Regulation of the Wnt/β-catenin pathway by steroid hormones in Danio rerio ovarian tissue

Macaulie Casey
Regulation of the Wnt/β-catenin pathway by steroid hormones in Danio rerio ovarian tissue

Macaulie Casey* and Alyce DeMarais
Biology Department, University of Puget Sound, Tacoma, WA
*mcasey@pugetsound.edu

Background

- Estrogens and progestins regulate the menstrual cycle, including regulation of the Wnt/β-catenin pathway.¹
- The pathway regulates genes involved in cell proliferation and apoptosis. It is implicated in ovarian cancer and it is used as a marker for Epithelial Ovarian Cancer (EOC)², where high levels of β-catenin may be involved in tumor development³

Objective

- Monitor the Wnt/β-catenin pathway’s activity following exposure to different steroid hormones by using β-catenin (ctnnb1) and GSK3β as markers, as well as p53 and c-MYC, target genes for this pathway

Methods

- Collect ovary tissue from zebrafish (Danio rerio) and incubate in 60% L-15 medium (with 1x antibiotic/antimycotic) and given steroid hormone (0, 1, 10, 100 ng/mL) at 26 °C for 4 hours
- Extract mRNA and convert to cDNA (50 ng/μL)
- Perform real time quantitative (rt-q)PCR to quantify relative concentrations of mRNA present in the ovary tissue

5α-dihydrotestosterone (DHT)

17β-Estradiol (Estrogen)

Bisphenol A (BPA)

Discussion

- DHT appears to inhibit the pathway, although p53 and c-MYC expression increase with DHT concentration
- Estrogen affects the pathway in a dose-dependent manner, with 10 ng/mL producing the largest response. The vehicle control (MeOH) also appears to have a significant impact. Expression of c-MYC decreases with an increasing concentration of estrogen, while the expression of p53 only mildly increases
- BPA exposure appears to stimulate the pathway, notably at 1 ng/mL, while p53 and c-MYC expression seem to be dose dependent

Acknowledgements: Thank you to the Mellam Foundation for funding this project, as well as the University of Puget Sound and University Enrichment Committee. Special thanks to my mentor, Alyce DeMarais; Amy Replige for rt-qPCR wisdom; and my fellow lab members.