Equine Research News
A digital presentation of Grayson-Jockey Club Research Foundation
Providers of Equine Research From 1940 thru 2017
Issue 9 • 2017

SERUM BIOMARKERS FOR EQUINE LAMINITIS

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Research Interests for Dr. Galantino-Homer include: Laminitis, Cell Biology, Protein Biochemistry, Theriogenology, Developmental Biology, Epithelial Stem Cells, Insulin Dysregulation and Proteomics

What first sparked your curiosity to explore this area of equine research? Have you studied this area of equine research before?

When I started the Laminitis Laboratory at New Bolton Center in 2007, my impression, as a cell biologist, was that the field was wide open for cellular and molecular investigation. Work had been published on the contributions of matrix metalloproteinases and inflammation to laminitis, but much less had been done regarding lamellar dysfunction and failure at the cellular and molecular levels. At about the same time, the first draft sequence of the equine genome was published, allowing, for the first time, much broader and more powerful molecular studies. The equine genome sequence allows us to identify equine genes by searching the genome sequence and comparing to known genes from mice, humans, and other species instead of going through the long and expensive process of cloning the equine gene.

In this edition:

GJCRF funded my first project, which was to apply proteomics to laminitis pathogenesis, an approach that used the equine genome information to gain a broader picture of the types of cellular processes affected by laminitis, such as cell and tissue mechanical strength and cell stress. That “discovery mode” study then provided data showing that several important structural proteins decreased in lamellar tissue during the early phases of laminitis and that the major structural proteins of the lamellae were a novel keratin pair. Keratin proteins form the cell (cyto-) skeleton of epithelial cells, like those that form the epidermal lamellae, and are responsible for most of the mechanical strength of those cells and tissues.

I’ve been very interested in characterizing those proteins as well as the other proteins that show changed expression during laminitis for two reasons: To understand laminitis pathogenesis and potential therapeutic targets and to better diagnose the onset and severity of laminitis. That was the basis for this project, to follow up on proteins that were differentially expressed in the proteomics study in relation to histopathology changes in natural cases of laminitis in our Laminitis Discovery Database and to see if those proteins could be detected in serum from the same cases as potential diagnostic biomarkers for lamellar tissue damage.

What was the most significant finding from this research? What, if anything, surprised you about your findings?

The most significant finding was that we confirmed, using PCR studies, that the novel keratins are only

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expressed in the epidermal lamellae. The significance of this is that those keratins, K42 and K124, are the best candidates for a serum biomarker of lamellar tissue damage because, unlike our other candidates, they are not expressed in skin or musculoskeletal tissues and are therefore specific to the hoof lamellae, besides being the most abundant proteins in the lamellae. Working with a collaborator at Lehigh University, Lynne Cassimeris, we have since more

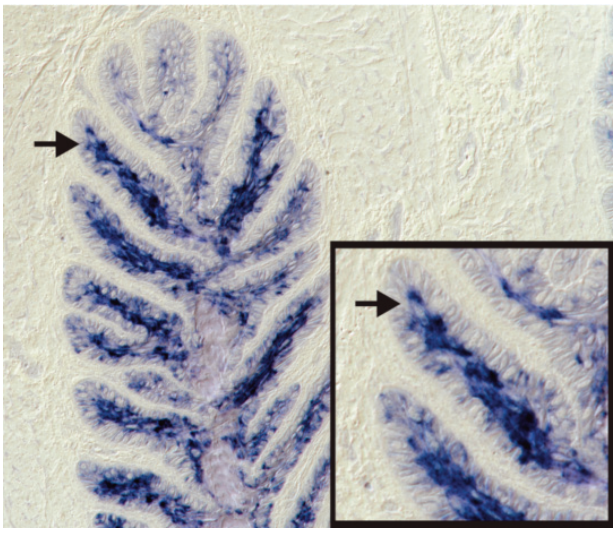
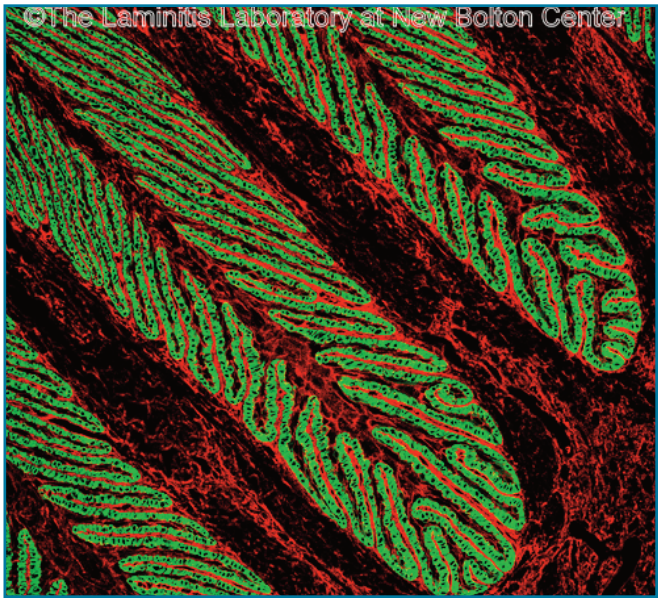


