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Dioxin and AHR impairs mesoderm gene expression and cardiac differentiation in human embryonic stem cells

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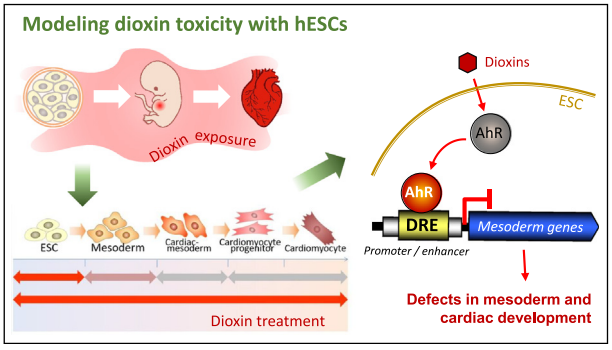
HIGHLIGHTS

- Established an in vitro model to study cardiac toxicity using human embryonic stem cells
- Defined the role of AhR in mesoderm development and lineage specification
- Uncovered TCDD toxicity and AhR function in early developmental stages in human

GRAPHICAL ABSTRACT



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ABSTRACT

Dioxin and dioxin-related polychlorinated biphenyls are potent toxicants with association with developmental heart defects and congenital heart diseases. However, the underlying mechanism of their developmental toxicity is not fully understood. Further, different animals show distinct susceptibility and phenotypes after exposure, suggesting possible species-specific effects. Using a human embryonic stem cell (ESC) cardiomyocyte differentiation model, we examined the impact, susceptible window, and dosage of 2,3,7,8 tetrachlorodibenzo p dioxin (TCDD) on human cardiac development. We showed that treatment of human ESCs with TCDD at the ESC stage inhibits cardiomyocyte differentiation, and the effect is largely mediated by the aryl hydrocarbon receptor (AHR). We further identified genes that are differentially expressed after TCDD treatment by RNA-sequencing, and genomic regions that are occupied by AHR by chromatin immunoprecipitation and high-throughput sequencing. Our results support the model that TCDD impairs human ESC cardiac differentiation by promoting AHR binding and repression of key mesoderm genes. More importantly, our study demonstrates the toxicity of dioxin in human embryonic development and uncovered a novel mechanism by which dioxin and AHR regulates lineage commitment. It also illustrates the power of ESC-based models in the systematic study of developmental toxicology.

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1. Introduction

Congenital heart defects (CHDs) are the most common type of birth defect, affecting close to 1% of live births worldwide (Junghare and Desurkar, 2017). They are thought to be caused by a combination of genetic and environmental factors (Catana and Apostu, 2017). However, only a small fraction of CHDs can be attributed to heritable gene mutations and the same CHD mutations can cause a variety of disease phenotypes, suggesting the involvement of modifier genes as well as non-genetic factors (Muntean et al., 2017; Sarmah et al., 2016; Fahed et al., 2013).

In human populations, epidemiology studies showed that occupational or accidental exposure to environmental chemicals such as dioxins can lead to increased congenital defects in newborns and ischemic heart diseases in adults (Humblet et al., 2008; Bertazzi et al., 2001; Revich et al., 2001; Ketchum and Michalek, 2005). In addition, epidemiology evidence linking prenatal dioxin exposure and birth defects, miscarriage, and stillbirth has also been well documented (Noel et al., 2015; Schmidt, 2016; Kishi et al., 2017). In a recent review of the Seveso cohort, it was reported that out of 52 Seveso children with prenatal dioxin exposure, 5 showed abnormalities in the heart and 3 showed abnormalities in the vascular system. But the small number of cases did not provide sufficient statistical power to draw strong correlations (Eskenazi et al., 2018). Thus, whether prenatal TCDD exposure directly contributes to congenital heart diseases remains unknown. In animals, experiments showed that the developing heart is sensitive to dioxins and dioxin-like polychlorinated biphenyls (PCBs) (Kopf and Walker, 2009), as dioxin impairs cardiac development, morphology, and/or function in different species (Cantrell et al., 1996; Hornung et al., 1999; Guiney et al., 2000; Antkiewicz et al., 2005; Walker and Catron, 2000; Sommer et al., 2005; Thackaberry et al., 2005; Aragon et al., 2008; Carreira et al., 2015a). While this is consistent with findings in human populations, the underlying mechanism has not been fully delineated.

The teratogenic and carcinogenic effects of dioxin are thought to be mediated by AHR. AHR is a ligand-activated transcription factor and is highly conserved from invertebrates to vertebrates. It contains a ligand binding domain, and can interact with many environmental compounds. Upon ligand binding, AHR binds to the dioxin-responsive elements (DREs) in gene promoters and enhancers, and regulates downstream transcription (Denison et al., 2011). In addition to xenobiotic response, AHR also plays critical roles in normal development and other physiological functions, as AHR deletion mice show defects in organ and vasculature development, reduced fertility, and cardiac hypertrophy (Fernandez-Salguero et al., 1997; Schmidt et al., 1996; Thackaberry et al., 2002). It has been proposed that AHR originally evolved to regulate embryonic development and only acquired the ability to bind xenobiotic chemicals during the evolution of vertebrates (Hahn et al., 2017).

