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Studying developmental neurotoxic effects of bisphenol A (BPA) using embryonic stem cells

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There is little to no toxicity information regarding thousands of chemicals to which people are exposed daily. In fact, of the 84,000 chemicals listed in the United States Toxic Substances Control Act Inventory, there is limited information available on their effects on neural development (Betts, 2010; US EPA, 2015). The number of chemicals tested remains low due to the high cost of conducting multi-generational animal studies and the lack of alternative testing methods.

In addition to high costs, inter-species variation has led to efforts to limit the use of animal models (mammalian and non-mammalian), and instead, turn to using alternative strategies to test toxicity. The goals of “Toxicity Testing in the 21st Century: A Vision and a Strategy”, the 2007 report of the U.S. National Research Council, are to use high-throughput assays to identify endpoints and pathways linked to toxicity and apply predictive models to prioritize chemicals of interest for further testing (Kavlock et al., 2012; NRC, 2007; Tice et al., 2013; Krewski et al., 2014). Ultimately, new testing paradigms based on computational models and *in vitro* assays have the potential to eliminate the need for traditional animal tests (NRC, 2007). The main challenge of traditional *in vitro* systems is to simulate

the complex nature of biological systems (Knudsen et al., 2015; Wu et al., 2014).

Studying the differentiation of stem cells is a promising model to measure the impact of environmental chemical exposure on embryonic development (Mori and Hara, 2013). Mouse embryonic stem cells (mESCs) were first isolated and cultured *in vitro* by Evans and Kaufman (1981). In 1998, Thomson et al. (1998) first isolated and characterized human embryonic stem cells (hESCs). Since then, embryonic stem cells (ESCs) have been used in a variety of fields to study tissue regeneration and to better understand normal development and to screen drugs and toxicants (Hoffman and Carpenter, 2005; Faiola et al., 2015). ESCs are derived from preimplantation stage embryos, a process that involves culturing embryos to the morula or blastocyst stage (Hoffman and Carpenter, 2005). ESCs are pluripotent, meaning cells can differentiate into the three germ layers: ectoderm, mesoderm, and endoderm (Thomson et al., 1998). During development, the endoderm eventually forms the gut epithelium; the mesoderm forms cartilage, bone, smooth muscle, and striated muscle; and the ectoderm forms neural epithelium, embryonic ganglia, and stratified squamous epithelium (Thomson et al., 1998), as shown in Fig. 1.

In a recently published study in the *Journal of Environmental Sciences*, Yin et al. from the State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, used mESCs to study the developmental neurotoxicity of bisphenol A (BPA). BPA, one of the most widely known environmental pollutants (Fromme et al., 2002; Jiang et al., 2012; Chen et al., 2014; Zhang et al., 2014), is a monomer found in polycarbonate plastics and in the lining of many food and beverage containers. Heat and contact with either basic or acidic compounds can accelerate leaching of BPA from food storage containers and cans (vom Saal and Hughes, 2005). In a literature review on BPA

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exposure, vom Saal and Hughes (2005) stated the need for new risk assessment methods to test the toxicity of BPA. The review emphasized the extensive literature of sub-lethal toxic effects, widespread human exposure due to leaching from food and beverage containers, high levels of BPA reported in human blood and tissue, and epidemiological studies linking BPA exposure to adverse effects in women as contributing to this need for further research.

One of the advantages of using ESC toxicity tests is the ability to observe the differentiation of mESCs via the formation of embryoid bodies (EBs). EBs are three-dimensional cell aggregates, which simulate the developing embryo *in vitro*, and are able to differentiate into all three germ lineages: endoderm, ectoderm, and mesoderm, and further differentiate into all the cell types (Desbaillets et al., 2000). Yin et al. (2015) first measured the gene expression of markers for each germ lineage to explore whether BPA affected differentiation of mESCs. Their results showed that BPA affected gene expression markers of the endoderm, ectoderm and trophoctoderm, but not of the mesoderm. The lack of effect on gene expression of the mesoderm was consistent with the observation of a previous study that focused on the mesoderm (Panzica-Kelly et al., 2013). However, the study of Yin et al. (2015) found that BPA affected the gene expression of the other germ lineages. To test whether BPA affected neurodevelopment, Yin et al. further examined the neural ectoderm specifically during EB differentiation. The expression of four neuroectoderm markers, *sox1*, *pax6*, *sox3*, and *nestin*, was significantly decreased in BPA-treated samples compared to the controls. These results showed direct evidence of BPA neurotoxicity.

