Lucas Beffa, Biology

University: University of Missouri-Columbia
Year in School: Senior
Hometown: Glencoe, Missouri
Faculty Mentor: Dr. Dawn Cornelison, Biological Sciences
Funding Source: Life Sciences Undergraduate Research Opportunity Program

Anti-canine Syndecan-4 as a tool to identify and analyze dystrophic dog satellite cells

Lucas Beffa and Dawn Cornelison

Adult skeletal muscle stem cells, known as satellite cells, have an important function in the human body to repair damaged or diseased skeletal muscle tissue. In Duchenne's muscular dystrophy, satellite cells are constantly repairing the damaged skeletal muscle, and thus become exhausted quickly, resulting in greatly increased disease severity. The canine model for muscular dystrophy is preferable to the mouse model because the disease progression is similar to humans. Currently, there is no good molecular tool for identifying satellite cells in dogs. Syndecan-4 is a transmembrane receptor that is expressed on all satellite cells and can thus be used as a satellite cell marker. Therefore, we set out to develop an antibody specific to the extracellular domain of the canine syndecan-4 protein. This involved: isolating RNA from canine satellite cells, transcribing it into cDNA, cloning the cDNA by PCR, ligating the DNA into a bacterial plasmid, sequencing the clone to confirm accuracy, moving the clone into a bacterial expression vector, and forcing the bacteria to express the foreign protein. The resulting polypeptide, which constitutes the extracellular domain of dog Syndecan-4, was sent to Aves Labs (Tigard OR) to be injected into chickens. This creates an immune response that produces polyclonal antibodies that are then isolated from the albumin of the eggs. Once the antibody pool is received, we will test it by Western blot on the expressed protein, then on whole-cell lysates from canine satellite cells. We will also see if it can be used to stain cells in applications such as section immunohistochemistry and FACS. Once its specificity has been verified, the antibody will be available as a tool in finding new therapies and analyzing results from experiments in canine muscular dystrophy.