

**North Dakota Tribal College Research Symposium 2019**  
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**Cankdeska Cikana Community College**

**An Analysis of the Expression of GAGE6, GAGE2A, GAGE12J, GAGE13, GAGE12C, and GAGE12B Genes in Thirteen Common Breast Cancer Cell Lines**

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The GAGE antigens are a member of the cancer/testis antigen group and are only expressed in the germ line cells of healthy individuals. In healthy individuals expression is limited to a subset of oocytes in the adult ovary, but expression is found to increase in a wide variety of cancers. Previous research demonstrated that the C-terminal domain of MT-3 induces the expression of the GAGE family genes. The expression of GAGE antigens was also differential in the presence of MT-3 or MT-1E suggesting unique roles for these genes in the development and progression of breast cancer. To further explore the role of the GAGE family genes this study measured the expression levels of GAGE6, GAGE2A, GAGE12J, GAGE13, GAGE12B and GAGE12C in thirteen commercially available breast cancer cell lines. Estrogen receptor positive cell lines MCF-7, T47D, ZR-75-1, and VP303. Basal/triple negative cell lines Hs578T, VP303, and MDA-MB-231 were characterized. Transformed breast epithelial cell lines SVCT and 1-7HB2 were used to represent non-tumor forming breast tissue. Breast cancer cell lines MDA-MB-157, MDA-MB-261, ZR-75-30, MT-M223 were also characterized. GAGE antigen expression was highly variable across the cell lines. GAGE12B had zero expression except in the T47D cell line. Future studies analyzing the expression of metallothioneins in these cell lines is needed to fully correlate GAGE antigen expression to metallothionein activity.

**Finding DNA Identifiers for the Different Sexes of the White-Tussock Moth (Lepidoptera: Lymantriidae)**

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*Orgyia leucostigma*, commonly known as the White-Marked Tussock Moth, is categorized as a pest insect, causing millions of dollars of damage to forests in North America. *Orgyia leucostigma* females are wingless; males have wings. Spread of the pest is limited because of wingless females, but other pests, like the Asian Gypsy moth, have females with the capacity to fly. Because of the economic and basic biological implications, an understanding of the underlying genetics of wingless females is of great importance. Previous studies have shown that the wings develop in the larval stages in Lepidoptera. Unfortunately, it is not possible to identify the different sexes of larvae; although, we can determine sexes visually during the pupal stage. A molecular identification method would facilitate future work and certain pest control methods.

In Lepidoptera the chromosomes determine the sex of the animal. Typically, females are ZW and males are ZZ. Our goal is to find a region on the W chromosome that detects females in *O. leucostigma*. In our project we used an assembled genome to identify regions that appeared to be unique to females. We designed primers from these regions and used PCR to see if regions were amplified in females, but not males. We also used restriction enzymes to identify possible polymorphic fragments (RFLPs) that differ between males and females.

### **Characterization of GAGE2B, GAGE2C, GAGE2E, GAGE12C, and GAGE12D Gene Expression in Breast Cancer Cell Lines**

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Previous research demonstrated that the C-terminal domain of MT-3 induces the expression of the GAGE family genes. The expression of GAGE antigens was also differential in the presence of MT-3 or MT-1E suggesting unique roles for these genes in the development and progression of breast cancer. The GAGE antigens are a member of the cancer/testis antigen group and are only expressed in the germ line cells of healthy individuals. In healthy individuals expression is limited to a subset of oocytes in the adult ovary, but expression is found to increase in a wide variety of cancers. To further explore the role of the GAGE family genes this study measured the expression levels of GAGE2B, GAGE2C, GAGE2E, GAGE12C, and GAGE12D in thirteen commercially available breast cancer cell lines. Estrogen receptor positive cell lines MCF-7, T47D, ZR-75-1, and VP303. Basal/triple negative cell lines Hs578T, VP303, and MDA-MB-231 were characterized. Transformed breast epithelial cell lines SVCT and 1-7HB2 were used to represent non-tumor forming breast tissue. Breast cancer cell lines MDA-MB-157, MDA-MB-261, ZR-75-30, MT-M223 were also characterized. GAGE antigen expression was consistently over 800 copies per microliter in the T47D cell line. All other cell lines exhibited moderate to low expression of the GAGE antigens investigated. This indicates that for most breast cancer cell lines GAGE antigen expression is highly heterogeneous in nature.

