA extraction, sequencing, and identification of differentially expressed genes (DEGs)

Three biological replicates were mixed as the same barcoded sample to do RNA-Seq with deep sequencing for the RNA-Seq, according to the previous studies (Zhang et al., 2016a, 2018). Total RNA of zebrafish larvae samples was extracted using a TRNzol Total RNA Extraction Reagents kit (Tiangen Biotech Co., Ltd., Beijing, China), following the manufacturer's instructions. RNA quality was assessed using NanoDrop™ (Thermo Fisher Scientific, Waltham,