The Effect of Overexpression of *pnpt-1* on Cell Proliferation in C. Elegans

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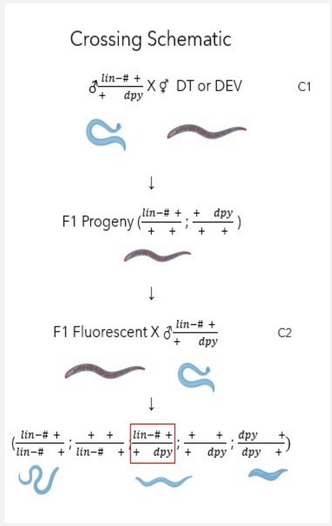
Abstract

Cancer is caused by uncontrolled proliferation of an organisms' cells, causing malignant tumors. The most well-known treatment, chemotherapy, may kill cancerous cells, but lacks specificity and often kills nearby healthy tissue. Gene therapy offers a more narrowly tailored avenue of treatment. Prior research found cell senescence is directed by controlled upregulation of the gene *pnpt-1* in HO-1 melanoma cells. This gene encodes for the exoribonuclease, PNPase, which degrades RNA. One way it affects proliferation is by PNPase downregulating *c-myc*, a growth-promoting gene. *C. elegans,* a roundworm, was chosen as an animal model to observe the effects of PNPase on cancerous cells due to its many conserved human genes and pathways. Two *C. elegans* mutation strains with intestinal cancer will be tested; the lin-4(e912) strain has a mutation in a noncoding RNA, and lin-23(1883) has a mutation in a F-box protein that causes excess cell divisions in the intestines. The test group needs to have fluorescently marked intestinal cells and a vector carrying *pnpt-1* under a heat shock promoter, and the control group needs fluorescently marked intestinal cells and an empty vector (heat shock promoter without the *pnpt-1* gene). The overexpression of the *pnpt-1* gene will be triggered by heat shock treatment. By counting fluorescently marked cells prior to and after the heat shock treatment, the effect *pnpt-1* will have on the number of intestinal cells in the mutated worms can be quantified. The experiment is ongoing and currently creating these lines of *C. elegans* with the necessary phenotypes. In order to properly screen the worms, new methods utilizing a microfluidic chip allow greater visualization of the fluorescent intestinal cells and vectors. By testing cancerous *C. elegans* models, the project aims to observe the effect of overexpressing *pnpt-1* as an alternative cancer therapy with greater specificity than current treatments. By overexpressing *pnpt-1* in *C. elegans*, it's expected that the cancerous models will have fewer intestinal cells and nuclei, similar to wildtype worms.

Methods

Experimental line:
Double Transgenic
- intestinal cells are marked with mCherry
- GFP marker marks pnpt-1 overexpression vectors under a heat shock promoter.

Control line:
Double Empty Vector
- intestinal cells are marked with mCherry
- no pnpt-1 gene in the GFP vector

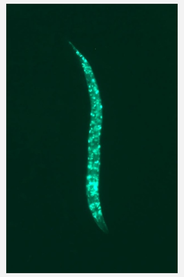


1. **Induce Males:** select worms in their most fertile stage and heat shock for four hours in a 31°C incubator
2. **Cross Worms:** follow schematic to create experimental double transgenic and control double empty vector worms in both *lin-4* and *lin-23* mutations
3. **Confirm Cancer Mutation Genotype:** observe all three phenotypes of wildtype, dumpy, and extra long worms in the progeny

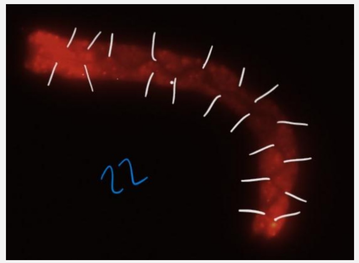
Methods

4. Verify Cancer in Worms: mount worms in mid-L4 stage under a ZeissAxio-Imager M2 microscope using DIC, GFP, and RFP fluorescence at 40X using the microfluidic chip

<u>If intestinal cells exceed 20 and the cell nuclei count exceeds 30-34, the worms are considered cancerous.</u>



GFP marker indicating empty vectors in a *lin-4* x DEV worm



mCherry marker indicating intestinal cells in *lin-4* x DEV worm. Cells are annotated and showing 22 cells.

