Effects of TCDD on MCF10A cells in 2D and 3D Cultures

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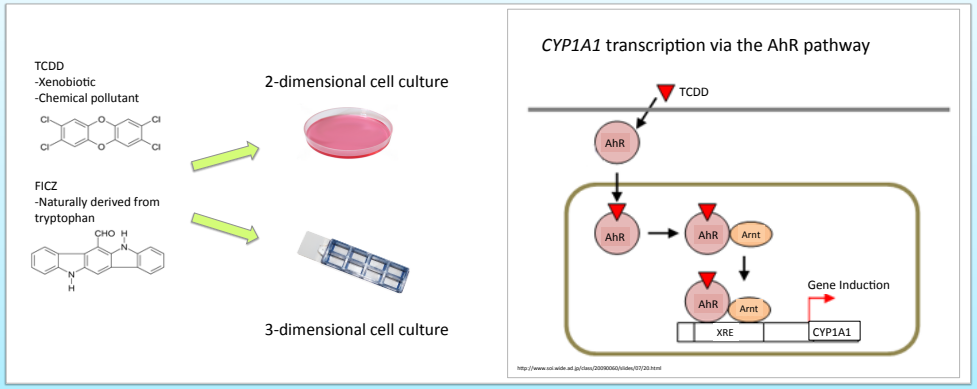
Abstract

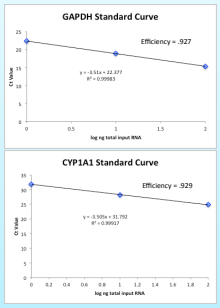
2,3,7,8 –tetrachlorodibenzo-*p*-dioxin (known as dioxin or TCDD) is a chemical pollutant that can be found in drinking water, soil, dust, and air. TCDD has a biological half-life of 7-10 years in humans. It is unethical to study the effects of TCDD on humans and the use of an animal model may not always be relevant to humans. A reasonable alternative is to use 3-dimensional human cell culture. The human breast epithelial cell line MCF10A represents normal breast tissue and can be grown in 3D culture. In contrast to traditional 2-dimensional culture, MCF10A cells form secondary structures, called acini, when grown in 3D culture. Studying acini offers a unique advantage because these structures more accurately portray tissue growth and development *in vivo*. TCDD binds to the aryl hydrocarbon receptor (AhR) and can induce cytochrome P450s and growth factors. In these experiments MCF10A cells were grown in 2D or 3D culture and were exposed to TCDD or left untreated. AhR mediated gene expression of one cytochrome P450s, CYP1A1, was measured.

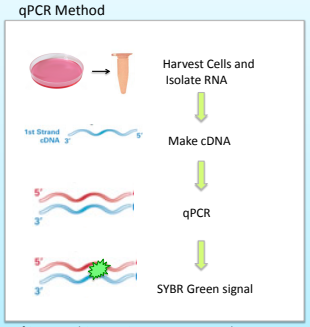
Background

Often to determine the toxic or carcinogenic potential of environmental chemicals, scientists have used animal testing. This approach is not viable for TCDD due to its varied toxicity. To understand the molecular mechanisms of toxicity, often two-dimensional (2D) cell culture is chosen as a model system due to its low cost and ease of use. However, cells grown in three-dimensional (3D) culture more accurately reflect how cells behave *in vivo* than cells grown in 2D culture. In addition, cell signaling and gene expression can differ dramatically depending on whether cells are grown in 2D vs. 3D culture. MCF10A cells are human breast epithelial cells that form a monolayer in 2D culture. In 3D culture, MCF10A cells form structures called acini that mimic *in vivo* duct structures. TCDD is a chemical pollutant and FICZ is naturally derived from tryptophan. Both are ligands for the Aryl hydrocarbon Receptor (AhR). The objective of my project will be to compare the effects of TCDD and FICZ on human mammary epithelial cells grown in 2D and 3D cultures.



