8-2017

Analysis of DLC-1 mediated regulation of the tumor suppressor protein GLD-1

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UGP Award Final Report
University of Montana Research Grant Program, 2016-2017
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DLC-1 is a developmental regulator that controls decisions related to cell proliferation and differentiation in *C. elegans*. This project focused on determining how DLC-1 promotes the RNA regulatory and tumor suppressor functions of GLD-1.

Progress Report

The specific aim outlined in the UGP Award project has been successfully completed and we have gained significant insight into the molecular mechanisms utilized by DLC-1 to facilitate GLD-1 function. Specifically, we have determined that DLC-1 is involved in mediating the translation of a subset of GLD-1 target mRNAs. For example, DLC-1 regulates the expression of *mex-3*, *mes-3* and *cye-1* mRNAs but not *puf-5* or *spn-4*. These mRNAs encode proteins that are cell cycle regulators and genes involved in cell division, chromatin modification and RNA regulation. GLD-1 helps regulate hundreds of target mRNAs during development and these results suggest that DLC-1 may direct the mRNA selectivity of GLD-1.

Previous research has shown that GLD-1 is enriched in perinuclear foci in the meiotic pachytene region of the *C. elegans* germline. We also assessed GLD-1 levels and subcellular localization in the germline by examining transgenic worms expressing GFP-tagged GLD-1 using a compound microscope. Additionally, we used Western blot analysis to quantitate the total level of GLD-1 in the presence and absence of DLC-1. The results showed that GLD-1 levels and subcellular localization were not disrupted when DLC-1 was depleted in worms using RNA interference. These results show that DLC-1 does not promote GLD-1 function by stabilizing GLD-1 and also suggest that the dynein motor related function of DLC-1 may not be involved in supporting GLD-1 activity.

Finally, we used transgenic worm methodology to determine if a direct DLC-1/GLD-1 binding interaction is required for DLC-1 to enable GLD-1 function *in vivo.* We had previously shown using a biochemical GST pull-down assay that DLC-1 binds the unstructured N-terminal domain of GLD-1. For the *in vivo* approach we created a transgenic worm that expresses the GFP tagged transgene of the mutated GLD-1 protein (GFP::(AAA)GLD-1). Next we crossed the homozygous GLD-1 mutant transgenic worm with a heterozygous *gld-1(-)* worm to obtain a worm strain homozygous for both *gld-1(-)* and the GFP::(AAA)GLD-1 transgene. Analysis of this mutant transgenic worm shows that the mutant worms exhibit increased sterility (~30%) compared to wild type. Also, the mutant worms exhibit a disorganized germline phenotype and the germ cells are defective in their ability to differentiate into oocytes.

Impact of Study

- UGP-funding allowed me to provide supplies for an undergraduate student's research and to train the student in genetic, molecular biology and biochemical laboratory techniques.
- Results of the UGP-funded research were presented at the *C. elegans* International Meeting (Los Angeles, CA, June 2017).
- I am currently preparing the research funded by UGP for publication.
- UGP funding helped me to generate preliminary results that will be used to secure NIH R01 funding in the upcoming year.