Exploring overlapping molecular mechanisms of aberrant cell outgrowth and cell death

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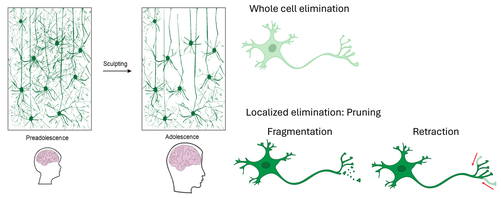
Abstract

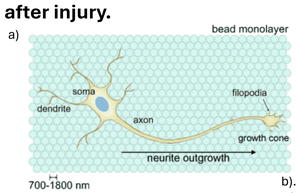
Normal brain development is achieved through both regressive and progressive events that must be precisely regulated. Part of this regulation is programmed elimination of neurons, which is especially intriguing given the diverse compartments of these specialized cells. Neurons can also be lost in neurodegenerative diseases or following injury. The *C. elegans* tail-spike cell is a differentiated cell found in the embryo that extends a long neurite-like process posteriorly to help form the tail tip. In the late embryo stage, the tail-spike cell normally dies via an elaborate cell death program called Compartmentalized Cell Elimination (CCE). CCE in C. elegans requires the main caspase protease CED-3 and its immediate upstream regulators. When CED-3/caspase activity is entirely lost, CCE fails and the tail-spike cell lives—even in larva—intact with its posteriorly directed process.
We have found that when *ced-3*/caspase mutants are aged, new additional processes extend from the cell body. We propose the application of the tail-spike cell to study aberrant cell outgrowth *in vivo*. We address the underexplored question of links between antithetical cell fates of elimination and outgrowth in differentiated cells like neurons, as neurons that survive inappropriately can form undesired connections that may be detrimental to nervous system function.

Background

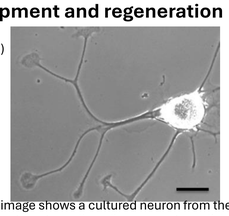
Neurons are specialized cells with diverse compartments. During brain development, regulatory mechanisms such as pruning serve the purpose of removing unnecessary connections.



Neurite outgrowth is critical for the wiring of the nervous system during both normal development and regeneration after injury.

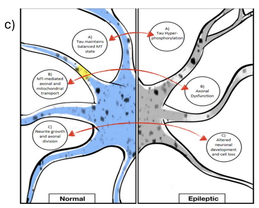


a). Schematic illustration of a neuron cultured on a silica-bead substrate (Kang, Kyungtae, et al, 2017).



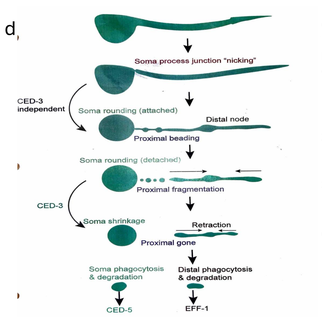
b). This image shows a cultured neuron from the adult CNS of an invertebrate mollusc (Lymnaea stagnalis) showing regenerated neurites emerging from the cell body. Growth cones can be observed at the tips of these regenerating neurite processes (Nasser. T, 2017).

Aberrant neurite outgrowth can be a consequence of impaired regulation, likely due to mutation of regulatory genes or as a result of age-dependent decline in synaptic integrity.



c). Schematic illustration of a normal versus epileptic neuron due to hyperphosphorylation of microtubule-associated protein , tau. In epileptic states, microtubules are implicated in altered neuronal development, eventually leading to neuronal loss, whereas in normal neurons MT contribute to proper neurite growth and axonal division (Gambino, Giuditta, et al, 2020)

Pruning in neuronal cells is relative to a novel program of cell death in *C. elegans* known as Compartmentalized Cell Elimination (CCE).



d). The shown model of CCE occurs in the Tail-spike epithelial cell (TSC) via a tripartite killing process at the embryonic stage whereby:

1. The cell soma appears morphologically apoptotic with nicking at the soma-process junction.
2. The proximal segment of the single posteriorly directed process (like dendrite) becomes fragmented.
3. The distal segment retracts into itself.

Therefore, we are left with remnants that are engulfed by separate phagocytes.

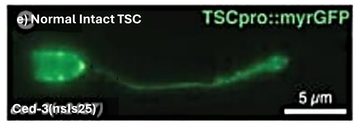
CCE in *C.elegans* TSC is a non-canonical form of apoptosis, therefore, occurs via CED-3/caspase signaling pathway.
When CED-3/caspase is entirely lost, CCE fails and the tail-spike cell lives—even in larva—intact with its posteriorly-directed process.