### **Creating Cankdeska Cikana Community College's Weathers Station Using Advanced Manufacturing**

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Advanced Manufacturing Initiative, Cankdeska Cikana Community College, 214 1st Ave, Fort Totten, ND

Advanced Manufacturing at Cankdeska Cikana Community College (CCCC) is joining with the 3D-PAWS (3D-Printed Automatic Weather Station) community by 3D printing and assembling over one hundred parts for each of two separate 3D-PAWS, at produced at CCCC. The Advanced Manufacturing lab personnel printed all the weather station parts on three different 3D printers. The Makerbot Z18 was used to build larger and taller weather station parts and a Makerbot 2x Replicator printed various medium sized parts. More complicated and detailed parts were created using the Mark forged 3D printer with Onyx plastic material including; small cases for electrical components, thin mesh filters and small detailed threads that could not be replicated to

specification on the Makerbots. University Corporation for Atmospheric Research (UCAR) staff member Martin Steinson held a 3-Day training at CCCC for advanced manufacturing teachers and technicians to assemble the 3D-PAWS including all of the wiring and installation of the components for Air Speed, Air Direction, Temperature, Barometric Pressure, UV radiation, and precipitation. All of the components also needed to be calibrated. The training and instructional materials can be used by CCCC to train other entities and TCU's. A modem on the 3D-PAWS will allow an ordinary user to check the weather at the station via their cell phones. 3D Chords.com allows users to check forecast and weather information at various 3D PAWS remote sites.

### **Exploring the Use of UAV in Natural Resources Applications**

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Advanced Manufacturing Initiative and natural resources departments, Cankdeska Cikana Community College, 214 1st Ave, Fort Totten, ND

The Cankdeska Cikana Community College (CCCC) Advanced Manufacturing Initiative (AMI) student laboratory technicians and faculty purchased the DJI Matrice 210, an unmanned autonomous vehicle (UAV). CCCC procured a Zenmuse XT camera, which provides precise thermal imaging, a Zenmuse Z30 camera which has 30x optical zoom ideal for precision inspections, and a Zenmuse X4S camera with a rapid leaf shutter speed. Finally, the team acquired a RedEdge-M Multispectral sensor which is a rugged, built-to-last, professional multispectral sensor. The next step was to learn the intricacies of the UAV, take necessary FAA remote pilot tests, and learn the functions of the cameras. Then, the AMI student lab techs learned to operate the UAV and the cameras. The ultimate vision is to be able to use the necessary sensors in a variety of environmental or agricultural applications. These include research of Harmful Algae Blooms on the Spirit Lake Reservation in collaboration with the Tribal EPA and Spirit Lake Fish and Wildlife. Additionally, the cameras and UAV can be utilized to assess the locations and distribution of invasive plants, determine crop and forest health, track populations of wildlife, and more.

### **CO2 on the Rise**

Janna Steen, Dr. Kerry Hartman  
Nueta Hidatsa Sahnish Collage

Numerous studies worldwide have proven that CO2 levels in the Earth's atmosphere are rising (IPCC). My Hypothesis is that CO2 emissions are on the rise in North Dakota due to oil flares and other oil related activity. In my study I have taken in situ reading at my home and other areas around New Town to gather base line data. I have utilized data from Dr. Aaron Kennedy PHD from the Department of Atmospheric Sciences at UND and multiple other sources to show that in North Dakota CO2 has risen in the past 15 years. With the online data and data I have gathered I find CO2 is higher especially in the areas with higher oil activity.

