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Cankdeska Cikana Community College

Expression of GAGE1 and GAGE2A in Estrogen Receptor Positive and Negative 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 GAGE1 and GAGE2A in six breast cancer cell lines. Estrogen receptor positive cell lines MCF-7 and T47D, basal/triple negative cell lines Hs578T and MDA-231-MB, and to represent normal breast epithelial cells MCF10A was used. Arsenite transformed MCF10 cells were also investigated for GAGE antigen expression levels. The expression of GAGE2A was significantly higher in MCF7 cells. The GAGE1 antigen was expressed in all breast cancer cell lines with no cell line being significantly different in expression. The increased expression of GAGE2A may correlate to the differential expression of metallothionein isoforms. Expression of metallothionein-1 and metallothionein-2 will be investigated in future studies.

Expression of GAGE2B and GAGE2C in Estrogen Receptor Positive and Negative 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 and GAGE2C in six breast cancer cell lines. Estrogen receptor positive cell lines MCF-7 and T47D, basal/triple negative cell lines Hs578T and MDA-231-MB, and to represent normal breast epithelial cells MCF10A was used. Arsenite transformed MCF10 cells were also investigated for GAGE antigen expression levels. When compared to the other five breast cancer cell lines the expression of GAGE2B was significantly higher in T47D cells. The GAGE2C antigen was expressed in all breast cancer cell lines with no

cell line being significantly different in expression. The increased expression of GAGE2B may correlate to the differential expression of metallothionein isoforms.

Expression of GAGE12C and GAGE12D in Estrogen Receptor Positive and Negative Breast Cancer Cell Lines Does Not Correspond to Estrogen Receptor Status.

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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 GAGE12D and GAGE12C in six breast cancer cell lines. Estrogen receptor positive cell lines MCF-7 and T47D, basal/triple negative cell lines Hs578T and MDA-231-MB, and to represent normal breast epithelial cells MCF10A was used. Arsenite transformed MCF10 cells were also investigated for GAGE antigen expression levels. The expression of GAGE12D and GAGE12C were similar in all cell lines. Expression of GAGE12C or GAGE12D was very low in MCF10, MCF10 As#B, and MDA-231-MB cell lines. Levels of expression of GAGE12C and GAGE12D were significantly higher in MCF7, T47D, and HS578T cells. These results suggest that GAGE12D and GAGE12C expression doesn't correlate to estrogen receptor status, and is variable amongst breast cancer cell lines.

Comparative Embryonic Mutation and Mortality Responses to Chronic Cadmium Exposure

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The highest concentrations of cadmium across the United States can be found in the Red River Valley. It is important to study the bioaccumulation of the metal through trophic levels to gain a full understanding of the environmental impact of cadmium. The purpose of this study was to observe the effects of cadmium bioaccumulation in two different snail species, *Stagnicola elodes* and *Physa acuta* and determine if the responses were dosage dependent. Adult snails were collected from an Island Park outlet in Mayville, ND (47.4997°N, 97.3256°W) and a drainage slough near North Golden Lake in Steele County, ND (47.5926°N, 97.6326°W). The breeding colonies of adult snails were maintained in 49.2 L plastic containers, holding 12 L of pond water. Each colony was exposed to predetermined concentrations of cadmium (0 µg/L [n=924, n=1391] 10 µg/L [n=1287, n=1029], 50 µg/L [n=994, n=1073], and 100µg/L [n=446, n=1296]) through all embryonic stages of development. Visual observations were conducted daily to determine survivorship of each embryo and phenotypic mutations. When looking at the total mortality rates, *Physa acuta* show a 12-17% increase from the control group to elevated cadmium

concentrations; this response is not noted in *Stagnicola elodes*. The overall effects of cadmium on the snails was significant for mutations and mortality in both snail species ($p < 0.05$). *Physa acuta* and *Stagnicola elodes* showed unique responses to chronic cadmium exposure. *Physa acuta* demonstrated a heightened sensitivity to environmental cadmium in comparison to *Stagnicola elodes*.

