Transdifferentiation causing Programmed Cell Death in *C. elegans*

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Abstract

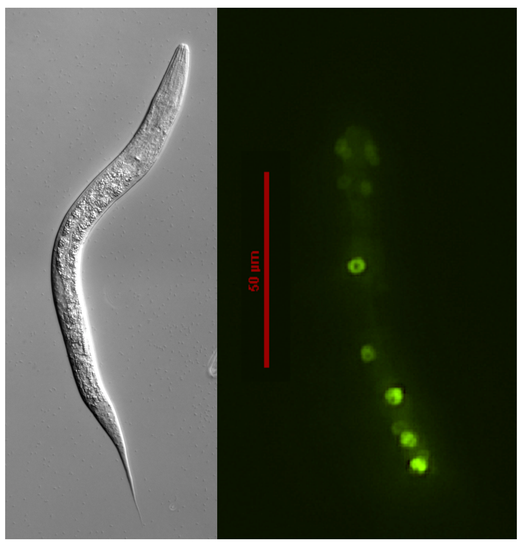
Cancer remains one of the leading causes of death worldwide. A way to understand how cells exhibiting initial cancerous characteristics are destroyed is by observing the programmed cell death (PCD) of a cell before it initiates tumorigenesis. The widely studied model organism Caenorhabditis elegans contains many homologous genes with humans and can therefore be studied and related to human cancers. Transdifferentiating specific cells in a particular worm strain (JR3642) by forcing the overexpression of the elt-7 transcription factor via heat shock puts stress on individual cells, possibly causing some to undergo PCD. To quantify the amount of transdifferentiate stress necessary to cause PCD, we heat-shocked JR3642 and quantified the post heat-shock corpses at different time points. When compared to a control, the difference in corpses found is not sufficient to provide conclusive results. The JR3642 strain was also crossed with strains containing fluorescent tags fused to the protein directly involved in corpse engulfment (coded by ced-1 gene), allowing for more accurate identification of cell corpses to provide more decisive results. We hypothesize that if the overexpression of the elt-7 transcription factor in C. elegans causes sufficient transdifferentiate stress within pharyngeal cells, then the cells will undergo programmed cell death.

Introduction

Around 1,806,590 new cases of cancer were diagnosed in 2020 in the US alone[1]. Due to the constant nature of human cell division, the body can produce a cancerous cell at any given time. Understanding programmed cell death and the mechanisms which trigger its initiation can indicate the methods a potentially cancerous cell uses to stop itself from becoming a tumor. I aim to define what level of stress is required for a cell to undergo programmed cell death and understand the system by which a cell triggers its suicide and subsequent engulfment. The stress placed on the cells will be driven by transdifferentiation caused by the overexpression of the elt-7 transcription factor[2]. If the overexpression of the elt-7 transcription factor in C. elegans causes sufficient transdifferentiate stress within pharyngeal cells, then the cells will undergo programmed cell death.

Methods

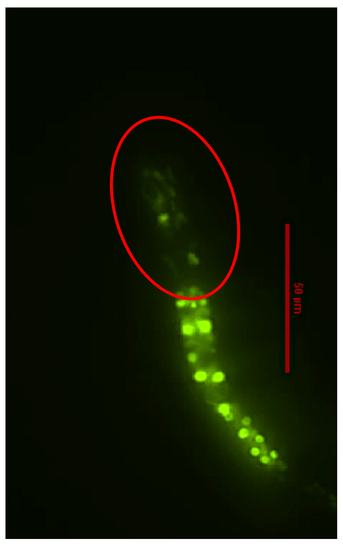
Transdifferentiation: the reprogramming of adult cells, used to control the amount of stress the cells are undergoing.



C. *elegans*, a widely studied model organism due to its genetic similarity to humans. Specific gene mutations found in worm strains can often be directly related to genes found in humans.

Heat-shock treatment: heating the worms to 33°C for 30 minutes to trigger transdifferentiation.