## **The Positive Effects of Recycling Shayla Gayton**

Jessica Marshall, Kerry Hartman Nueta Hidatsa Sahnish College

Recycling is one of those things everyone talks about and wants to do but never does. Everyone knows that recycling is good for the environment but I don't think they know the exact figures and how much recycling helps make a change. That is the reason I have decided to do this research. For my research study, I have obtained data from various literature works, websites, and interviewed Recycling Center Directors and have used that data to compile charts and graphs of the positive effects of recycling. My data includes methods of other recycling programs and how I can implement those tactics into something similar at my college, Nueta Hidatsa Sahnish College and throughout the Fort Berthold Reservation.

## **Mercury Levels in Bait Fish**

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The Cannonball and the Grand are the two main rivers located on the Standing Rock reservation; both are tributaries of the Missouri River. The Cannonball River is located on the North Dakota side of the reservation and the Grand River is located on the South Dakota side of the reservation; both rivers have similar land-use characteristics with minor differences like the Cannonball having a little more farming around it; while the Grand has more livestock surrounding it. The purpose of this research was to address the mercury levels in bait fish on both rivers. Fish samples were collected at various locations on each river using mainly a seine and a few nets. All fish were identified by species and those determined large enough to extract a tissue sample for mercury analysis were kept for the study. Length and weight of the collected fish were measured in the lab. Overall eight species of fish were collected with each river having 3 species of fish that were unique to it. A tissue sample of a quarter of a gram was taken from each fish and was analyzed on the Hydra-C mercury analyzer. Initial analysis reveals that the mean Mercury concentrations are higher in the fish collected from the Cannonball River. Agriculture may play a role in the higher Mercury levels; further analysis is required to determine if this is true.

## **Water Quality in a Typical Bison Pasture**

Kylee M. Harrison\*

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Water is essential to everything that is living on our planet. Even more important, is the quality of the water as opposed to its quantity. Poor water quality can lead to poor productivity and low health outcomes in livestock. This study assessed the question of whether there is a difference in water quality at various times of the year. It was hypothesized that there was a difference in water quality throughout the year based on many environmental factors. The study took place on a stream located in the Sitting Bull College buffalo pasture near Selfridge, ND, during the months of August, September, and October. Water and macroinvertebrate samples were

collected during these months. A bio-assessment, chemical and nutrient analysis, and microbial analysis were completed with the samples to determine water quality status. The findings indicate that water quality changed only slightly throughout the sampling period. It was quantitatively determined that the water quality was good based on the assessment. All of the elements were within livestock water quality standards except for iron, manganese, and sodium. These 3 nutrients have no major health problems listed for livestock on a pasture beyond taste, smell, and color. Total E. Coli and total fecal coliform counts decreased throughout sampling time. There was also a decrease in total number of macroinvertebrate species and total number of individuals throughout sampling time. This may be due to seasonal fluctuations and the decrease in temperatures.

**Assessing Arsenic Concentrations Around Devil's Lake Waters: Public Health Implications**  
**Moriah J. Thompson\*** Environmental Science Department, 9299 Highway 24, Sitting Bull College, Fort Yates ND 58358

The Spirit Lake Tribe resides in the north-east-central area in North Dakota around Devil's lake. It shares its water boarder with the City of Devils Lake. The community relies on the lake basin waters for its daily life from a social, economic, cultural and spiritual perspective. For many years the members of the reservation that still use well water as their main source of water were unaware of the concentration levels of Arsenic in their water. The current study hypothesizes that there is a significant level of Arsenic in the waters of the Spirit Lake Nation beyond allowable limits; and that there is a difference between the Arsenic level of the lake water and the levels in the residential houses that use well water. The study areas included three residential houses that use well water, the Devils Lake Basin, Sweetwater Lake, Spring Lake, and Wood Lake. Fifteen water samples (12 lake samples and 3 residential samples) were collected and processed using standard methods and protocols. The samples were then analyzed for Arsenic using a PE Analyst 800 Atomic Absorption Spectrometer and Graphite Furnace. Findings indicate that half of the samples were above the MCL of 10 ppm set by the EPA. However, these levels were not statistically significant. The findings also showed no significant difference between Arsenic levels in lake waters and residential well waters. Given the health status of the community, it is important to identify a correlation between these concentration levels and health outcomes.

