Targeting RGD cell-binding motif of LAM-3 and its effects on ECM

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Introduction

Caenorhabditis elegans (*C. elegans*) is a free-living soil nematode that is an excellent genetic model organisms due to its short generation time (3 days at 25° C), small genome size (100 Mbase), and easily observable mutant phenotype. *Lam-3* is a gene within *C. elegans* involved in forming laminin, a protein essential in the composition of the basement membrane of tissues. The ECM is a major component of living systems allowing for the structure, formation, and migration of cells and tissues, while also providing a strong connection between the cell and its surroundings. The specific ECM interaction studied is the alpha subunit within the laminin protein comprising of integrin receptors of *C. elegans*. The purpose of the experiment is to change the RGD amino acid sequence in the laminin protein to RGE using CRISPR-Cas9 as well as the Co-CRISPR, DPY-10. These targeted our RGD binding sequence (5"-AGAGGTGAT-3") and induced the mutant phenotype.

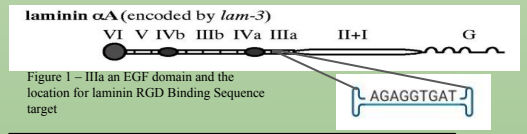
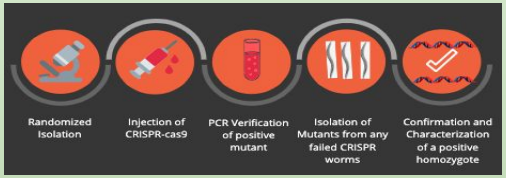


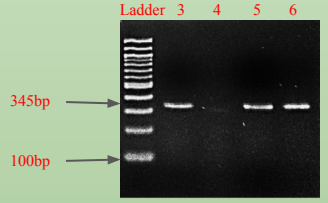
Figure 1 – IIIa an EGF domain and the location for laminin RGD Binding Sequence target

Materials and Methods

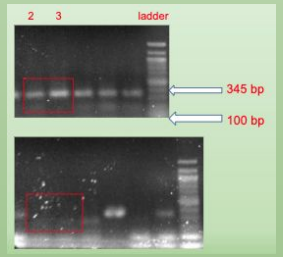
Starting the experiment, the N2 strain of *C. elegans* was utilized as the wild type. From the N2 strain, 49 worms were isolated and injected with CRISPR-Cas9. The Co-CRISPR, DPY-10, was used to initially phenotypically determine the *C. elegans* that have been mutated. Subsequently, a PCR was run to determine if the worms were genetically mutated at *lam-3*, signified by a band of 345bp. Worms that tested positive were used to breed another progeny. The adults from this progeny were used in a double PCR with the intention of differentiating between a heterozygous and homozygous mutation in the *lam-3* gene.



Results



Top Image: PCR round 1 (ID mutant)



Bottom Image: PCR round 2 (ID homozygote)

Experimentation achieved one positive PCR clone that yielded a size of 345 bp. A single positive band indicates that a mutation was successfully induced. In other experiments of lam-3, observed mutants have complex terminal phenotypes, including ruptured tissues, ectopic cell adhesions, and abnormally-positioned adherens junctions.

Discussion

The observed phenotype of *lam-3* is structurally classified as "superficially wild type" and calls for further assays and testing to further assess the positive mutation and its developmental effects. These may include thrashing and touching assays-to detect any neural and muscle defects underlying the superficial WT - and the NEMA-FLEX: can tell us exactly how strong their muscle cells are and if any mutants possess weaker muscles. It is known from previous research that laminin mutations that result in its total removal are embryologically lethal, therefore, further research is needed to determine if the RGD motif itself plays any significant or contributing role to laminin's production and maintenance in *c. elegans.* Understanding epithelial polarity and alterations in the RGD binding motif that allows integrins to closely associate with the ECM can further our understanding of the cause of muscular dystrophy in both nematode and human models with the LAMA-2 mutation. If found significant, the manipulation of this complex within the field of medicine may be useful for patients with this disease for possible treatment and cures.

Left Image: "Superficially Wild type" mutant *C. elegans,* Right Image: Normal N2 strain *C. elegans*

References

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Acknowledgments

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