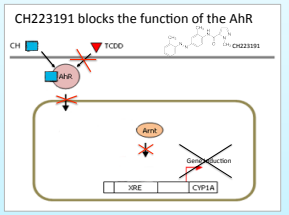
Quantitative PCR





qPCR primer standard curves- Standard curves of CYP1A1 and GAPDH primers were constructed to confirm our qPCR assay was optimal To construct the standard curves, serial dilutions of MCF10A cDNA were tested with each primer. The slope of each curve is a measure of reaction efficiency, a slope of -3.32 corresponds to a reaction efficiency of 100%. The *y*-intercept represents the limit of detection, Ct values above this number are not valid. Results indicated that our primers have similar reaction efficiencies, allowing GAPDH to be used as a reference gene for CYP1A1 induction.

AhR Antagonist



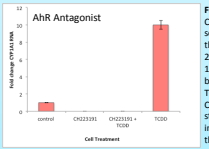


Figure 1. The AhR Antagonist, CH223191, inhibits the ability of some ligands to bind and activate the Ah Receptor. MCF10A cells in 2D culture were incubated with 10 μM CH223191 for 30 minutes before being exposed to 10 nM TCDD for 4 hours. The ability of CH223191 to block TCDD-stimulated CYP1A1 induction indicates that TCDD is acting through the AhR in MCF10A cells.

2D Culture

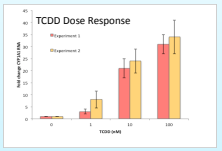


Figure 1. MCF10A cells in 2D culture were exposed for 3 hours in experiment 1 and 4 hours in experiment 2. As the concentration of TCDD increases induction of CYP1A1 increases.

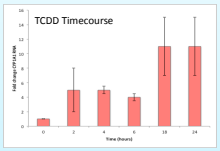


Figure 2. MCF10A cells were exposed to 10 nM TCDD for time periods indicated. Maximum CYP1A1 induction occurs at 18 hours and is sustained at 24 hours.

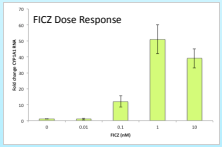


Figure 4. MCF10A cells were exposed for 3 hours. Induction of CYP1A1 was maximal at 1 nM FICZ.

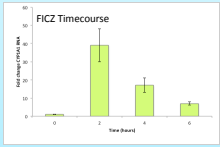


Figure 5. MCF10A cells were exposed to 1 nM FICZ for the periods indicated. Maximum CYP1A1induction occurred at 2 hours and decreased over time.

3D Culture

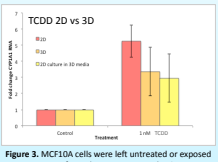


Figure 3. MCF10A cells were left untreated or exposed to 10 nM TCDD for 24 hours. CYP1A1 induction was highest in 2D culture but there was not a significant difference between the different cell culture conditions.

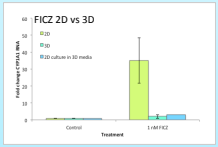


Figure 6. MCF10A cells were left untreated or exposed to 1 nM FICZ for 2 hours. CYP1A1 induction was maximized in 2D culture. The addition of 3D media to 2D culture blocked CYP1A1 induction.

Conclusions

- Experiments with the AhR antagonist demonstrate that both FICZ and TCDD signal through the AhR pathway.
- In 2D culture TCDD causes maximum CYP1A1 induction at late, 18-24 hour, time points. In contrast, FICZ stimulates CYP1A1 induction at early time points of 2-3 hours.
- In 3D culture, FICZ did not stimulate CYP1A1 induction. FICZ stimulated CYP1A1 induction was also blocked in 2D culture when 3D assay media was used in the monolayer system.

Future Experiments

- Repeat experiments using 3D assay media in 2D monolayer culture.
- Measure induction levels of other genes regulated by the AhR.
- Determine CYP1A1 induction due to TCDD and FICZ in different cell lines that can be grown in 3D culture.
- Use compounds that do not act through the AhR to investigate potential differences in gene expression in 2D vs 3D cultures.

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