**Implementing Bio-Rad Labs and Meeting State Teaching Standards as a High School Science Teacher**

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Incorporating Bio-Rad lab activities and content into a high school science class, while meeting North Dakota state teaching standards, is hypothesized to increase student understanding and retention of content as well as increase their enjoyment of the learning process and overall science literacy. Experience with these kits showed some challenges including minimizing the length of each lab to fit class periods, along with the students' ability to proficiently complete each lab. The Bio-Rad kits we studied included: pGLO Transformation, Biofuel Enzyme, ELISA Immuno

Explorer, and Forensic DNA Fingerprinting. Learning objectives for each activity were matched to North Dakota state teaching standards. After running all kits with high school students and current high school science teachers, labs best suited for a high school classroom setting were identified. Factors considered were the lab prep time and adequate time for content mastery. Giving a pre and post assessment of the understanding of the content will allow the teacher to adapt instruction as needed. Preparation and equipment required may be limiting factors, therefore grants and/or partnerships may need to be pursued. Teachers in AP and general science classes can best gauge which labs can be incorporated to enhance their students' learning and overall science literacy.

### **SPARC Expression at Tumor Initiation and After Tumor Formation in a Bladder Urothelial Carcinoma Model System**

\*Emily R. Biggane<sup>1</sup>, Seema Somji<sup>2</sup>, Scott H. Garrett<sup>2</sup>, Don A. Sens<sup>2</sup>, Xu Dong Zhou<sup>2</sup>, Andrea Nore<sup>2</sup>, Jane R. Dunlevy<sup>1</sup>

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Bladder cancer has a strong link to environmental exposure to toxicants. Our system uses a urothelial carcinoma model due to heavy metal cadmium (Cd) exposure to examine potential biomarkers. UROtsa bladder cells exposed to long term, low doses of Cd resulted in 7 independent malignantly transformed cell lines. The resulting microarray analysis identified SPARC as the most repressed gene. In this study, SPARC repression via alternative transcription factor binding to the promoter was assessed using Chromatin Immunoprecipitation (ChIP). An *in vitro* cell attachment and spreading assay determined SPARC's role in these two processes. Finally, serial heterotransplant tumors were analyzed for SPARC expression using RT-qPCR with human or mouse specific primers. ChIP results indicated alternative transcription factor, SOX5, binding to the SPARC promoter in Cd transformed cells compared to the control. Results also showed SPARC promotes cell spreading *in vitro* on collagen. Finally, serial heterotransplant results showed SPARC expression was not maintained nor increased within tumor cells. However, stromal SPARC expression was found to increase. The stromal SPARC expression was found to be of mouse and not human origin, indicating that the mouse tumor stroma and not the human tumor cells were responsible for the SPARC expression. Further *in vitro* experiments showed that SPARC expression in Cd transformed SPARC transfected cell lines was repressed when exposed to exogenous human or mouse SPARC. Overall, SPARC expression appears to play a vital role in bladder tumor formation where high stromal SPARC may repress expression by tumor cells.

## **The $\alpha_{1A}$ -adrenergic receptor alters seizure susceptibility and epileptiform frequency**

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This study aims to further our understanding of modulatory neurotransmitter, norepinephrine, in epilepsy. Specifically, we were interested in the role of the  $\alpha_{1A}$ -Adrenergic Receptor ( $\alpha_{1A}$ -AR) subtype. This receptor subtype has been shown to positively modulate cognition and mood, properties opposite that of most other antiepileptic therapies. We hypothesized that the  $\alpha_{1A}$ -AR would exhibit antiepileptic effects upon activation. The experiments in this study included behavioral observations, hippocampal slice electrophysiology, and electroencephalographic measurements of induced and spontaneous epileptiform activity. Behavioral observations showed that  $\alpha_{1A}$ -AR knockout mice were vulnerable to spontaneous seizures, while neither wild-type control or  $\alpha_{1B}$ -AR knockout mice exhibited seizures. In induced seizure models, constitutively active mutant  $\alpha_{1A}$ -AR mice showed significant resistance to the onset of flurothyl-induced seizures, while mice treated with the  $\alpha_{1A}$ -AR selective agonist, cirazoline, showed a trend towards resistance of the onset of kainate-induced seizures. Electrophysiological recordings showed that several  $\alpha_{1A}$ -AR agonists reduce epileptiform event frequency in pharmacological isolation, but  $\alpha_{1A}$ -AR expression is requisite for this effect. Interestingly, we found that  $\alpha_{1A}$ -AR knockout mice exhibited higher baseline epileptiform frequency than either wild-type controls or  $\alpha_{1B}$ -AR knockout mice. We can conclude that  $\alpha_{1A}$ -AR activation can reduce epileptiform frequency and increase latency to the onset of seizure activity, while loss of this receptor subtype results in vulnerability to epileptiform intensification and spontaneous seizures. This research may lead to the development of novel antiepileptic therapies, where only two-thirds of epilepsy patients have effective treatment and all current therapies have significant negative side-effects.

