RESEARCH PROGRESS REPORT SUMMARY

Grant 02309-T:
Targeting Cancer Epigenome Epigenome: The Effect of Specific Histone Lysine Methyltransferase Inhibition in Canine B-Cell Lymphoma

Principal Investigator:
Angela McCleary-Wheeler, DVM, PhD

Research Institution:
University of Missouri

Grant Amount:
$21,321

Start Date: 09/1/2018
End Date: 06/30/2020

Progress Report:
Mid-Year 3

Report Due:
06/30/2019
Report Received:
10/01/2019

(The content of this report is not confidential and may be used in communications with your organization.)

Original Project Description:
Canine lymphoma is one of the most common cancers in dogs. While some breeds appear more at risk than others, all can be affected. Although it is often treatable, canine lymphoma can rarely be cured. A continued understanding of the mechanisms causing lymphoma in dogs and identification of novel therapies are needed to improve survival in dogs with lymphoma. One area of research that has been actively explored and provided exciting breakthroughs for human lymphoma is epigenetics, or alterations in how genes are turned on and off independent of the DNA sequence. One way in which this occurs is due to modifications of the proteins that interact with DNA called histones. Various modifications to these histones can result in genes being turned on or off, leading to the development of cancer. One particular enzyme that modifies histones, EZH2, has been found to play a role in some human lymphomas. However, this has been unexplored in canine lymphoma. Given the striking similarities between human and canine lymphoma, the objective of this work is to characterize the function and role of EZH2 in canine lymphoma. The investigators will utilize an EZH2 inhibitor to study EZH2 in canine lymphoma cells. The information obtained from this study will help guide the future development of this targeted inhibitor for use as a novel therapy to treat canine lymphoma.

Publications: None at this time.

Presentations: The work was presented as a poster presentation at the Veterinary Cancer Society Annual Conference in October 2017.

Report to Grant Sponsor from Investigator:
Lymphoma, particularly the large, B-cell subtype, is one of the most common malignancies in dogs. Canine lymphoma can be treated, but it is rarely cured. Novel therapeutic strategies are necessary to improve outcomes in dogs diagnosed with lymphoma. Recently, advances in the understanding of human lymphomas have focused on the area of epigenetics. One area of this research involves understanding how genes are turned on or off based on different modifications to histone proteins, a specific group of proteins that interact with DNA. Specific enzymes that modify these histone proteins have altered activity that can lead to lymphoma development in human lymphomas. One of these enzymes is EZH2. Increased activity of EZH2 has been shown to play an important role in the development of some human lymphomas. Data from a Phase I study of an EZH2 inhibitor, tazemetostat, in relapsed or refractory human B-cell, non-Hodgkin lymphoma has shown to be a safe, oral therapy with potential clinical benefit. The role of EZH2, however, has not been evaluated in canine B-cell lymphomas to date. Given the similarity between human and canine B-cell lymphoma, we seek to investigate whether EZH2 activity plays a role in canine B-cell lymphoma. To do this, we use canine lymphoma cells and specific EZH2 inhibitors, including the tazemetostat used in early human studies, to evaluate the effect of EZH2 inhibition on cell growth and survival. Our data suggest that this inhibitor is highly potent and effective for inhibiting EZH2 effects on histone modification in canine lymphoma. This is important as this inhibitor is an orally bioavailable drug with a good toxicity profile in humans, making this inhibitor a candidate for clinical trials in dogs with lymphoma. Initial data suggests that EZH2 inhibition may not impede lymphoma cell proliferation or survival. However, we have confirmed that some genes that regulate the ability of canine lymphoma cells to replicate are altered with EZH2 inhibition. Specifically, one gene, CDKN1a, is turned back on when EZH2 is inhibited. The activation of CDKN1a is repeatable and profound. We will be continuing this work with a sequencing approach to further understand what genes are regulated by EZH2 in canine B-cell lymphoma cells. Our findings are suggesting an importance for EZH2 in canine lymphoma and for continued investigations into cell cycle regulators that may be abnormal. By understanding the genes regulated by EZH2, we can characterize the role this histone modifying enzyme has in canine lymphoma. Moreover, we can assess how inhibition of EZH2 activity may be utilized clinical in dogs with lymphoma. We appreciate the continued generous support provided by this grant from the CHF and its supporting donors.

RESEARCH PROGRESS REPORT SUMMARY

Grant 02597:
Molecular Epidemiology of Methicillin-resistant Staphylococcus pseudintermedius *in the United States*

Principal Investigator:
Stephen Kania, PhD

Research Institution:
University of Tennessee

Grant Amount:
$47,082.00

Start Date: 05/1/2019
End Date: 04/30/2021

Progress Report:
Mid-Year 1

Report Due:
10/31/2019
Report Received:
10/23/2019

(The content of this report is not confidential and may be used in communications with your organization.)

