Characterization of Hemicentin in C. elegans

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In the nematode, *Caenorhabditis elegans*, the *him-4* gene linked to the X chromosome encodes hemicentin protein, a component of the extracellular matrix (ECM), which is characterized by 45 immunoglobulin repeats, and fibulin-like domains. The ECM is a three-dimensional network composed of proteins and sugars deposited outside of the cell. ECM proteins are typically large, glycosylated, and contain repeats and motifs for cell binding. Hemicentin is specifically present in the basement membrane (BM), a special sheet-like ECM, that plays an important role in cell migration and tissue attachment, and stability of mitotic germ cells. *HIM-4* contains six RGD motifs, a protein sequence specific to the integrin binding receptor. In the following study, CRISPR gene editing was used to create mutations in *him-4* at two of the six RGD sequences. These sequences were targeted to replace the D amino acid (Aspartic Acid) with the E amino acid (Glutamic Acid). This mutation in *him-4* causes defective phenotypes related to cell binding. We have isolated several targeted animals with tissue fragility, suggesting that the RGD sequence is vital for the function of the protein; the gene editing may interfere with hemicentin binding to the integrin receptor. The disruption of the ECM causes improper attachment of the gonad BM to epithelial BM leading to the hemorrhaging of the gonads and the intestines in *C. elegans*. The observation of the hemorrhaging phenotype and the single-worm PCR will be used to detect CRISPR-induced homozygous alleles. This research may allow for further studies on gonad development and human orthologs of the hemicentin protein. The connection between the hemicentin protein and the ECM deformities may offer insight into diseases associated with tissue fragility.