## **Differential Behavior Response in C57BL/6 and BALB/c Mice Induced by Food Allergy**

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Allergic hypersensitivity is often found comorbid with neuropsychiatric disorders, including attention deficit hyperactivity, autism spectrum, and anxiety disorder. Though classic allergic symptoms are well defined, it is evident that subclinical allergic hypersensitivity exist and remain undiagnosed. A central factor in the establishment, provocation, and peripheral to central signaling of allergic symptoms are helper T-cells and their cytokines. To understand how differing T-cell immune bias can alter clinical presentation of allergy we sensitized helper T-cell type 1 biased C57BL/6 and helper T-cell type 2 biased BALB/c mice to the milk protein  $\beta$ -Lactoglobulin (BLG). Starting at 4 weeks of age, the mice were sensitized via weekly oral gavage of BLG in the presence of cholera toxin as an adjuvant. Utilizing behavioral tests, clinical anaphylaxis scoring, and serum BLG-specific IgE ELISA we profiled the lasting effects in sensitized mice post-allergen exposure. BLG-sensitized C57BL/6 mice present no anaphylactic

symptoms, but increased serum BLG-specific IgE. Additionally, C57BL/6 mice display subtle anxiety-like behavior when tested with an elevated-zero maze. In contrast, BALB/c mice exhibit anaphylactic symptoms and anxiety-like behavior orders of magnitude great than matched C57BL/6 mice in both elevated zero maze and open field tests. From these results, we see that mice will differing immune predisposition have differing responses to allergic challenge.

**CD133+CD24+ cells isolated from the human proximal tubules have proliferative and regenerative potential in response to insult by the environmental toxicant cadmium**

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The proximal tubules of the kidney are a major site of toxic insult, cell death and regeneration, and the development of renal tubular diseases. Toxic insult can result from exposure to heavy metals, pharmaceuticals, diabetic induced nephropathy and ischemia. Cadmium an environmental nephrotoxicant accumulates in the proximal tubule cells leading to overt renal damage. Previous studies from our laboratory have shown that the human proximal tubules contain a population of cells co-expressing the cell surface markers CD24 and CD133. This CD24+ CD133+ double positive population is scattered throughout the kidney cortex and is capable of regenerating. Under cell culture conditions, these cells can be expanded and they retain their phenotype and their potential for self-renewal as well as the capacity to differentiate. The goal of this study was to determine the response of this progenitor population to the environmental toxicant cadmium and determine their ability to regenerate and differentiate. Our data suggests that the CD24+CD133+ proximal tubule cells are more resistant to the toxic effects of cadmium with increased ability to proliferate and regenerate in response to cadmium exposure when compared to the cells that only express CD24, which are more sensitive and lack the proliferative capacity. In addition, the CD24+CD133+ cells show in-vitro tubulogenic, osteogenic, adipogenic and neurogenic differentiation, whereas the CD24+ cells lack the ability to differentiate. In conclusion, our study demonstrates that the CD24+CD133+ cells are the progenitor/stem cells that have proliferative and regenerative capabilities and exhibit multi-lineage differential potential, whereas the CD24+ cells are the differentiated cells that are sensitive to toxic insults and lack proliferative and regenerative capacities.