Original Project Description:
The bacterium *Staphylococcus pseudintermedius* is the most common cause of canine skin infections as well as other important canine diseases. Disfigurement caused by skin infections and treatment failures is an important problem. Resistance to antibiotics is becoming increasingly widespread with few or no antibiotic options left for some cases. Alternative therapeutic approaches being investigated include vaccines, small molecule virulence factor inhibitors and bacteriophage lytic enzymes. In order for new products to be effective against the broadest spectrum of wildtype bacterial strains as possible, it is important to determine which strains of *S. pseudintermedius* clinically predominate in the United States today. A genetic typing method for *S. pseudintermedius* was previously developed by the research team along with a survey of bacterial strains in the United States in which they sequenced the genomes of the most common strains. This analysis provided a snapshot of predominant strains and suggested a potential for emergence of new, highly antibiotic resistant organisms. Identifying the current strains in the US and sequencing their genomes will provide a basis for developing the next generation of treatments as well as important information about changes that occur in the bacterial population in response to selective pressures.

Publications: None at this time.

Presentations: None at this time.

Report to Grant Sponsor from Investigator:
This projected is designed to study the molecular epidemiology and characteristics of *Staphylococcus pseudintermedius* in the United States. This bacterium is the major cause of skin infections in dogs and has become widely resistant to antibiotics over the past 15 years. With data from about one-third of the samples we plan to collect, we have found widespread antibiotic resistance and emergence of new strains previously only associated with canine disease in other parts of the world. This information is important to understand the spread of antibiotic resistance and for the development of new strategies to treat and prevent this important disease.

RESEARCH PROGRESS REPORT SUMMARY

Grant 02651:
Discovery of Novel Biomarkers of Canine Atopic Dermatitis through Lipid Profiling

Principal Investigator:
Harm HogenEsch, DVM, PhD

Research Institution:
Purdue University

Grant Amount:
$99,105

Start Date: 05/01/2019
End Date: 10/31/2020

Progress Report:
Mid-Year 1

Report Due:
10/31/2019
Report Received:
10/28/2019

(The content of this report is not confidential and may be used in communications with your organization.)

Original Project Description:
Canine atopic dermatitis (CAD) is a common allergic skin disease of dogs with a strong genetic basis. CAD can severely affect the health and well-being of dogs and current diagnosis of CAD requires time-consuming and expensive procedures for the owner. Furthermore, the molecular mechanisms underlying this condition are not well understood. Evidence from human studies suggests that several variants of atopic dermatitis (AD) exist with different mechanisms and responses to treatment. Therefore, new approaches to identify molecular markers that can help with better diagnosis and management are warranted. CAD and human AD are associated with changes in the composition of lipids in the epidermis which may precede the inflammation or result from the inflammation. The investigators will analyze the lipid composition of the epidermis and blood of healthy dogs in comparison to dogs with CAD using a novel analytical method developed by their interdisciplinary team. The results of this work could lead to new, minimally-invasive tests for the diagnosis of CAD and for the prediction and monitoring of the response of CAD patients to treatment.

Publications: None at this time.

Presentations: None at this time.

Report to Grant Sponsor from Investigator:
Canine atopic dermatitis (CAD) is a common allergic skin disease of dogs with a strong genetic basis. Evidence from human studies suggests that several variants of AD exist with different mechanisms and responses to treatment. Current diagnosis of CAD requires time-consuming procedures that involve a considerable cost to the owner. Therefore, new approaches to identify molecular markers that can help with better diagnosis and management of the disease are warranted. In this study, we are using our tailored methodology for lipid biomarker discovery in CAD. Thus far, 19 atopic dogs and 13 healthy dogs have been recruited. Patients are males and females of several different breeds and ages with seasonal or year-round itch. CAD patients enrolled so far are being treated with either Apoquel®, Cytopoint® or prednisone and followed for 2 months to evaluate the lipid changes in their skin and blood. Using non-invasive sampling procedures, we have collected samples from the skin of healthy controls and from affected and non-affected areas of the skin of CAD patients. Preliminary statistical analysis demonstrates that the lipid fingerprint of the skin accurately classifies samples from healthy dogs and CAD patients, and can distinguish between affected and non-affected skin of CAD patients. MRM-profiling approach allows an unbiased analysis of the lipids that may result in new diagnostic biomarkers to classify disease phenotypes that drive the development of new therapies.

(Journal Scan continued from page 13)

What's Next

Association is not causation, so more research is needed to determine whether pruritus causes stress in dogs with AD or if other factors are to blame. As the researchers conclude, "Given the potential importance of stress in AD development and the implications of the behavioral problems we have found for dog and owner bonds, further studies need to be carried out."

To read the full study, *https://www.mdpi.com/2076-2615/9/10/813/htm#B10-animals-09-00813*

Harvey ND, Craigon PJ, Shaw SC, et al. Behavioural differences in dogs with atopic dermatitis suggest stress could be a significant problem associated with chronic pruritus. Animals 2019;9(10):813.

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