RESEARCH PROGRESS REPORT SUMMARY

Grant 02152: Translation of MicroRNA into an Early Diagnostic Test for Chronic Kidney Disease

Principal Investigator: Mary Nabity, DVM, PhD

Research Institution: Texas A&M AgriLife Research

Grant Amount: $26,988

Start Date: 1/1/2015 **End Date:** 6/30/2019

Progress Report: FINAL

Report Due: 6/30/2019 **Report Received:** 9/20/2019

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Original Project Description:

Chronic kidney disease (CKD) is a significant cause of illness and death in dogs and is often due to glomerular diseases. Dogs with glomerular disease often have poor outcomes with standard therapy, and specific treatment recommendations are difficult without performing a kidney biopsy to determine the type of glomerular disease present, since treatment and outcome among these diseases differs substantially. Even then, there lacks an understanding of the mechanisms driving these diseases, limiting the ability to optimally treat these dogs. Therefore, tests to non-invasively diagnosis the type of glomerular disease would help veterinarians more appropriately treat these patients and provide insight into the mechanisms that cause the diseases. This could lead to better therapies that slow disease progression and improve quality and length of life in dogs with CKD.

One area of emerging importance in CKD is the role of microRNAs (miRNAs) in disease pathogenesis and progression. miRNAs are small molecules that can regulate gene expression by up or down regulation of messenger RNA transcripts and proteins in target tissues. Many studies have found that increases or decreases in miRNAs can serve as biomarkers of diseases, including human CKD. They also contribute to the development of diseases. The goal of Dr. Nabity's study is to identify miRNAs in serum and urine of dogs that are specific for the three major causes of glomerular disease in this species. They also aim to identify miRNAs associated with disease progression for each of these diseases. Successful completion of these goals will support the translation of miRNAs into diagnostic tests and viable targets for future drug development.

Publications:

1) Chu CP, Nabity MB. 2019. Comparison of RNA isolation and library preparation methods for small RNA sequencing of canine biofluids. Vet Clin Pathol. 48(2):310-319. doi: 10.1111/vcp.12743. Epub 2019 May 11.

2) Technical Aspects in Isolating and Profiling Circulating and Urinary MicroRNAs for Domestic Animals (in revision for resubmission to Vet Clin Pathol).
This manuscript is intended to be a review that can serve as a resource for veterinarians interested in miRNA research. It is in the process of being substantially revised based on the reviewers' suggestions to focus on urinary microRNAs.

3) MiRNA profiling of serum and urine in dogs with chronic kidney disease caused by glomerular diseases (in preparation)

Presentations:

Dr. Candice Chu, who completed her PhD in my laboratory, has presented one abstract and will be presenting two additional abstracts based on the data from this study:

1) "Comparison of methods for preparation of biofluids in dogs for small RNA sequencing" (oral presentation), ACVP/ASVCP Concurrent Annual Meeting, Vancouver, BC, November 4-8, 2017. She received the 2017 ASVCP Young Investigator Award for this presentation. She also presented this talk at our TAMU-CVM graduate student symposium and won the 'People's Choice Award' for her presentation.
2) "Biofluid microRNA expression patterns in three types of naturally-occurring canine models for glomerular disease" (poster), ASN Kidney Week, Washington DC, November 5-10, 2019. This abstract was accepted by the American Society of Nephrology for a poster presentation at the 2019 Kidney Week. Dr. Chu was also selected to participate in the ASN Kidney STARS program, a highly selective program for research trainees in the nephrology field. This program has not had a DVM participant since 2015.
3) "Urinary microRNA profiling in dogs with chronic kidney disease caused by glomerular diseases" (oral), ACVP/ASVCP Concurrent Annual Meeting, San Antonio, TX, November 9-13, 2019. Dr. Chu will be competing for the 2019 ASVCP Young Investigator Award at this conference.

Dr. Chu also presented much of this work during her dissertation defense, June 2018.

Dr. Mary Nabity presented an invited talk at the Society of Toxicologic Pathology Annual Symposium entitled "Traditional renal biomarkers and new approaches to diagnostics" in Indianapolis, IN, June 17-21, 2018 as well as an invited talk entitled "MicroRNAs as biomarkers of renal disease in dogs" at the ECVIM congress in Rotterdam, Netherlands, Sept 6-8, 2018. A brief summary of the results obtained from the sequencing data was included in these presentations.

Report to Grant Sponsor from Investigator:

MicroRNAs (miRNAs) are small, non-coding RNAs that can alter gene expression, and they can serve both as biomarkers of disease and instigators of disease progression. The goal of this project was to identify miRNAs as new biomarkers in the serum and urine of dogs with kidney disease due to primary glomerular diseases. In particular, our goal was to identify miRNAs that can non-invasively (i.e., with a blood or urine sample) distinguish among the 3 most common glomerular kidney diseases in dogs. Currently, such non-invasive identification of these diseases is not possible; yet, their treatment and prognosis vary considerably.

The first objective of this project was to identify and quantify all miRNAs present in the serum and urine from dogs with each of the 3 glomerular diseases, both early and late in the disease process. This was performed using a technique called small RNA-sequencing. Data analysis revealed a number of miRNAs that were either increased or decreased in the urine and serum of dogs with kidney disease compared with healthy dogs. Additionally, several miRNAs were increased in the urine of dogs with late disease versus early disease. Our most exciting finding was that there were 3 miRNAs that appeared to differentiate among the 3 most common categories of glomerular disease in dogs.

The second objective aimed to verify the sequencing results using a faster, less expensive, more clinically feasible technique (PCR) and a larger number of samples. Based on testing of 25 urine samples from dogs, most with CKD due to glomerular diseases, 2 of the 3 urinary miRNAs identified in the first objective appeared promising to identify immune complex glomerulonephritis and amyloidosis. This is impactful because immunosuppressive therapy is currently recommended when immune mediated disease is identified. For amyloidosis, however, there are no additional therapies beyond supportive care that currently appear to impact disease progression and survival. It is important to recognize that testing additional samples is still necessary to verify these results. We have obtained additional funding to continue this investigation.

We are optimistic that the results from this study will provide a way for veterinarians to determine whether immunosuppressive therapy is warranted in a dog with proteinuric kidney disease without obtaining a kidney biopsy. While non-invasive tests are unlikely to ever provide as much information as a kidney biopsy, being able to accurately determine appropriate therapy using a urine sample would benefit those patients that are poor candidates for having a kidney biopsy or for owners who can't afford it.

Overall, this project has provided my laboratory and graduate students with substantial experience with methods for RNA evaluation in urine and serum. For instance, throughout this project, we tested 10 different urine and serum RNA isolation methods. This resulted in a method comparison study that, in the future, can contribute to the development of a standard method for RNA isolation and preparation from urine and serum samples. The experience gained through this project also helped us

improve upon our original study design, which was critical in generating results that could provide statistically significant differences among groups and therefore allow us to more carefully select miRNAs for further testing. Through this project, we have also investigated several methods for qRT-PCR data normalization, which is an ongoing area of difficulty with biofluids. Our efforts will contribute to the veterinary literature on this subject. Additionally, the substantial data generated in objective 1 can provide the basis for a number of projects in the future, which we anticipate will further contribute to biomarker identification for canine CKD.

Training of the future generation of veterinary researchers is an important aspect of my work, and this project has provided research experience to 2 graduate students, serving as a significant portion of a PhD thesis for one of these students who is now a veterinary clinical pathology resident and desires to be a clinician scientist after her residency training. This student won awards at both local and national conferences for her presentations of data generated during this study.