Utilizing Photovoice Research as a Mechanism to Explore Environmental Health on a Rural Northern Plains Tribal Reservation

Background: American Indians face several environmental hazards that affect their health status, such as living in remote and isolated locations that expose residents to severe climatic conditions, hazardous geography, and disease-carrying insects and rodents. Other factors include limited availability of housing and extensive use of sub-standard housing, unsanitary methods of sewage and waste disposal, and unsafe water supplies (Indian Health Service, 2016, June).

Purpose: To explore the barriers and facilitators to a healthy environment on a rural reservation in the upper Midwest.

Method: Qualitative research design photovoice was used with eight tribal community college students. Participants learned about photovoice as a participatory research method, which included a hands-on lesson using digital cameras. Participants were instructed to capture five photos of their perceptions of health and environment. The eight students were divided into two focus groups. The students were asked questions on how each of their five photos related to health and environment within their Nation. Focus group interviews were verbally recorded, transcribed verbatim, and coded for themes.

Results: All eight students self-identified as American Indian. Three (38%) were male and five (42%) were female. Ages ranged from 20-29 ($n=2$), 30-39 ($n=3$), and 40-49 ($n=3$). Seventy-five percent were enrolled in a GED program, while two students (25%) were currently enrolled in college courses. The seven environmental themes identified to either facilitate or hinder being healthy on the reservation were rising water, water safety, weather safety, trash, road conditions, physical activity, and beauty of nature.

Conclusions: Through this photovoice project, American Indian students discussed their environmental concerns but also shared the beauty of their environment. The themes identified by the students can be used as a framework to address environmental health concerns, increase awareness and foster change across American Indian reservations. However, the results also reflect opportunities for physical activity and the resilience of the environment.

Co-authors: Heather Marxen, Instructor, Ann Wadsworth, Next Steps Program Assistant Director

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Sustainable Energy Efficiencies Applications

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Sustainable technologies are necessary in order to curtail our growing power usage/consumption trends. The college has been expanding power consumption dramatically since 1997. Older buildings were utilizing inefficient T-12 fluorescent lighting. Demand charges accounted for half of the power bill and cold air leaks could be felt around doors and walls. The energy efficiency infrastructure approach is to utilize multiple methodologies. The AICF funded a grant that has enabled NHSC to utilize wind, solar, insulation, and lighting efficiency upgrades. The college completed all of these upgrades in November 2017. Preliminary Kwh production numbers indicate an estimated minimum savings on electricity bills of approximately \$400/month or 4800/annual. This does not include large reductions in carbon footprint or insulation and lighting efficiency impacts. Data collection will be expanded in the future to better document and validate energy savings. NHSC plans to expand its use of sustainable technologies and energy conservation practices in the future.

Rangeland Management on Fort Berthold Indian Reservation, Culturally Relevant Social Mechanisms Behind Management Practices

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Native plant communities are at risk due to oil and gas development. As with any development, the negative impacts on plant communities is an unavoidable disturbance. The Fort Berthold Indian Reservation (FBIR) is located at the center of the area currently being overrun by the development of the Bakken oil reserve. This explosion of development has caused widespread destruction of thousands of acres of land. The correlation of soil and plants has been greatly overlooked and the potential impacts that result from poor design and construction include reducing the capacity of environment to support vegetation and wildlife. Production of oil and gas causes habitat conversion and fragmentation. The challenge is to preserve and manage the small amount of remaining natural land cover and to sustain viable populations of the remaining endemic and native species while critical resources for their survival fluctuate in response to climatic, ecological, social, and economic forces. The purpose of this study was to quantify the disturbance of native grasslands impacted by the ongoing oil development on the FBIR. The remaining native grasslands were emphasized due to the cultural relevance of this pristine vegetation. GPS, GIS, and Remote Sensing were utilized for locating, mapping, and determining area of oil well pad sites in multiple sample areas to implement management practices. This area has predominantly been rangeland which interim and final reclamation outlines best management practices in which they have to follow. There are multiple studies that have been done on using indigenous influences within rangeland management. The results of these studies showed there is better success in maintaining sustainability.

