hpo has sexually dimorphic gene expression in male and female *Drosophila Melanogaster.*

Murakami K.[1], Yang K.[2], Kim E.[3], Ghosh M.[4], Suresh-Kumar N.[5,6]

[1]Oxford Academy, [2]Abraham Lincoln High School, [3]Portola High School, [4]Homestead High School, [5]Boz Life Science Research and Teaching Institute, [6]UC San Diego Extension.

Summary

- *Drosophila melanogaster* (fruit flies) were exposed to 1000 mg/kg-day GenX for 7 days
- Measured gene activity *hpo* in *Drosophila melanogaster* (fruit flies) using qRT-PCR
- Females express significantly less *hpo* compared to males at baseline

Abstract

Male and female organisms often have different responses to stressors, but most science experiments are performed on male organisms. GenX is a chemical with no conclusive evidence as to its toxicity. The purpose of this study is to determine the sexually dimorphic expression of the *hpo* gene and its expression in response to GenX exposure. Female fruit flies upregulated *hpo* 2.96-fold (p=0.074) while males downregulated *hpo* 1.86-fold (p=0.256) after exposure to GenX. Males express *hpo* 16.98-fold more relative to females at control conditions (p=0.007). We conclude that females significantly downregulate *hpo* compared to males at baseline.

Introduction

Hypothesis

- After exposure to GenX, *hpo* will be upregulated in fruit flies
- *hpo* will not have different expression between males than females.

Drosophila melanogaster (fruit flies)

- Sexually dimorphic
- Short life cycle (10-12 days) & easily observable developmental stages
- High conservation between fruit flies and humans

hpo

- plays role in controlling cell proliferation
- codes for serine/threonine kinase, which is part of the first step to a phosphorylation cascade that leads to cellular responses like apoptosis [1]
- connected to tumor microenvironment, cancer growth & progression

Figure 1. Female and male adult fruit flies.

GenX

- chemical within the PFAS (per & polyfluoroalkyl substances) group found in food packaging, household appliances, and industrial products [2]
- Unknown health effects and toxicity

Materials & Methods

Fly Maintenance: Flies were raised on BDSC cornmeal food. Male and female virgin flies were separated ≤4 h post-eclosion.

Treatments: Control flies were fed BDSC cornmeal food for 7 days and GenX-exposed flies were fed BDSC cornmeal food dosed with 1000 mg/kg of GenX per day for 7 days.

Sample collection: After 7 days, flies were sacrificed via freezing at -80°C. Fly brains were pooled by sex, with 100 brains per sample. RNA was extracted using the RNeasy kit (Qiagen).

qRT-PCR: *hpo* was targeted by the following primers
Forward primer:
3' CTGGAGTCGAACTTGGGCAC 5'
Reverse Primer:
3' GGCCATCTCCCGCATTTTTG 5'

Data Analysis:

- STRING protein interactions and gene homology analysis was done to learn more about the gene function of *hpo*.
- *hpo* was normalized to *Actin*.
- Fold difference in gene expression was calculated using $2^{-\Delta\Delta CT}$
- ΔΔCT was found for the different conditions that were compared by taking the ΔCT difference between control female and control males, as well as between experimental condition and control condition for both males and females.
- A Student's t-test was conducted using the ΔCT values, where a p-value of less than 0.05 was considered significant.

Figure 2. Experimental Overview

Results

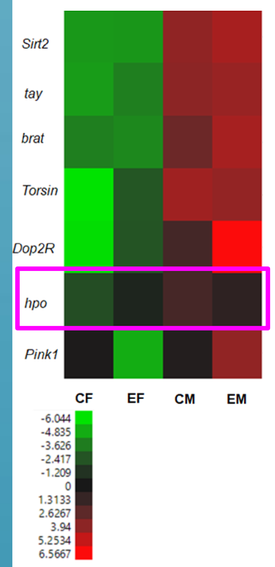
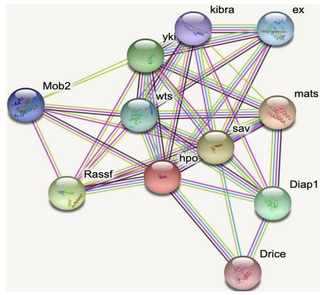


Figure 3. Heat map of gene expression in fly brains based on ΔCt values from qRT-PCR. Green indicates downregulation of a gene, and red indicates upregulation of a gene. CF represents Control Female, EF represents Experimental Female, CM represents Control Male and EM represents Experimental Male. *hpo* was upregulated 2.96 fold in experimental females compared control females (p-value= 0.0739), downregulated 16.9 fold in control females compared to control males (p-value=0.00699) and downregulated 1.859 fold in experimental males compared to control males (p-value=0.256).

Figure 4. *hpo* protein interaction chart. *hpo* interacts with other proteins in the Hippo/SWH pathway. Generated through STRING (https://string-db.org)



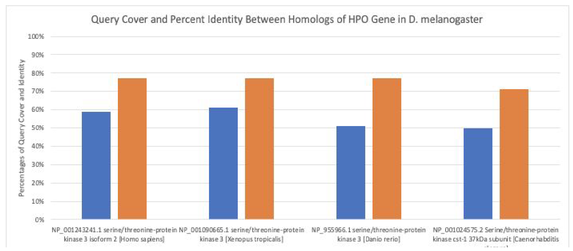


Figure 5: Gene homology chart showing query cover & percent identity to *hpo* in *D. melanogaster* from NCBI BLAST calculations. Blue represents Query cover and orange represents percent identity

Discussion

- *hpo* is involved in a pathway that when activated, leads to a slow down in cell growth and an increase in cell death, allowing for regulation of tissue size.
- *hpo* shows sexually dimorphic gene expression, with a 16.9 fold difference in male and female flies (p-value = 0.0069).
- *hpo* expression is not significantly altered when male and female flies are exposed to GenX.
  - experimental females compared control females (p-value= 0.0739)
  - experimental males compared to control males (p-value=0.256)
- The increased *hpo* expression in male fruit flies may be a reason why male flies are smaller in size compared to females as it would reduce tissue growth in males.
- Exposure to GenX likely did not significantly alter cell proliferation or apoptosis in experimental flies.

Limitations & Future Directions

- The experiment only used one dosage of GenX (HFPO-DA) on *Drosophila melanogaster*. Varied doses should be used in the future to observe potential changes in gene expression.
- Only one model organism was used in this study. For future research on GenX we can use other organisms and compare the effects of GenX on gene expression to understand if these findings are consistent.
- *D. Melanogaster* was exposed to GenX in its adult life stage. Future experiments can observe the effects of GenX exposure on earlier life stages to understand the effects GenX may have on development or growth.

Sources

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2. Basic Information on PFAS. EPA, Environmental Protection Agency, 6 Dec. 2018, www.epa.gov/pfas/basic-information-pfas.
3. Hpo hippo [*Drosophila melanogaster* (fruit fly)] - Gene - NCBI. (n.d.). Retrieved November 27, 2020, from https://www.ncbi.nlm.nih.gov/gene/37247