To improve the precise assessment of BPA developmental neurotoxicity, Yin et al. (2015) utilized two protocols to drive the differentiation of mESCs specifically into neural ectoderm first, and then into neural progenitor cells (NPCs). The first protocol was via the formation of EBs, and the second

was via cells grown under monolayer conditions (Fig. 2). The results showed again that BPA significantly reduced the expression of the four neural ectoderm markers based on the EB formation method. BPA also impaired the expression of *sox1* and *pax6* based on cells grown in monolayer conditions. These data further supported the conclusion of the developmental neurotoxicity of BPA.

In summary, Yin et al. (2015) demonstrated that mESCs are a good model to detect developmental toxicity of environmental substances. One advantage of their method is its higher sensitivity, enabling the detection of BPA neurotoxicity at lower concentrations (*e.g.*, 10 $\mu\text{mol/L}$). The concentrations of BPA used by Yin et al. are significantly lower than the median lethal concentration (IC_{50} , 29 $\mu\text{g/mL}$ or 127 $\mu\text{mol/L}$) reported by Kong et al. (2013). The other advantage is that they observed the effects of BPA on gene expression over a time course, rather than at a single time point that is usually used in other toxicological studies (Mori and Hara, 2013). In fact, gene expression during ESC differentiation is a dynamic process, and not all the genes are expressed at the same time.

The probable daily intake (PDI) of BPA, as estimated by the Health Products and Food Branch (HPFB), Health Canada in 1995, is 0.18 $\mu\text{g/kg}$ body weight (bw)/day for adults and 2.63 $\mu\text{g/kg}$ bw/day for infants (Health Canada, 2008). The provisional tolerable daily intake (pTDI) established by Health Canada in 1996 is 25 $\mu\text{g/kg}$ bw/day. The U.S. Environmental Protection Agency (US EPA) and the European Food Safety Authority (EFSA) have also established pTDI values for BPA. The US EPA expresses this as an “oral reference dose (RfD)” set as 50 $\mu\text{g/kg}$ bw/day (US EPA, 1993) and the EFSA has recently revised the value of the “tolerable daily intake” from 50 $\mu\text{g/kg}$ bw/day to a “temporary tolerable daily intake” of 4 $\mu\text{g/kg}$ bw/day (EFSA, 2015). The estimated daily intake by Canadians is much lower than these guideline or reference values. However, BPA has since been characterized as an endocrine disrupting agent and has shown

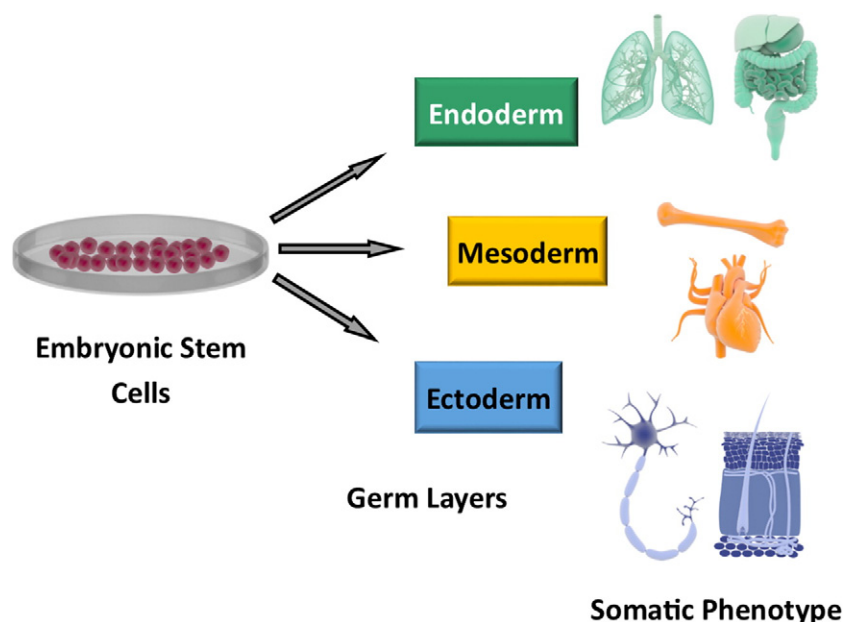


Fig. 1 – Differentiation of embryonic stem cells into three primary germ layers: endoderm, mesoderm, and ectoderm.

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