An Investigation Study of Juneberry (*Amelanchier anifolia*) Phenology on Fort Berthold Indian Reservation

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Restoration of the Juneberry plant on the Fort Berthold Indian Reservation has led to many different aspects on the success of rejuvenating the plant. Due to different variables of yield in previous research and other ecological facets that needed to be connected, a phenology trail has been established through this research. Soil samples, vegetation inventories, and animal habitats have been logged. Color infrared imagery (CIR) was used extensively for soil moisture and vegetation health. Bloom times and yields were significantly different from cultivated to uncultivated and also to locality of the Missouri River. Findings have proved the depletion of Juneberry plant communities, pollinators (birds, bees, bats), and animal habitats are changing either from onslaught of industrialization and climate change.

An Investigation into the Presence of Mercury in Lake Sakakawea

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Mercury is a powerful neurotoxin that comes in many forms and people exposed to high levels may experience adverse effects. Humans are usually exposed methyl mercury through the consumption of fish. Lake Sakakawea on the Fort Berthold Indian Reservation in North Dakota is a very large reservoir with many different recreational fishing areas. So, I and a few colleagues collected fish samples from two different sights: 4 Bears Bay vs. Indian Hills Bay in Lake Sakakawea to test their mercury levels and compare them to each other. My hypothesis is that the levels will not be different based on area. Meat samples were taken from harvested fish (exclusively walleye) during fishing derbies. Local fisherman donated samples during the fillet process and then we stored them in a freezer. Samples were then shipped to Sitting Bull College (Fort Yates, ND) where they were analyzed by thermal decomposition, amalgamation, and atomic absorption spectrophotometry (EPA method 7473). Next, the results were sent back to us via e-mail. The results suggest that Indian Hills Bay walleye have a higher concentration of mercury in their meat than 4 Bears Bay walleye. Also, the results suggest that larger walleye tend to have larger amounts of mercury than smaller ones. These results will be used by both Nueta Hidatsa Sahnish College and the Mandan Hidatsa and Arikara Nation Game and Fish for further study and possible walleye consumption guidelines.

Climate Change Effects on Pollinators

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Climate change has been discussed and studied at length for the past few decades. The finds have provided a wide range of conclusions. It seems there are proponents for both views of climate change, and its effects on the populace of the planet earth. One is that there is no real effect that can be directly correlated to climate change, the other is that climate change is something that is real, and will affect all aspects of life on the globe. It is a widely accepted concept that climate change does effect the short term living conditions for all life on the planet earth. Many of these climate change effects are as follows; global warming, ice ages, human pollution, and oceanic conditions. Most of these conditions have a history to study, but some don't. Such as human pollution , this factor alone is being studied very intensely at the current time. But only time will tell the true effects of this area of interest based on real time data collection. It could and should warrant more studies in the area of climate change. So that if we can avoid a disaster of our own making, we would have the ability to do so. On behalf of all life on this ever changing planet.

An Investigation of Indoor Air Quality in the Welding Shop at Nueta Hidatsa Sahnish College

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This Study is focused on the characterization of indoor particles generated from in the welding shop at Nueta Hidatsa Sahnish College. Our intention for this study is to establish scientific evidence regarding the extent of unhealthy particulate matter in our welding shop. Four portable MiniVol TAS (tactical air samplers) from Airmetrics, were strategically placed in various locations, in and out of the welding shop to obtain findings and controls. Preliminary results of the unprocessed filters reveal obvious observable evidence between the differences in the samples and controls. It is anticipated that subjective and objective analysis of the filters will reveal the presence of unacceptable levels of particulate matter hazardous to health. Particulate Matter generated from welding has shown to cause detrimental health conditions. The results of this study will be used to document needed improvement or replacement of the colleges welding shop ventilation system.