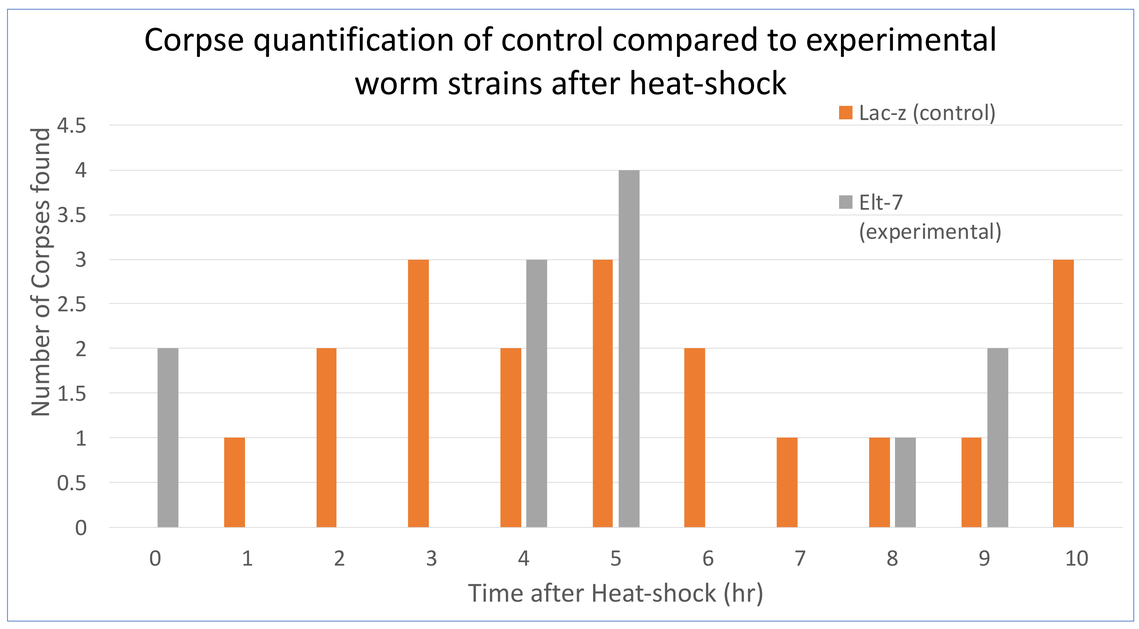
Nikon fluorescent microscope used for imaging and analysis following heat-shock treatment



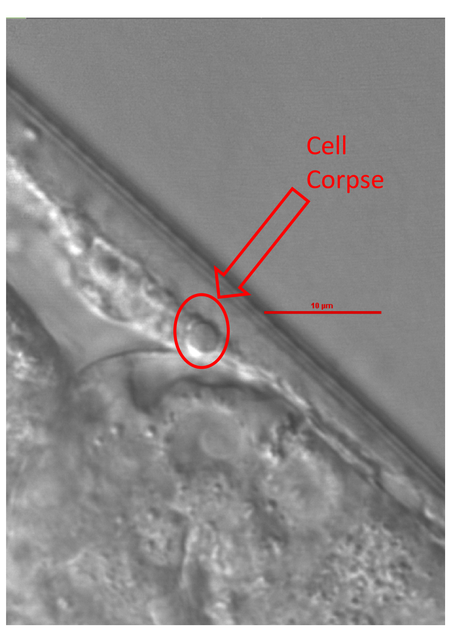
Transdifferentiated worm. Green fluorescence in head indicates adult pharyngeal cells are reprogramming into gut cells.

Results

Analysis following heat shock and transdifferentiation proved to be difficult due to cell corpse identification being tedious and inaccurate because of minimal distinction between corpses and vacuoles. Fluorescent tags were crossed into the strain with reprogrammable pharyngeal cells to allow for more accurate corpse quantification.

