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

Kyle Dinh, Mark Han, Matt Lishewski, Erika Nichols, Jessica Root, Meg Taylor, Zhongqiang Qiu
Baylor University Biology Dept.

Laminin is a protein composed of α, β, and γ chains and is present in basement membrane ECM. *lam-3* is an α subunit which is essential in the formation of the basement membrane. It is highly expressed in the cell surroundings of the alimentary, epithelial, excretory, muscular, and nervous tissues. Mutations in the LAM-3 of *C. elegans* may cause pathological conditions of improper cell adhesion, migration, and signal-receptor pathways.

Integrins are the extracellular matrix receptors that facilitate bidirectional signaling at the membrane surface. The specificity of this ligand-receptor interaction is based upon specific characteristics of the cell binding motif. The RGD is a binding motif synthesized of a tripeptide including Arginine-Glycine-Aspartate. Mutations in the RGD binding site will result in obvious inhibition of cell binding to the protein.

A mutant *C. elegans* strain containing RGD to RGE mutation in *lam-3* gene has been created by injecting a CRISPR-Cas9 mutation specific to the RGD site. This mutation induced a change from aspartic acid (D) to glutamic acid (E). We injected forty-nine N2 *C. elegans* and obtained one positive homozygote clone verified with a single-worm PCR. Further confirmation will be conducted in the coming weeks with comparison by thrashing assays as well as DNA sequencing of the positive homozygotic mutant. The identification of this homozygotic phenotype may directly relate to the human ortholog of LAMA-2 and potential treatment of the human muscular dystrophy.