Bison Health: Bovine Viral Diarrhea (BVD) in Bison herds on Standing Rock Sioux Reservation

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Bison are a keystone species on the Great Plains of North America. In their natural setting, the impact of the large bison can be both positive and negative; but in modified environments the key is how the species is managed. Bison are variously managed as wildlife and as domestic herds. The bison is sacred to many Native American tribes of the Great Plains. Several factors have contributed to the decline of the bison as an ecosystem modifier. Among these factors, prevalence of diseases that historically did not impact bison populations. Diseases have a measurable impact that can inhibit production and growth. Bovine Viral Diarrhea has been reported as affecting bison. The clinical symptoms, spread mechanisms and prevalent strain types are similar to what has been reported in other bovine species. Blood samples were collected from 4 herds over a 2 year period. These were submitted for IgM antibody titer testing by ELISA to determine the presence of viral antibody to establish herd exposure status. Results show 1 herd had relatively high BVDV antibody titers in 8 animals tested in year 1 of sampling. Recommendations post-test were to remove animals testing positive for BVDV. Sampling in year 2 showed no animals testing positive for BVDV in any of the herds sampled. Exposure to endemic bovine infectious diseases that influence reproductive rates was confirmed on Standing Rock however it was not established whether this was an active/persistent exposure or residual exposure. Identifying sound health programs are paramount to improving herd productivity.

The Great Plains Turnip (*Pediomelum esculentum*)

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Pediomelum esculentum or prairie turnip is found in short and mixed-grass prairie. There is a need to study this plant for future prairie restoration due to the fact it takes 3-4 years for the tuber to become large enough for a food source. Experiments in the hilltop habitats and bison pastures will give greater insight into what conditions prairie turnips prefer. The hilltop experiment was set up to test whether prairie turnips would germinate and survive better in natural prairie soil or commercial soil. Germination in prairie soil was higher than in commercial soil. Seedling survival in prairie soil was higher than in commercial soil but predation from rodents was seen only in prairie soil. The hypotheses that turnip seeds would have a higher germination and higher seedling survival in prairie soil were supported. In the bison pasture two experiments were set up. One experiment tested whether a prairie turnip would regenerate from a piece of the tuber or root. The other experiment was set up to test germination and seedling success in bison manure. The bison pasture experiments will not show results till spring 2018.

Bison (*Bison bison*) Grazing Effect on Plant Diversity

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There are different natural occurrences that can shape a prairie. One of those occurrences is grazing by bison (*Bison bison*). My question was what effect does bison grazing have on plant diversity in a mixed-grass prairie? I hypothesized that plant diversity would be greater in the grazed pastures compared to the ungrazed pastures. The study took place in Sitting Bull College's buffalo pasture near Selfridge, North Dakota during the months of June and July, 2017. Plant samples were taken in a total of 5 pastures; 2 heavily grazed, 2 ungrazed, and 1 moderately grazed. The surveys were done along a 30 meter transect; 3 total samples were taken with each transect at 10, 20, and 30 meters. A one meter squared (1m^2) quadrat was used for the survey; within the quadrat, the number of species were counted and percent cover of each was visually estimated. Species richness and diversity were calculated for each pasture. With a total of 86 species in all 5 pastures, species richness was highest in a heavily grazed pasture and species diversity was highest in an ungrazed pasture and lowest in a heavily grazed pasture. The data did not support my hypothesis. The surrounding pastures and cropland, soils, and other things like grazing regime may affect the results.

Effects of Sustainable Bio-based Polymers on Microbial Growth

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Polymers define our modern society because of their broad range of usage. They are macromolecules built from smaller units called monomers. Polymers are classified as natural or synthetic. Bio-based polymers derived from sustainable agricultural plant materials are being tested as replacements for synthetic polymers. These have the potential of being safer for the environment throughout the product life cycle and are renewable. However, their resistance to biotic and abiotic processes of transformation and degradation in usage situations is an issue. This study tested the ability of microorganisms to use biopolymers bonded to nitrogen groups as a growth resource in the environment. Studies show microorganisms will readily use nitrogen containing compounds as a ready substrate source for growth and development. A treatment-control experiment was designed to measure how varying concentrations of biopolymers in nutrient media affected the growth of microbes. FDCA and a model polymer created from chemically bonding FDCA to a known nitro-photo trigger, were used as biopolymers in the study. *Bacillus subtilis* was used as test microbe because it enhances the biodegradation of hydrocarbons and it plays a role in the breakdown of nitrogen-containing compounds such as TNT. Preliminary findings show bacteria growth on plates with FDCA bonded to the nitro-photo trigger. No growth occurred in plates with only FDCA. Increased polymer concentration reduced microbial growth, though this disparity was not statistically significant. Inserting nitrogen containing groups into polymers may facilitate their usage as substrate by *B. subtilis*. It enhances the biodegradation potential of the polymer.

