The effects of the overexpression of *pnpt-1* on cell proliferation in *C. elegans*

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Introduction

Sustained cell proliferation describes the continued division of cells which occurs through the blocking of developmental signals meant to engender senescence; cells contain genes meant to maintain cellular homeostasis by regulating these signals and pathways, one such gene is *PNPT1. PNPT1* is a human gene that synthesizes PNPase, an enzyme implicated in a myriad of diverse and important biological effects, in order to restrain cellular proliferation. What are the biological consequences of the overexpression of PNPase in normal versus cancer cells? That question is key to being able to safely overexpress proteins in order to mitigate cell proliferation. The effects of overexpressing these genes that regulate proliferation in cells is needed to understand how we can seek to limit cell proliferation in cancers.This question can be answered by overexpressing PNPase in a model organism, *C. elegans* to give insight on how overexpressing PNPase in humans will affect cancerous cells. In *C. elegans*, the ortholog to the human *PNPT1* gene is *pnpt-1*. The lin-23 gene mutation in *C. elegans* causes sustained proliferation of cells causing an increase in the number of intestinal cells. If PNPase is overexpressed in *C. elegans* with the lin-23 gene mutation excessive cellular proliferation will be mitigated.

Methods

Worm Strains:

- **I-VC2010 elt-2:: mCherry:** Red fluorescence marking intestinal cells
- **VC2010 FSN 3D1**: GFP marking pnpt-1 under a heatshock promoter
- **I-VC2010 PPD 4C2**: GFP marking empty vector
- **Double Transgenic (DT) (experimental line):** I-VC2010 elt-2 mCherry:: VC2010 FSN 3D1
- **Double Empty Vector (DEV) (control line)**: I-VC2010 elt-2 mCherry :: I- VC2010 PPD 4C2
- **lin-23**: contains e1883 mutation; increased gene expression in meiosis by coding for F box/WD repeat protein

Methods

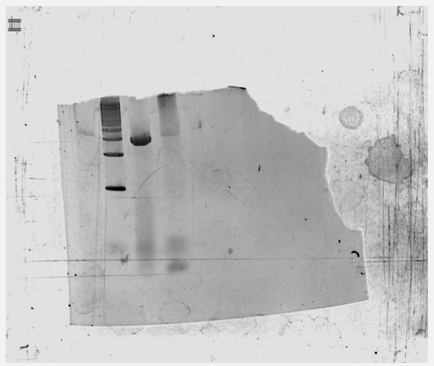
1. **Performed crosses to produce an experimental strain and a control strain:** The transgenic worms with GFP/pnpt-1 are bred with lin-23 worms with mcherry to produce double transgenic (DT) lin-23 worms. Then, the GFP/empty vector of worms are bred with lin-23 worms with mCherry to produce double empty vector (DEV) strain (control).

1. **Genotype worms for lin-23 by PCR**: The conditions for PCR, annealing temperature and concentration of DNA, were tested. Afterwards, *C. elegans* from the crosses will be tested for the lin-23 mutation using PCR.

2. **Overexpressed *pnpt-1* in worms with the lin-23 mutation:** The experimental worms that contain the lin-23 mutation are heat-shocked to overexpress *pnpt-1*.

1. **Intestinal cells are counted:** Since *pnpt-1* has been overexpressed in the worms with the cancer phenotype, the intestinal cells are counted to determine if cell proliferation was mitigated.

Results



Gel of PCR testing annealing temperature of 60 °C and DNA concentration of 40 ng/µL. At these conditions, the DNA primers work effectively.

Discussion

Now that the genotype of the lin-23 mutation can be screened, the intestinal cells of that specific genotype should be counted to verify the phenotype of lin-23. Additionally, after the phenotype has been verified, overexpression trials should begin to determine if the overexpression of PNPase can mitigate the overproliferation of cells. Furthermore, overexpression trials should be conducted on other model organisms closer to humans to gain a better understanding of overexpressing PNPase in humans.

References

Hur, B. J. (2019). *Phenotypic Characterization of PNPase Mutation and Overexpression in C. elegans* (thesis).

Sarkar, D., & Fisher, P. B. (2006). Polynucleotide phosphorylase: An evolutionary conserved gene with an expanding repertoire of functions. *Pharmacology & Therapeutics*, *112*(1), 243–263. https://doi.org/10.1016/j.pharmthera.2006.04.003

Kipreos, E. T., Gohel, S. P., & Hedgecock, E. M. (2000). The*C. elegans*F-box/WD-repeat protein Lin-23 functions to limit cell division during development. *Development*, *127*(23), 5071–5082. https://doi.org/10.1242/dev.127.23.5071

Corsi, A. K. (2015). A transparent window into biology: A Primer on caenorhabditis elegans. *WormBook*, 1–31. https://doi.org/10.1895/wormbook.1.177.1

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