Objectives & Approach

- **Confirm tail-spike cell persistence in aged CED-3 mutants.**
- **Examine outgrowth of additional processes and understand the direction of growth.**
- **Examine novel mechanisms of mitochondrial involvement in cell outgrowth.**
- **Understand the molecular players in aberrant cell outgrowth.**

Results

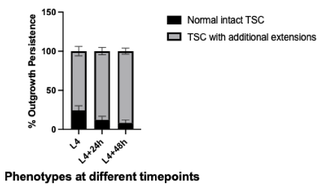
We observed preservation of the tail-spike cell in adult CED-3 mutants.



e). Persisting TSC in L4 larvae ced-3(nsIs25)

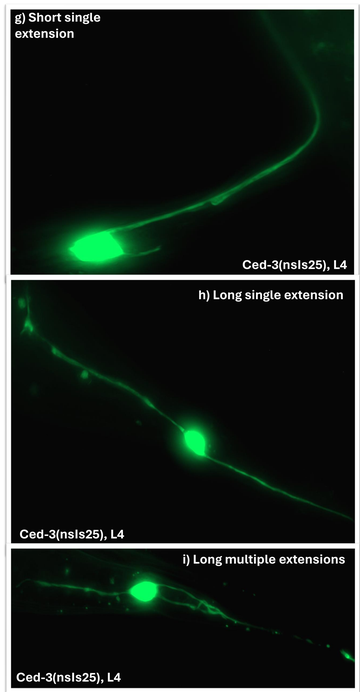
New processes emerge with age, in addition to baseline posterior process. Comparison of outgrowth vs normal TSC at different timepoints after L4 stage reveals normal TSC phenotype begins to diminish with age.

f)



f). Worms were observed every 24 hours past the L4 stage and found a decrease in normal TSC phenotype in ced-3(nsIs25) with age.

Outgrowth is randomly directed, therefore, categorized as short/long single extension, short/long multiple extensions, and other.

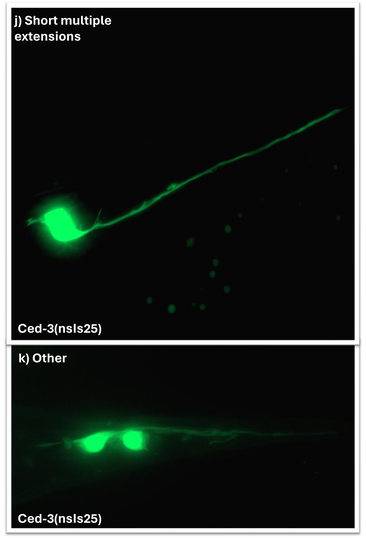


g). Outgrowth appears to be posteriorly directed like the original posterior process. However, it is shorter in length in comparison, therefore, classified as a short single extension.

h). Outgrowth appears to be anteriorly directed and long, therefore, classified as a long single extension.

i). Outgrowth appears in both anterior and posterior directions, in addition to baseline posterior process. Therefore, we classified this as long multiple extensions.

Results Continued

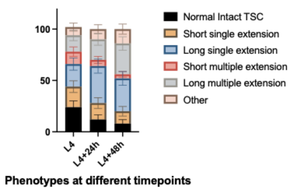


j). This image shows one short anteriorly directed outgrowth and a second outgrowth budding from the posterior process. We then classified this as short multiple extensions.

k). This image shows an anteriorly directed outgrowth, but also two somas, which we classified as "other," as it suggests a separate/additional molecular mechanism in comparison to plain outgrowth.

Further analysis of outgrowth phenotypes in aged CED-3 mutants reveals a higher persistence of the "long single extension" phenotype.

l)



l). Worms were observed every 24 hours past the L4 stage and found increasing persistence of long single and multiple extension phenotypes. The decrease in short extensions suggests continuous elongation, with age, in shorter outgrowths to reach the "long" stage.

Future direction

- **Observe ced-3 mutants beyond L4+48hrs and determine whether there is a peak in %outgrowth persistence, as well as the different phenotypes.**
- **Examine novel mechanisms of mitochondrial transport in cell outgrowth.**
  - **Examine the transportation and localization of mitochondria via motor proteins/microtubules network, in the new TSC process(es) of aged CED-3/caspase mutants.**
  - **Determine microtubule polarity in the new process(es): We have found that tail spike cell posterior process, like a dendrite, has mixed microtubule polarity, i.e., both + ends in and out.**
- **Study the interplay between microtubules and the endoplasmic reticulum in terms of regulation of cell outgrowth.**
- **Understand the associations between the endoplasmic reticulum and mitochondria in preserving the new process(es).**
- **How mechanotransduction from the surrounding environment guides outgrowth.**

References

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