Coyote Food Habits in the North Dakota Mixed-Grass Prairie

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The North American native coyote, *Canis latrans* has an extraordinary adaptability to its surroundings and eats anything that is available. My original question was to assess if season and location affected the coyote diet. I hypothesized that the coyote's diet would be dominated by plants and insects during the summer and that the location of my preliminary study would have more insect prey. My preliminary study took place in a bison pasture managed by Sitting Bull College in June and July 2017. I surveyed for coyote scat by walking through a large percent of the pasture. The evidence that coyotes were utilizing this area was very scarce. A total of 4 scats were collected through a 2 month period. The data did not support my hypothesis that the coyotes diet was plant and insect based.

The Effects of Bison Grazing on Soil Properties in a Mixed-Grass Prairie

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Bison grazing is a problem in the mixed-grass prairie as it affects soil properties, plant growth, and food availability for wildlife. Overgrazing by bison has negative effects like compaction, nutrient deficiencies, and low soil moisture. The purpose of this study was to see what the effects of bison grazing are on soil properties. It was hypothesized that as bison grazing intensity increased, soil moisture and nutrient levels will decline due to higher compaction and low plant cover. The study was conducted in the summer of 2017 in a mixed-grass prairie grazed by bison (*Bison bison*). The pasture is located south of Selfridge, ND, and managed by Sitting Bull College. The pasture was split into five sub-pastures, then further subdivided based on grazing intensity characteristics identified as: ungrazed, moderately grazed, and heavily grazed. A total of 60 soil core samples were collected, 12 from each sub-pasture. Samples were analyzed for percent soil moisture, texture, nutrient content (total N and NO₃), and mineral content (P, Cu, Se). Initial findings show that percent soil moisture was significantly lower with increased grazing. Soil texture was sandy across the pasture which could contribute to low water retention in the pasture. Mineral and nutrient content may also have an effect because they were lower than required for a healthy pasture for bison. Minerals such as copper are essential for bison productivity and wellbeing. Instituting programs that manage bison grazing, increase mineral and nutrient supplementation, improve soil water retention and structure to encourage plant growth are recommended.

Genetic variants (RS1342326, RS1837253, RS7444910) are not associated with asthma among Native American Children

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Background & Objectives: Asthma is recognized as a complex, multifactorial condition. While considerable information is available regarding genetic factors associated with asthma in majority populations, there is relatively little known about these factors among Native American children. We tested 3 single nucleotide polymorphisms (SNPs) for association with asthma, rs1342326, rs1837253, and rs7444910. Interleukin-33 (IL-33) is one of the last discovered members of the human IL-1 family. It is involved in the pathogenesis of many inflammatory diseases. The IL-33 rs1342326 polymorphism was associated with a lower childhood asthma risk in the Tunisian population and a higher IL-33 protein expression. rs1342326 was associated with a lower risk of asthma in a case/control study among the Tunisian children. Rs1837253 was also associated with asthma, rs744910 was not.

METHODS: Electronic medical records were screened for a clinical diagnosis of asthma among children between ages 6 and 18 (N=108). After informed consent, detailed medical records were reviewed for case defining criteria. Control children (N=215), matched for age, were identified. Salivary DNA was genotyped for rs1342326, rs1837253, and rs7444910. Genotypes were determined by TaqMan (ThermoFisher Scientific) assay. Appropriate Student's t test, chi-square statistics and logistic regression methods were employed for analysis. Additive genetic models were considered.

RESULTS: No significant difference in allelic frequency was found between cases and controls. BMI shows a positive, independent and significant association with asthma in this cohort. No significant difference in allelic frequency was found between cases and controls risk of asthma in an additive genetic model, using multivariate logistic regression modeling, with simultaneous adjustment for age and body mass index (BMI). In the multivariate analysis, BMI continues to show a positive, independent and significant association with asthma in this cohort.