5. Overexpress pnpt-1: both experimental line and control line are heat shocked

6. Observe Reduction of Intestinal Cell and Nuclei: repeat mounting protocol to record and compare the effect of pnpt-1 overexpression in an EXCEL sheet.

In previous research, it was found that when verifying cancer in the worms, there was no significant difference in the number of cells in the cancerous worms lines and in wildtype worms. It was determined that the method of attaining the intestinal cell and nuclei counts led to inaccuracies. In order to gather more accurate data, a new method of screening C. elegans and their intestinal cells was developed.

Original Method: Worms are sedated through NaN3 droplets on glass slides. About 10 worms are mounted onto each slide. Pictures are taken under a ZeissAxio-Imager M2 microscope using DIC, GFP, and RFP fluorescence at 40X.

Photo of lin-23 x DEV worm under RFP fluorescence taken using original NaN3 sedation method

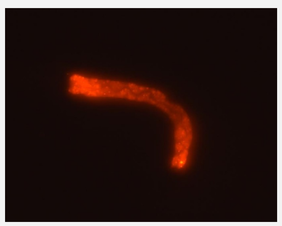
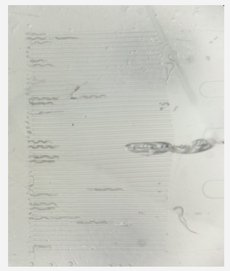


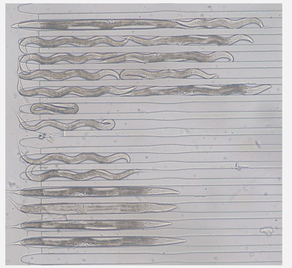
Photo of lin-4 x DEV worm under RFP fluorescence taken using original NaN3 sedation method

New Method: Worms are collected at exact mid-L4 stage and are placed in a solution of 1% Pluronic and Sbasal M9. Supernatant is injected into microfluidic chip in which worms are held in channels. Photos are taken under a ZeissAxio-Imager M2 microscope using DIC, GFP, and RFP fluorescence at 40x.

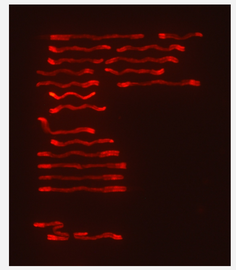
Results



First attempt of injecting *lin-23* x DEV worms in microfluidic chip



Recent attempt of injecting Double Transgenic worms in microfluidic chip



Recent attempt of Double Transgenic worms in microfluidic chip under RFP fluorescence

Unlike, the original method, the microfluidic chip allows the C. Elegans to be held straight when taking photos allowing more accurate data collection when counting their intestinal cells and nuclei. The microchip can potentially hold up to 60 worms at one instance unlike the original method where only 10 can be mounted at once. This method also successfully keeps the C. elegans alive while taking pictures allowing for further analysis of worms.

Conclusion

The microfluidic chip method proves to be a successful improvement to the experiment's procedure in obtaining intestinal cell and nuclei counts. This method not only will allow more accurate data when verifying cancer in the C. elegans, but also will prove to be effective in observing the cells after overexpressing *pnpt-1*. The method's ability to hold up to 60 worms at once while keeping the C. elegans alive will allow for more effective further research. In order to continue to improve the accuracy in data collection, procedures are being developed to strengthen the brightness of the fluorescent markers, furthering removing the possibility of error.

Acknowledgements

Thank you to the MSIP program and its directors and sponsors for this opportunity as well as Dr. Shiang and her lab members for their guidance and help throughout this project.

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