Image shows the localization of keratin-124 gene expression to secondary epidermal lamellae. Photo credit: Lynne Cassimeris

precisely localized K124 to the secondary epidermal lamellae (figure above) and we are still working with Bettina Wagner at Cornell University to generate monoclonal antibodies to one or both proteins so that we can see if we can detect one or both in serum from horses with lamellar tissue damage and laminitis. Tissue-specific keratins are used for markers of other types of organ damage, such as liver disease. Since these keratins are lamellar-specific, they would also potentially serve as laminitis-specific biomarkers, in contrast to the other markers that we investigated, which could be released due to dermatological or orthopedic conditions.

A surprising finding is that the histopathology, protein localization, and keratin gene expression studies revealed just how much the epidermal lamellar cells change in their structure and, we assume, function. We also found that some proteins may not change in expression level, but they change in distribution within the tissue. My interpretation of this, as a cell biologist, is that these cells are changing their identity, which also changes their ability to "do their job" (i.e., transfer weight-bearing and force of impact between the hoof and digital skeleton). Also, although much emphasis has been placed on mechanical failure occurring at the basement membrane, the histopathology studies of our Laminitis Discovery Database laminitic and control cases with Julie Engiles (U Penn) found that changes in epidermal lamellar cell shape, death of epidermal lamellar cells, and loss of cell-cell adhesion are probably at least as important, if not more important, than basement membrane destruction in the mechanical failure of the lamellae.

A very surprising side finding from this project was that Robert Clark (Cumberland County College) discovered that the lectin-binding protein, wheat germ agglutinin (WGA) can be used as a beautiful and effective counterstain for fluorescence microscopy studies of lamellar tissue. Dr. Clark joined the lab for a sabbatical to help with the GJCRF project. Sam Black (UMass-Amherst) had reported about changes in proteoglycan localization during laminitis and we thought that they might potentially serve as serum biomarkers for laminitis. Dr. Clark was investigating whether lectins, which bind to specific chains of sugars that are attached to proteoglycans, would show changes in proteoglycan expression or distribution during laminitis when he discovered that WGA is a perfect counterstain. We now use this counterstain in all of our fluorescence microscopy studies as it makes it much easier to visualize dermal vs epidermal lamellae and to localize the outlines of epidermal cells and the basement membrane.



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ELIZABETH LOCKE JEWELS

The elegance of Elizabeth Locke Jewels again has supported Grayson-Jockey Club Research Foundation. The artist designated a percentage of sales to the Foundation during her recent show at Keeneland. Ms. Locke is generous enough to extend that percentage program through Christmas for all sales in which the customer mentions the Foundation. We thank all who patronized the show. Elizabeth Locke Jewels' neo-classical, hand-made, 19k gold designs reflect the beauty of antique jewelry of the Etruscans, Greeks, and Romans. Elizabeth Locke Jewels has two flagship stores: One in Manhattan and another in Boyce, Virginia. For more information on the collection visit her website at elizabethlocke.com.

