Characterization of Hemicentin in C. elegans

Department of Biology, Baylor University, Waco, TX 76708
BIO 4108 Dr. Myeongwoo Lee

Emma Haworth, Kylie Ross, Halee Sowinski, Keerthi Antony, and Abraham Ahumada

OBJECTIVE

Use of CRISPR-Cas 9 in C. elegans to edit the *him-4* gene and hemicentin protein in order to measure the effects on the basement membrane

BACKGROUND

C. Elegans are free-living, soil nematodes with short generation times and easily observable mutant phenotypes. C. elegans are cultivated on Agar plates with short generation times for lots of turnover. The him-4 gene in the C. elegans produces the protein hemicentin which is a structural protein located in the ECM. The *him-4* gene is located on the X chromosome and encodes the Hemicentin protein. Hemicentin is characterized by 45 immunoglobulin antibody repeats. Hemicentin has unique N and C terminal domains and contains fibulin proteins. Functions of hemicentin include tissue attachment, placement of gonads, chromosome stability in mitotic germ cells, and it plays a part in cell contractions and adhesion. Extracellular matrix (ECM) is a 3-D network, consisting of large, glycosylated proteins. Integrin in the ECM binds RGD sequences of other ECM proteins. Editing the RGD sequence will inhibit hemicentin binding to the ECM. The disruption of the ECM causes improper attachment of the gonads to epithelial cells and

MATERIALS AND METHODS

Materials used: C. elegans, agar plates, picks, microscope, flame, micropipette, primers, buffers, dNTPs, polymerase, PCR thermal cycler and electrophoresis equipment

Methods: CRISPR gene editing was used to create mutations in *him-4* at two of the six RGD sequences: RGD sequence number 3 and 5. These sequences were targeted to change the D amino acid (Aspartic Acid) to the E amino acid (Glutamic Acid). A coCRISPR was used to help identify the affected animals. The assumption was made that if the animal presented with the coCRISPR dumpy phenotype then the RGD sequences were also edited.

DATA AND DISCUSSION

64 N2 C. elegans were injected with CRISPR Cas-9 and the co-CRISPR at the amino acid 3352 to induce the *him-4* phenotype. Another 58 N2 C. elegans were injected with CRISPR Cas-9 and the co-CRISPR at the amino acid 3519 to induce the *him-4* phenotype. This was in order to induce the changing of the cell binding loop to RGE and the overall phenotype of the nematode to dumpy. Both strands (3352 and 3519) displayed hemorrhaging. Since hemicentin is an extracellular protein that creates long, thin tracks for tissue attachment and gonad movement often using the cell binding loop RGD, the disruption of this protein affected the overall stability of the basement membrane and resulted in hemorrhaging.

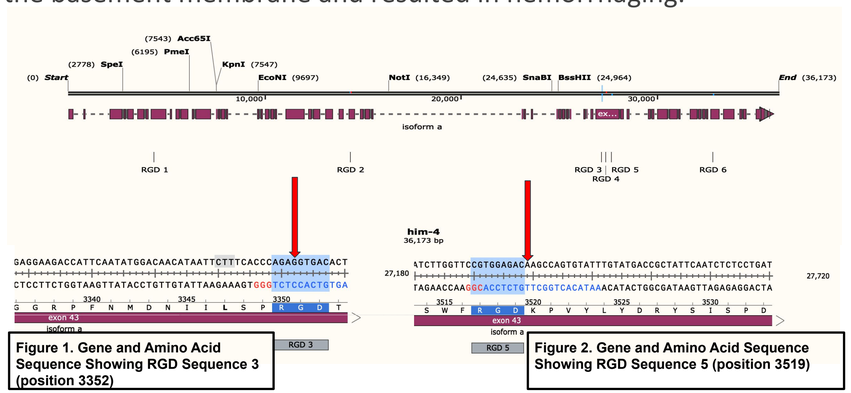
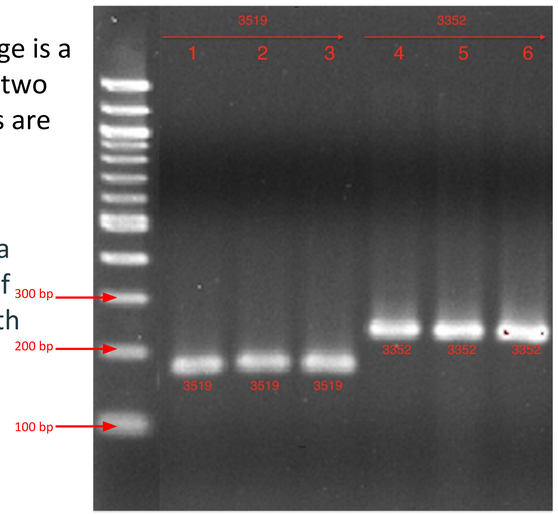


Figure 1. Gene and Amino Acid Sequence Showing RGD Sequence 3 (position 3352)

Figure 2. Gene and Amino Acid Sequence Showing RGD Sequence 5 (position 3519)

Figure 3. The above image is a gene map of *him-4.* The two targeted RGD sequences are pictured.



PCR Product Results:

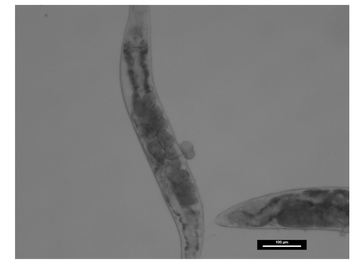
The PCR results display a homozygous genotype of the C. elegans picked with the hemorrhaging phenotype.

RESULTS

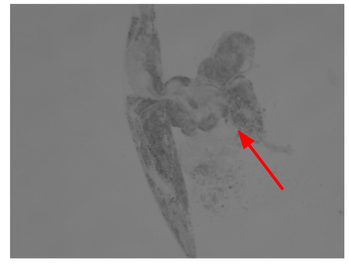
The effect of editing the RGD sequence of the *him-4* gene in C.elegans caused an inhibition of the binding of the hemicentin protein which lead to hemorrhaging of the basement membrane. The hemorrhaging was due to the improper attachment of the gonads to the basement membrane

In conclusion, we were able to create a mutation in the *him-4* gene of C.elegans using CRISPR-Cas9 targeting 2 RGD sequences. Since our research found the link of hemicentin to tissue fragility, our research may allow for further studies of gonad development and extracellular membrane deformities.

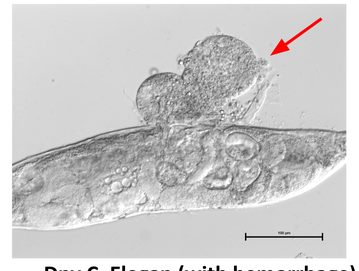
N2 C. Elegan



Dpy C. Elegan (No hemorrhage)



Dpy C. Elegan (with hemorrhage)



Dpy C. Elegan (with hemorrhage)

ACKNOWLEDGEMENTS

We would like to give special thanks to our lab assistants, Zhongqiang Qiu, and Shiva Ramani for preparing all our materials. We would also like to thank Dr. Myeongwoo Lee for giving us the opportunity to conduct this research.