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Transcriptomic and methylomic analysis reveal the toxicological effect of 2,3,7,8-Tetrachlorodibenzodioxin on human embryonic stem cell

Keng Po Lai [a], Jing Woei Li [a, b], Ting Fung Chan [b], Andy Chen [c], Cherie Yin Lau Lee [c], William Shu Biu Yeung [c], Chris Kong Chu Wong [d, *]

[a] Department of Chemistry, City University of Hong Kong, China
[b] Partner State Key Laboratory of Agrobiotechnology and School of Life Sciences, The Chinese University of Hong Kong, China
[c] Department of Obstetrics and Gynaecology, The University of Hong Kong, China
[d] Partner State Key Laboratory of Environmental and Biological Analysis, Croucher Institute for Environmental Sciences, Department of Biology, Hong Kong Baptist University, China

HIGHLIGHTS

- Dioxin exposure altered transcriptomic profile of hESCs.
- Dioxin exposure altered epigenetic pattern of hESCs.
- Dioxin exposure caused hepatic and cardiac toxicological effects in hESCs.
- Dioxin exposure predisposed metabolic disease in offspring.

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ABSTRACT

Cumulating epidemiological studies demonstrated that environmental exposure to endocrine disrupting chemicals (EDCs) during the early stages of fetal development is associated with the increase in disease susceptibility in later life. The fetal developmental plasticity is considered as a protective mechanism against an undesirable prenatal environment. Dioxin is one of the environmental contaminants and is considered a diabetogenic factor. Experimental animal and human epidemiological studies have revealed that dioxin exposure was associated with insulin resistance and altered beta cell function. But the effect of dioxin exposure in early stage of fetal development is still largely unknown. In this report, we used the human embryonic stem cell (hESC) line, VAL-3, as a model, together with Methyl-CpG Binding Domain (MBD) protein-enriched genome sequencing and transcriptome sequencing (RNA-seq), in order to determine the dynamic changes of the epigenetic landscape and transcriptional dysregulation in hESC upon dioxin exposure. The bioinformatics analyses including the Database for Annotation, Visualization and Integrated Discovery (DAVID) analysis and Ingenuity Pathway Analysis (IPA) highlighted the predisposed neural, hepatic, cardiac and metabolic toxicological effects of dioxin during the fetal development.

1. Introduction

The World Health Organization and cumulating studies have reported that environmental pollution is one of the major contributing factors (Gluckman et al., 2010) for the prevalence of human diseases. Approximately a thousand new chemicals are produced every year (Judson et al., 2009), and over 358 industrial chemicals and pesticides have been detected in the cord blood of American infants (Environmental Working Group (EWG), 2009). Moreover, the transfer of environmental contaminants across placenta to cord blood/tissue and human breast milk were documented in a cohort study of over 2000 paired maternal-fetal samples in the Faroe Islands (Needham et al., 2011). More importantly, epidemiological evidence increasingly implies that environmental exposure to EDCs during the early stages of fetal

* Corresponding author. Partner State Key Laboratory of Environmental and Biological Analysis, Croucher Institute for Environmental Sciences, Department of Biology, Hong Kong Baptist University, China.
E-mail address: ckcwong@hkbu.edu.hk (C.K.C. Wong).

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development play a role in the observed increase in disease susceptibility in later life. Fetal life is a critical stage for structural and functional development, which can be influenced by both genomic (epigenetic and genetic) and environmental factors (Bateson et al., 2004; Gluckman et al., 2005a, 2005b, 2008, 2009; Hanson and Gluckman, 2008; Gluckman et al., 2007). The concept of fetal developmental plasticity has been thought to be a protective mechanism against an undesirable prenatal environment and developed as a predictive adaptive response to prepare for a similar undesirable situation in postnatal life (Gluckman et al., 2005c). Intriguingly, emerging evidence supports the hypothesis that the modulatory effects of different EDCs on epigenetic programming, cell signaling and tissue functions would lead to chronic and/or *trans*-generational effects on the exposed animals or increase disease susceptibility in their offspring (Diamanti-Kandarakis et al., 2010; Ho et al., 2012). For instance, numerous reports have demonstrated the effects of EDCs on DNA methylation in the early development of mice (Anway and Skinner, 2008; Bernal and Jirtle, 2010; Dolinoy et al., 2007), abnormal embryonic karyotype and chromosome synapsis defects in humans (Susiarjo et al., 2007; Sugiura-Ogasawara et al., 2005), spine synapses formation in non-human primates (Leranth et al., 2008), fetal mouse mammary gland development and stromal-epithelial signaling (Soto and Sonnenschein, 2010; Vandenberg et al., 2007), Hox gene expression and genitourinary development. (Block et al., 2000; Yiee and Baskin, 2010).

