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Effect of high water temperature on apoptosis in Zebrafish (Danio rerio) ovarian tissue

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Background

Elevated water temperature has been shown to induce apoptosis, or programmed cell death, in various tissues of fish. In ovarian tissue, high levels of apoptosis could affect the reproductive capability of fishes.

My project addresses the effects of high water temperature on apoptosis in the ovarian tissue of zebrafish. Initial results suggest that apoptosis may actually have been reduced in the high water temperature treatment, though the results were variable and more replications are needed.

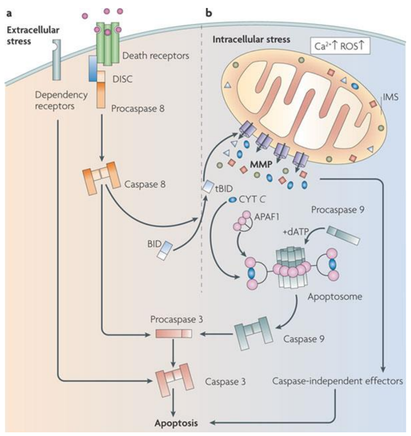

Figure 1. The extrinsic (a) and intrinsic (b) apoptotic signaling pathways (Galluzzi et al 2009). Caspase-3 functions as an executioner caspase, and is involved in the catabolic processes of the end stages of apoptosis. p53 (not shown) induces apoptosis after cellular damage through either the intrinsic or extrinsic pathways.

Methods

- Female zebrafish were kept in 10-gallon aquaria for 2 weeks in one of two treatments: high temperature (~32°C) or control temperature (~23°C)
- Fish were euthanized by decapitation and ovary tissue was surgically removed
- mRNA was extracted from tissue (using a Qiagen RNAeasy kit) and converted to cDNA (using a Bio-Rad kit) and was stored at -20°C until use
- Quantitative (q)PCR was used to measure the relative expression of *p53, caspase-3*, and *β-actin* (a reference gene) in the control and high temperature samples

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Results and Conclusions

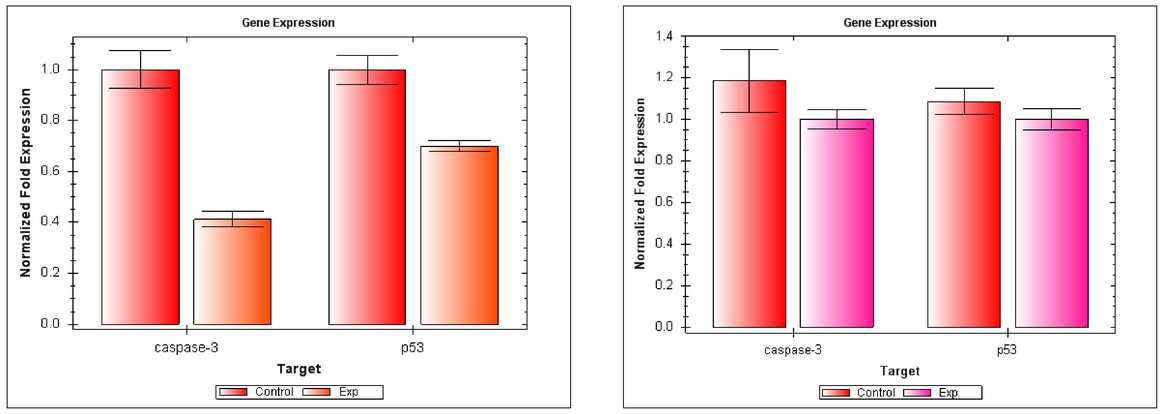

Figure 2. Expression of *caspase*-3 and *p53* relative to a reference gene *β-actin* in the first biological replicate (left) and second biological replicate (right). Both the control and treatment samples were composed of ovarian tissue from 3 fish, and were exposed to control or high water temperatures for 2 weeks. Relative expression was measured using qPCR. Data are the mean ± SE.

Figure 3. Expression of *caspase*-3 and *p53* relative to the reference gene *β-actin* in the combined first and second biological replicates. Each bar represents 2 samples of tissue (each from 3 different fish). Fish were exposed to control or high water temperatures for 2 weeks. Relative expression was measured using qPCR. Data are the mean ± SE.

- In the first replicate, expression of both *caspase*-3 and *p53* appears lower in the high water treatment, suggesting that apoptosis may have been reduced. However, there was no clear difference in expression of *caspase*-3 or *p53* in the second replicate or overall study
- Environmental stressors typically increase apoptosis in fish, so the water temperature used may not have been high enough to actually stress the fish. This suggests that our control temperature (23°C) might actually be too low. The Zebrafish Book suggests a normal temperature of 28.5°C, but does add that over 31°C, abnormal development may occur.

Future Research

- Complete a third biological replicate
- Use TUNEL assay to verify presence/absence of apoptotic cells
- Run experiment again using a higher control temperature and higher experimental temperature

Citations

Westerfield, M. (2000). *The Zebrafish Book: A guide for the laboratory use of zebrafish* (Danio rerio). 4th ed., University of Oregon Press, Eugene.

Galluzzi, L., Blomgren, K., and Kroemer, G. (2009). Mitochondrial membrane permeabilization in neuronal injury. Nature Reviews Neuroscience *10*, 481–494.