CONCLUSION: As found in other populations, BMI is associated with asthma among Native American children; but these genetic variants do not seem to be associated with asthma in this community.

The expression of keratin 6 is regulated by activation of the ERK1/2 pathway in arsenite transformed human urothelial cells

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Urothelial cancers have an environmental etiological component, and previous studies from our laboratory have shown that arsenite (As^{+3}) can cause the malignant transformation of the immortalized urothelial cells (UROtsa), leading to the expression of keratin 6 (KRT6). The expression of KRT6 in the parent UROtsa cells can be induced by the addition of epidermal growth factor (EGF). Tumors formed by these transformed cells have focal areas of squamous differentiation that express KRT6. The goal of this study was to investigate the mechanism involved in the upregulation of KRT6 in urothelial cancers and to validate that the As^{+3} -

transformed UROtsa cells are a model of urothelial cancer. The results obtained showed that the parent and the As⁺³-transformed UROtsa cells express EGFR which is phosphorylated with the addition of epidermal growth factor (EGF) resulting in an increased expression of KRT6. Inhibition of the extracellular-signal regulated kinases (ERK1/2) pathway by the addition of the mitogen-activated protein kinase kinase 1 (MEK1) and MEK2 kinase inhibitor U0126 resulted in a decrease in the phosphorylation of ERK1/2 and a reduced expression of KRT6. Immunohistochemical analysis of the tumors generated by the As⁺³-transformed isolates expressed EGFR and tumors formed by two of the transformed isolates expressed the phosphorylated form of EGFR. These results show that the expression of KRT6 is regulated at least in part by the ERK1/2 pathway and that the As⁺³-transformed human urothelial cells have the potential to serve as a valid model to study urothelial carcinomas.

The Effect of Combined Exposure of Hyperglycemia and Cadmium on Human Proximal Tubule Cells

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Diabetic nephropathy (DN) is a complicated microvascular disease, where prolonged exposure to hyperglycemia induces damage to proximal tubule (PT) cells of the kidney. Ultimately, this disease will progress to an end-stage renal disease (ESRD) as the severity of toxic insult is increased. Hyperglycemia has been shown to induce the expression of aldose reductase (AR) enzymes to assist with the extra glucose metabolism. Global microarray analysis indicated an AR isoform, AKR1B10, has also been shown to be induced upon cadmium (Cd²⁺) exposure. Since both glucose and Cd²⁺ have similar effects on the PT cells, which is also a target of Cd²⁺ toxicity, their co-exposure may increase the disease severity. PT cells chronically exposed to hyperglycemia, alone, induced morphological changes and increased mesenchymal marker expression. Results from a global gene array analysis conducted on primary PT cells exposed to hyperglycemia showed an induction of thioredoxin-interacting protein (TXNIP) which may be a downstream target of both toxicants. The goal of this project was to investigate the effects of dual exposure of glucose and Cd²⁺ toxicity on PT cells. For this purpose, PT cells were exposed to 0, 4.5, 9 μ M Cd²⁺ for 48 hours alone then 5.5, 7.75, 11, or 16 mM glucose was added for a dual exposure of 72 hours. TXNIP is significantly induced by hyperglycemia and the combined toxicant exposure at the mRNA and protein levels. Since both toxicants induced AKR1B10, sorbitol accumulation and glucose utilization was measured. These results suggest prolonged Cd²⁺ exposure may contribute to DN disease progression.

The Association of Metallothioneins Expression and Cisplatin Treatment in Human Bladder Cancer

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INTRODUCTION: Arsenite (As³⁺) and Cadmium (Cd²⁺) are known carcinogens that are commonly consumed through cigarette smoking, agricultural products, and contaminated water sources. Previous studies from our laboratory have shown that exposure of UROtsa cells to either

As³⁺ or Cd²⁺ can cause malignant transformation of cells grown in culture. Cisplatin is a small heavy metal complex that can inhibit DNA synthesis and transcription by enhancing alkylating agents. Cisplatin makes the DNA strands unable to uncoil and separate therefore triggering cell cycle arrest. Cisplatin may be susceptible to mediating factors, such as Metallothioneins (MT). MT are a family of low molecular weight proteins that have metal binding antioxidant activity. Through cysteine residues MT's have a high binding affinity for heavy metals, such as As³⁺ and Cd²⁺.