SUCCESSFUL KENTUCKY DOWNS DAY FOR GRAYSON

Sponsorships were sold out for all of the six races Kentucky Downs made available for the Fourth Annual Grayson-Jockey Club Research Foundation Day on Saturday, Sept. 2nd. Because of extreme weather from the remnants of Hurricane Harvey, Sept. 2nd card was cancelled and the slated races were held on Sept. 6th &7th.

Under the unique arrangement offered by Kentucky Downs President Corey Johnsen, Grayson-Jockey Club Research Foundation was invited to solicit sponsorships for six of the individual races on the Sept. 2 card, with sponsorship fees retained by the foundation. "We appreciate the innovative program Kentucky Downs has offered," said Edward L. Bowen, president, Grayson-Jockey Club Research Foundation. "We are always pleased to support charities within the horse industry," said Johnsen of Kentucky Downs, which provides trophies for the sponsored races, "and we admire the work of the Grayson-Jockey Club Research Foundation, which is contributing $1.5 million this year for important research projects."

Along with the sponsor of each race, below is the complete list of the winning horses and their connections:

Grayson-Jockey Club Research Foundation Days at Kentucky Downs:

Six organizations made donations to the Foundation to sponsor races in their names.

Grayson-Jockey Club Research Foundation expresses gratitude to Kentucky Downs and to the individual sponsors.

Summary of Results of the races follow:

SPONSOR	Winning Horse	Owner	Trainer	Jockey
Breeders' Cup	Voluptuous	Patricia's Hope LLC	Larry Rivelli	Jose Valdivia, Jr.
Equibase	My Impression	Stuart S. Janney III	Claude McGaughey III	Tyler Gaffalione
Godolphin	Proud Reunion	Brereton C. Jones	Thomas Proctor	Feargal Lynch
Great American Insurance	Tyler U	Silverton Hill LLC	Troy Wismer	Robby Albarado
Hagyard Equine Medical Hospital	Vanilla Cat	W.B. Harrigan & Mike Pietrangelo	William Harrigan	Julie Burke
Rood & Riddle Equine Hospital	Run Time	Bloom Racing Stable & Allen Racing LLC	Michael Maker	Tyler Gaffalione

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What observations do you have about the research process as it relates to this research?

This project and follow-up projects have emphasized how important people and creativity are to research. The keratin expression studies have been a group effort involving Samantha Brooks (U FL), my technician, Caitlin Armstrong, Robert Clark (Cumberland), Julie Engiles (U Penn), Bettina Wagner (Cornell), and Lynne Cassimeris (Lehigh). Besides the technical skills that these people bring to the project, the discussions and trouble-shooting have been invaluable and invigorating.

How will this research improve equine health and welfare?

Laminitis is such an incredibly complex and only minimally understood disease. This research adds to our knowledge of the basic physiology of the hoof lamellae and laminitis pathophysiology, both of which are necessary steps in any advances in the prevention or treatment of laminitis. If we are able to generate monoclonal antibodies to K42 or K124, they will allow us to continue to develop a serum diagnostic assay for lamellar tissue damage. Such assay could be used to detect and rapidly treat subclinical or recurrent laminitis and might also help in determining the severity of lamellar tissue damage, and hence prognosis.

Has this research led to additional projects?

We currently have projects funded by the Animal Health Foundation and American Association of Equine Practitioners Foundation to investigate endoplasmic reticulum (ER) stress in hyperinsulinemia-induced and supporting limb laminitis, respectively. Those studies are direct extensions of the GJCRF projects as the idea to look at ER stress came from the proteomics studies that revealed that protein synthesis and cell stress pathways were up-regulated. ER stress is important in several human diseases, including type 2 diabetes and several neurodegenerative diseases, and is therefore a topic of intense investigation and pharmaceutical development. If we determine that this process is important in laminitis pathogenesis, horses at risk for or suffering from laminitis could benefit from those advances. I am looking forward to extending the serum biomarker and cell stress studies and others relating to my GJCRF projects to collaborations with Andrew van Eps, now at U Penn, on his limb overload, lamellar microdialysis, and cryotherapy studies.

Equine Research News is the digital newsletter of the **Grayson-Jockey Club Research Foundation** *501(c)(3) organization*

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