Among different EDCs, dioxins are one of the environmental contaminants with the highest potency on biological activities at concentrations of picomolar (pM) levels. In a review study of blood concentrations of dioxins in 29,687 subjects of general populations in 26 countries, the mean dioxins level (WHO-Toxic Equivalency (TEQ)) was about 7 pM (Consonni et al., 2012). From a recent study of measuring dioxins in blood and breast milk samples from pregnant women in Japan, the mean levels of dioxins (WHO-TEQ) were found to be 13.1 and 8.6 pM respectively (Todaka et al., 2011). The mechanistic actions of dioxins are known to be mediated mainly via the cytosolic receptor, aryl hydrocarbon receptor (AHR), which belongs to a member of the basic helix-loop-helix/Per-Arnt-Sim (bHLH/PAS) family of transcription factors (Mimura and Fujii-Kuriyama, 2003). An evolutionary analysis on the physiological significance of AHR in diverse species suggested its role in both normal cell homeostatic and developmental functions (Sartor et al., 2009; Bock and Kohle, 2006; Fernandez-Salguero et al., 1997). A genome-wide analysis of the TCDD-AHR-mediated pathway revealed its important role on gene clusters associated with morphogenetic and developmental programs (Sartor et al., 2009). Since dioxin is a diabetogenic factor, both experimental animal and human epidemiological studies have revealed that dioxin exposure was associated with insulin resistance and altered beta cell function. In the current report, we used the human embryonic stem cell (hESC) line, VAL-3, as a model, together with Methyl-CpG Binding Domain (MBD) Protein-enriched Genome Sequencing and transcriptome sequencing (RNA-seq), in order to determine the dynamic changes of the epigenetic landscape and transcriptional dysregulation in hESC upon dioxin exposure. The combined toxicogenomic analysis will provide better understanding on the predisposed toxicological effects of dioxin during the fetal development.

2. Materials and methods

2.1. Human embryonic stem cell culture

Human embryonic stem cell line, VAL-3 was cultured on Matrigel (BD Biosciences) coated plate with mTeSR1 medium (Stemcell technologies). VAL-3 was passaged every 4–5 days using Accutase (Stemcell technologies) with the supplementation of ROCK inhibitor (Y-27632, Stemcell technologies) for the first day after cell seeding. The cells were maintained at 37 °C in a humidified incubator in an atmosphere of 5% CO2 – 95% air. For the TCDD treatment, cells were exposed to 10 pM TCDD (Sigma-Aldrich) (paralleled with 0.05% of DMSO as control) for 3 weeks.

2.2. Methyl-CpG binding domain (MBD) protein-enriched genome sequencing

MethylMiner™ Methylated DNA Enrichment Kit (Invitrogen) was used to enrich and fractionate the methylated double-stranded DNA according to the manufacturer's instruction. Briefly, the whole genomic DNA was fragmented into sizes enriched at range of 400–600 bp using the Covaris® M220 Focused-ultrasonicator™ Instrument. The sizes of fragmented DNA were verified using Agilent Bioanalyzer 2100 systems plus high sensitivity DNA chip. Methylated DNA was isolated from fragmented whole genomic DNA (100 μg) via binding to the methyl-CpG binding domain of human MBD2 protein, which was coupled to paramagnetic Dynabeads® M – 280 Streptavidin via a biotin linker. Finally, the methylated DNA fragments were eluted as a single enriched population with a 2000 mM NaCl elution. The DNA was precipitated in ethanol and resuspended in DNase-free water. The methylated DNA was subjected to DNA library construction using the Illumina TruSeq DNA LT Sample Prep Kit according to the manufacturer's instruction. Paired-end reads, each of 150 bp read-length, were sequenced on the Illumina MiSeq sequencer. The sequence reads were dynamically trimmed according to the Burrows-Wheeler Aligner (BWA) –q algorithm with a parameter of 20 (Li and Durbin, 2009). A running sum algorithm was executed. Quality-trimmed reads were aligned onto human genome GRCh38 (GCA_000001405.15_GRCh38_full_analysis_set) using BWA-MEM (version 0.7.10) (Li and Durbin, 2010). Duplicate reads were removed by Piccard Tools (version 1.77). Peaks were called using MACS (version 1.42) (Feng et al., 2012) and annotated using HOMER (Heinz et al., 2010).

2.3. RNA sequencing and data analysis

Four RNA (cDNA) libraries of hESC were constructed (2 for the control group, 2 for the TCDD treatment group) using the TruSeq Stranded RNA LT Sample Prep Kit (Illumina), each prepared from 300 ng of total RNA as previously described (Wang et al., 2016a). Paired-end reads, each of 150 bp read-length, were sequenced on the Illumina MiSeq sequencer. The STAR aligner v.2.3.0e (Dobin et al., 2013) was used to align the quality-trimmed (method as described above) transcriptome data onto the 1000 Genome hs37d5 reference. Gene level read-count were summarized to the GENCODE gene annotation v19 with the use of HTSeq version 0.6.0 (Anders et al., 2015) (parameter used are as follows, featuretype: exon; mode:union stranded: reverse). Differential gene expression was determined using edgeR 3.0.8 (Robinson et al., 2010), and samples with identical treatments were considered to be biological replicates. Genes with B&H corrected p-value < 0.05 and |log2 (fold change)| >1 were classified as differentially expressed genes (DEGs).

3. Results

3.1. TCDD caused differential gene expressions in human embryonic stem cell

Using RNA sequencing, we obtained 13.98 Mb and 11.85 Mb