Male fruit flies express Guardian of Genome, *p53*, 4.19-fold more than females

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Summary

- Do males and females suppress tumors differently?
- mRNA expression of *p53* in virgin fruit flies was quantified.
- Male fruit flies have 4.19-fold more of the tumor suppressor *p53* than female flies, suggesting sex differences in the formation of tumors.

Abstract

The gene *p53* encodes a protein that regulates the cell cycle and acts as a tumor suppressor. The differential expression of *p53* in the brains of male and female *Drosophila melanogaster* (fruit flies) was investigated. After pooling 100 brains from each sex, RNA was extracted, and qRT-PCR was performed on the target gene, *p53*. Results show a 4.19-fold higher expression of *p53* mRNA in males compared to females, indicating differences in tumor suppression mechanisms by *p53* between the sexes.

Introduction

Hypothesis: *p53* expression will be higher in female relative to male fruit flies.

Drosophila melanogaster

- Share 75% of disease-associated genes with humans (1).
- Reproduce quickly and are inexpensive to keep.
- Age-controlled populations are easy to maintain.
- Developmental stages and sex differences are easy to distinguish.

Target gene

- ***p53*** codes for the protein **p53** that regulates the cell cycle.
- Downregulation of ***p53*** is linked with the development of brain tumors (2). Upregulation of ***p53*** decreases the risk of tumor development (2).
- Overexpression of ***p53*** in *D. melanogaster* has shown to increase lifespan in females but decrease lifespan in males, indicating sexually dimorphic functions (3).

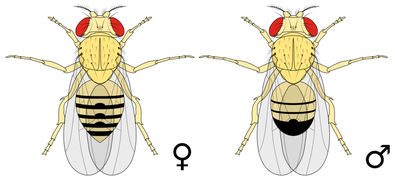

Figure 1: Female and male *D. melanogaster*.
On the left is a female, on the right is a male fruit fly. Females have a lighter, pointed abdomen, while males have a darker, rounder abdomen and sex combs on their forelimbs

Methodology

Sex Determination
Flies were separated by differentiation of sex-specific physical characteristics as shown in Figure 1.

Figure 2. Virgin Fruit Fly.
Recently eclosed fruit fly (4).

Virgin Collection
Within 4 hours post-eclosion, virgin flies (Figure 2) were anesthetized using CO_2 and isolated.

Sucrose Exposure
To establish control conditions, flies were fed 5% sucrose on Whatman paper for 72 hours.

Brain Isolation
Fly heads were dissected under a microscope, and brains were isolated using forceps.

RNA Extraction
RNA was extracted from the fly brains using TriZol and chloroform and treated with DNase

qRT-PCR
Using iTaq universal SYBR® Green reaction mix and iScript reverse transcriptase, qRT-PCR was performed with the following primers:

FWD: 5' GCGATTACCGTCTGGCTATT 3'
REV: 5' CCCACAGATGCAACACATTTC 3'

Data Quantification
Calculated the fold difference ($2^{-\Delta\Delta Ct}$) in expression of *p53* between female to male fruit flies. The inverse provided the fold relationship from male to female gene expression.

Results

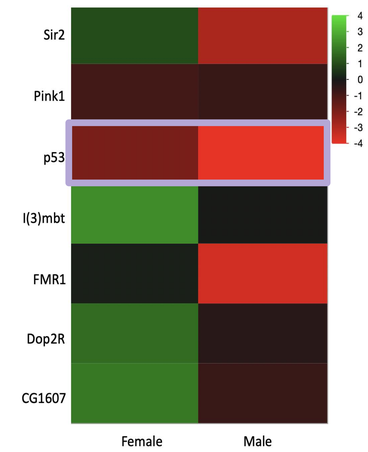

Figure 3: *p53* is expressed 4.19-fold higher in males compared to female fruit flies. Red indicates higher expression and green indicates lower expression. *p53* mRNA levels are normalized to *gapdh1* mRNA levels.

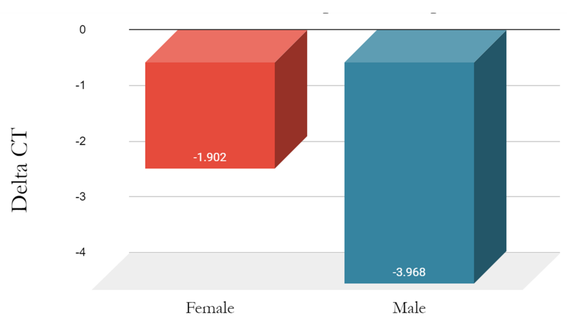

Figure 4: Male fruit flies have a lower ΔCT value compared to females for *p53* expression: A lower ΔCT value correlates with a higher mRNA expression levels. *p53* was normalized to *gapdh1*.

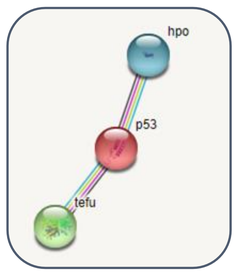

Figure 5: Similarities between p53 and other proteins.

***hpo*:** Plays a role in tumor suppression by restricting cell growth and promoting programmed cell death (5).

tefu: Suppresses spontaneous programmed cell death in multiplying cells (5).

Conclusion

Female and male fruit flies differentially express the tumor suppressor gene *p53*.

- Male fruit flies expressed *p53* 4.19-fold higher relative to females.
- Higher levels of *p53* have been shown to decrease lifespan in males (3). Lower levels of *p53* are linked to decreased tumor-suppression (2).
- These results suggest post-eclosion male fruit flies may have sex-specific differences in regulation of lifespan and increased tumor suppression compared to females due to higher *p53* gene expression.

These results indicate a sexually dimorphic difference in regulation of the cell cycle, tumor suppression, and lifespan from an early life-stage of development.

Study Limitations

- No replicates for statistical analysis
- Only one life stage analyzed (post-eclosion)
- Fruit flies are evolutionarily distant from humans

Future Directions

- Analyze expression of *p53* in females and males when exposed to stressors (extreme temperatures, starvation, toxicants)
- Measure gene expression at different stages of development (larvae and adults)
- Repeat the experiment in a higher order organism
- Cancer research: mutations in *p53* are linked to abnormal cell growth, and thus cancer. Our results may be useful in future research in cancer risk between the sexes.

References

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