Embryonic stem cells (ESCs) are pluripotent stem cells that can differentiate into all cell types in the adult body. In vitro differentiation of ESCs into the cardiac lineage has been found to largely mimic the early steps in heart development, and has been successfully used as a culture model to investigate the regulatory mechanisms during cardiogenesis. In addition, it has also been used to test the developmental toxicity of environmental chemicals (Jiang et al., 2016). Here, we employed an advanced monolayer-based human ESC cardiomyocyte differentiation protocol (Burridge et al., 2014) to investigate the role of dioxin and AHR in human cardiac development. Our results indicate that dioxin impairs the induction of mesoderm genes via AHR, thereby inhibiting mesoderm and cardiac differentiation. Our study provides experimental support for the toxicity of dioxin in human cardiac development, and uncovers a novel epigenetic mechanism by which AHR regulates developmental gene expression. It also illustrates the power of ESC-based in vitro models in the systematic study of environmental health science questions.

2. Materials and methods

2.1. Cell culture and cardiomyocyte differentiation

Human ESC lines H1 (WA01) and H9 (WA09) were obtained from WiCell Research Institute. Human ESC line Mel1 was kindly provided by Dr. Edouard G Stanley. In this cell line, EGFP expression is driven by the regulatory elements of the cardiac progenitor gene Nkx2.5, and can thus be used to monitor the differentiation process. Cells were routinely maintained in TeSR™-E8™ (Stemcell Technologies) on growth factor-reduced Matrigel (Corning) following published protocols (Chen et al., 2011).

Human ESCs were cultured in E8 to 90–100% confluency before induction. To induce cardiac differentiation, the medium was replaced with cardiac differentiation basal medium 1 (RPMI1640 containing 1 × B27 minus insulin supplement) with 5 μM GSK3 inhibitor CHIR99021 (SelleckChem) for 2 days, and changed to basal medium 1 without the inhibitor for another 2 days. Cells were then cultured in basal medium 1 with 5 μM Wnt inhibitor IWR-1 (SelleckChem) for 2 days, followed by basal medium 1 only for 2 additional days. After that, cells were cultured in cardiac differentiation basal medium 2 (RPMI1640 containing 1 × B27 supplement) for 6 more days with medium refreshment every 2 days. During this differentiation process, ESCs were induced to cardiomyocyte through the mesoderm, cardiac mesoderm, cardiomyocyte progenitor, and cardiomyocyte stages.

2.2. Chemical exposure

2,3,7,8 Tetrachlorodibenzodioxin (TCDD, Sigma), AHR inhibitor CH223191 (Sigma) and induction reagents were dissolved in DMSO. In dose titration, TCDD is added to a final concentration of 0.1, 1, 2, or 10 nM as indicated. In other experiments, TCDD is added to a final concentration of 2 nM based on a previous study (Grumetto et al., 2015). The AHR inhibitor CH223191 is added to a final concentration of 1 μM. For control experiments, equivalent volume of DMSO is added. The final DMSO concentration in both the control and experimental groups were kept at 0.1% to minimize the impact on cell growth.

2.3. Immunofluorescence staining and western blotting

Immunofluorescence staining was carried out as described before (Zheng et al., 2016). Cells at different stages were stained with primary antibodies against T (TBXT) (Santa Cruz sc17745, 1:200), ISL-1 (ISLET1) (Developmental Studies Hybridoma Bank 39.4D5, 1:200), ACTN2 (ACTININ2) (Abcam ab9465, 1:300), TNNT2 (TROPONIN T2) (Abcam ab8295, 1:300) and cell nuclei were counterstained with DAPI (Invitrogen). Images were taken with Zeiss Axiovert 40 inverted microscope.

Cells were lysed in cell lysis buffer (Thermo Scientific 78501) containing 1× protease inhibitor tablet (Sigma 11836170001) and blotted using standard protocols. Antibody against AHR (b-11, Santa Cruz) was used at 1:1000 dilution.

2.4. RNA isolation, reverse transcription, and RT-qPCR

Total RNA was isolated using the GeneJet RNA purification kit (Thermo Scientific), and reverse transcribed using the iScript cDNA Synthesis Kit (Bio-Rad) to generate cDNA. qPCR was performed using the SsoFast EvaGreen Supermix (Bio-Rad) on the Bio-Rad CFX-384 Real-time PCR Detection System. Primers used in the study are listed in Table S5. Actin (ACTB) was used for normalization, and values were plotted as mean ± standard error.