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5α-dihydrotestosterone and the Wnt/β-catenin pathway in Danio rerio ovarian tissue following 2 and 4 hour incubations

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Introduction

This study investigated whether exogenous 5α-dihydrotestosterone (DHT) utilizes the Wnt/β-catenin pathway by monitoring β-catenin and GSK-3β mRNA expression through quantitative (q)PCR analysis. Data showed that the concentrations of β-catenin and GSK-3β mRNA varied between both time points and concentrations of DHT.

Background

- The Wnt/β-catenin pathway regulates genes involved in cell proliferation and apoptosis, hallmarks of cancer cells[1]
- This pathway is implicated in ovarian cancer and is used as a marker for Epithelial Ovarian Cancer (EOC)[1]
- High levels of β-catenin may be involved in tumor development[2]
- Ovarian epithelium displays high quantities of androgen receptors and thus may be more susceptible to changes in testosterone concentration[3]

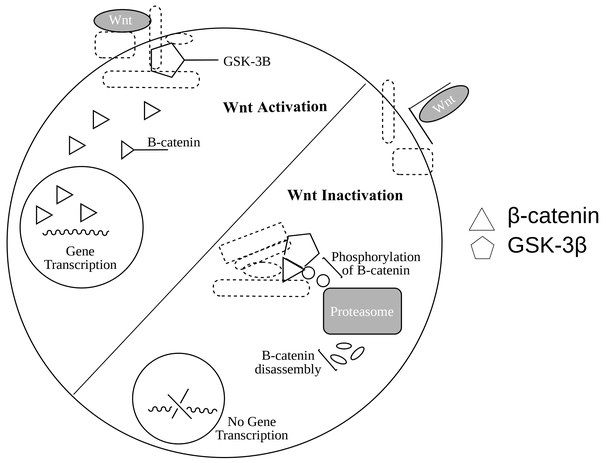

Figure 1. The Wnt/β-catenin signaling pathway in cells. When Wnt is activated, it interacts with other proteins in the cell to inhibit GSK-3β, which causes an increase of β-catenin in the cell. Excess β-catenin translocates to the nucleus, where it increases gene transcription for cellular proliferation and decreases gene transcription for apoptosis. When Wnt is not activated, GSK-3β phosphorylates β-catenin, causing it to be degraded by a proteasome. Dashed figures indicate structures involved in the pathway, but not analyzed in this project. Figure adapted from Arend *et al.,* 2013.[4]

Methods

- Collect ovary tissue from zebrafish (*Danio rerio*) and incubate in 60% L-15 medium (with 1x antibiotic/antimycotic) and 5α-dihydrotestosterone (0, 1, 10, 100 ng/mL) at 26 °C for 2 or 4 hours
- Extract mRNA and convert to cDNA (50 ng/μL)
- Perform qPCR to quantify relative concentrations of β-catenin and GSK-3β mRNA present in the ovary tissue

Objectives

- Learn qPCR technique and design/optimize primers that identify β-catenin and GSK-3β mRNA
- Monitor the effect of DHT on the Wnt/β-catenin pathway at different time points using qPCR analysis

Results

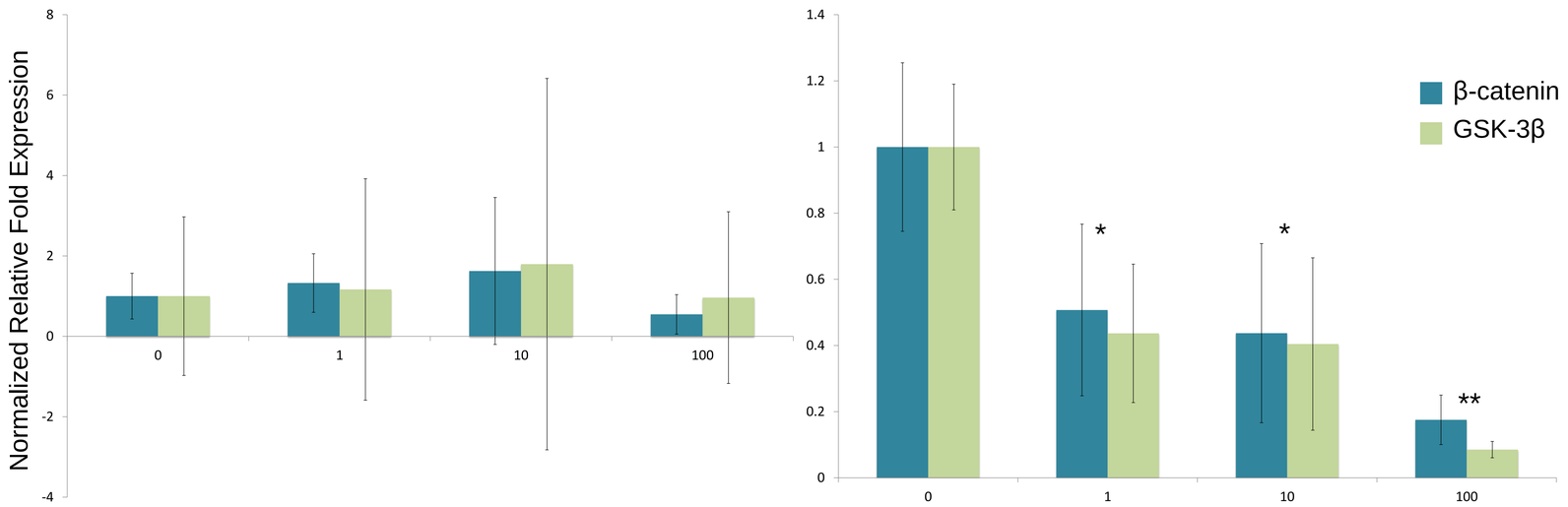

Figure 2. Expression of β-catenin and GSK-3β mRNA in ovarian tissue following a 2 hr (left) and 4 hr (right) incubation in different concentrations of DHT. Three fish were collected per experiment, with three technical replicates per specimen. Extensive error bars may be due to the biological variance among specimens, as well as the diversity of tissue present in ovarian tissue. Expression was normalized to the reference gene β-actin. T-tests were used for statistical analysis, where statistical significance compares treated tissue (1, 10, 100 ng/mL) to untreated tissue (0 ng/mL). Untreated tissue was arbitrarily set at 1 to demonstrate fold differences in expression. *p<0.05; **p<0.01

Discussion

- Longer (4 hr) exposure to DHT may lead to inhibition of the Wnt/β-catenin pathway
  - Significantly less β-catenin and GSK-3β in all 3 treatments
- Shorter (2 hr) exposure produced no statistical significance, possibly due to…
  - Biological variance among specimens
  - Too short of an exposure time, where the effects of DHT have yet to take place
- Future directions include…
  - Quantifying the relative concentrations of β-catenin and GSK-3β proteins in ovarian tissue at 2 and 4 hrs using Western Blot technique
  - Designing primers for genes regulated by the Wnt/β-catenin pathway and performing qPCR to investigate if any regulation is occurring in the presence of DHT

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