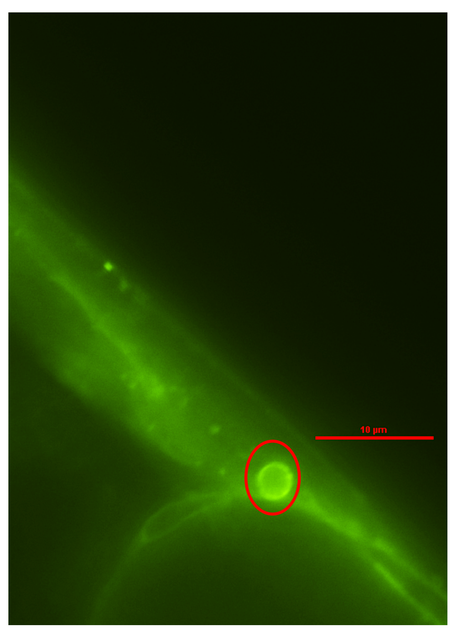


Corpses quantified at specific time intervals following 30-minute heat shock treatment showed a peak of corpses at 5 hours after treatment for the experimental worm strain and various peaks for the control strain. Y-axis shows the total number of corpses found, with three worms being scored from each strain at each time point. A potential source of error is misidentification of corpses due to very slight differences between corpses and other cells.



Using GFP to identify cell corpses

CED-1, a gene which codes for a protein required for corpse engulfment is linked with a Green Fluorescence Protein (GFP). This strain will be crossed with JR3642 to fluorescently tag cells undergoing PCD. These images portray a corpse in the germline of an adult worm, with the same cell being compared under brightfield and fluorescent light to display the outline surrounding a cell corpse tagged for engulfment using the proteins coded by the ced-1 gene[3].



Discussion

The preliminary data acquired when comparing the number of corpses found in the experimental (JR3642) and control strains gave inconclusive results due to the minimal difference between the two corpse counts. The transdifferentiation was successful in the experimental strain starting at hour 3 following heat-shock, eliminating transdifferentiation inconsistency as a source of error. The corpse formation starting an hour after transdifferentiation began hints that the transdifferentiation could be the driving factor of the programmed cell death. However, because the lac-z control strain also had corpses, we cannot yet determine whether transdifferentiate stress causes programmed cell death in *C. elegans* or not. The results remain inconclusive because of the level of error involved in scoring corpses which are not fluorescently tagged. Looking into the future, successful crossing of the JR3642 (transdifferentiating) strain with the ced-1::gfp strain will allow for more accurate corpse quantification. The results from this experiment will likely provide a conclusive outcome by eliminating the main source of error. Additionally, a cross will be performed to knockout the ced-1 gene in the JR3642 strain to limit corpse engulfment, allowing for more time to quantify the corpses before they are engulfed. Although the results were inconclusive, they provided a key foundation into further experimentation which could lead to a better understanding tumorigenesis and how to stop it.

References:

1. Cancer Statistics. Cancer.gov. 2015 Feb 4 [accessed 2021 Aug 8]. https://www.cancer.gov/about-cancer/understanding/statistics
2. Riddle MR, Weintraub A, Nguyen KCQ, Hall DH, Rothman JH. Transdifferentiation and remodeling of post-embryonic C. elegans cells by a single transcription factor. Development (Cambridge, England). 2013;140(24):4844–4849.
3. 3. ced-1 (gene) - WormBase : Nematode Information Resource. Wormbase.org. [accessed 2021 Aug 8]. https://wormbase.org/species/c_elegans/gene/WBGene00000415

Acknowledgments:

A very special thank you to Dr. Sammy Davis, Dr. Joel Rothman, Tsunghan Yeh, as well as the NIH MARC Scholars Program and the Center for Science and Engineering Partnerships (CSEP) for aiding me throughout my research project and providing me with this priceless opportunity.

Further Information:

1. Please see https://www.cancer.gov/about-cancer/understanding/statistics for further information on cancer statistics.
2. Please reference https://cbs.umn.edu/cgc/what-c-elegans for more information on *C. elegans* and why it is so widely used as a model organism.