SIGNIFICANCE: The goal of this study is to determine if there is an induction in MT expression levels when the UROtsa cells are malignantly transformed with heavy metals, As³⁺ or Cd²⁺. Furthermore, to establish if MT expression influences resistance of the UROtsa parent and transformed isolates to the toxic effects of Cisplatin.

METHODOLOGY: Performed RT-qPCR and western blots on the UROtsa parent and the transformed cell lines to determine the mRNA and protein expression levels respectively of MT isoforms after 72 hours of treatment with Cisplatin. Cell viability was determined using the MTT assay.

RESULTS: The data suggests that the UROtsa parent and Cd #1 cell lines are more resistant to the toxic effects of Cisplatin when compared to Cd #4, As #1, and As #5. The MT-2A isoform demonstrated the highest expression when compared to the other MT isoforms. Treatment with Cisplatin did not induce or repress expression levels of the MT isoforms in the UROtsa parent or the transformed cell lines.

CONCLUSIONS: The data shows that treatment with Cisplatin in the UROtsa parent and the transformed cell lines does not increase the expression of MT. Thus, resistance to Cisplatin that occurs in some human bladder cancers may not occur via the induction of MT.

As³⁺- and Cd²⁺- Transformed UROtsa Isolates Characterized into Basal and Luminal Muscle-Invasive Bladder Cancer Subtypes

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The characterization of breast carcinoma based on the gene expression of molecular markers in order to provide better management of the disease and treatments has led to the classification of muscle-invasive bladder cancer (MIBC) into basal-like and luminal-like subtypes. Being classified as human carcinogens, arsenic and cadmium have been implicated to play a role in the development of bladder cancers via exposure through contaminated water sources and cigarette smoking, respectively. With an interest in studying toxicant-specific carcinogenesis, our laboratory has developed malignantly transformed arsenite (As³⁺) and cadmium (Cd²⁺) cell lines from a normal human urothelium, UROtsa. Microarray analysis was performed to find molecular markers to characterize MIBC into basal-like and luminal-like subtypes. Real-time qPCR of subcutaneous heterotransplant tumors and spheroids from As³⁺- and Cd²⁺- transformed UROtsa cells showed increased expression levels of the basal marker CD44 and decreased expression levels of luminal markers CD24, ERBB2, FABP4, KRT7, KRT18, KRT20, and XBP1 in the majority of the As³⁺- and Cd²⁺- transformed isolates. Initial gene expression

data of all the basal and luminal markers shows that the subcutaneous transplant tumors and spheroids derived from As³⁺- and Cd²⁺- transformed UROtsa cells exhibit more basal-like characteristic features. The ability to characterize the UROtsa transformed isolates into basal-like and luminal-like will enhance the applicability of this *in-vitro*, toxicant-specific carcinogenesis model to specific types of human bladder cancers.

SPARC in a Cell Culture Model of Heavy Metal Induced Bladder Transitional Cell Carcinoma

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Our lab studies biomarkers using a bladder cancer model based on environmental exposure to cadmium in a cell culture system that can also be used to generate heterotransplant tumors in immunocompromised mice. The biomarker SPARC is the most repressed gene in all of the independent malignantly transformed UROtsa cell lines. It has been shown to play a role in many contradictory cell biological functions and has been described as both an oncogene and a tumor suppressor dependent upon which cancer is being studied. Its role in bladder cancer is yet to be determined. This study focuses on the matrix-associated protein SPARC and its role in cell attachment and spreading using cell culture assays and microscopy techniques as well as its role in tumor initiation using mouse studies and immunohistochemistry. Results show that cell spreading of non-SPARC expressing cadmium transformed cells was decreased compared to SPARC expressing cells. Immunohistochemical analysis shows that with successive tumor inoculations SPARC expression within the tumor is not maintained nor does it increase; however, stromal SPARC expression does increase. These results support the literature in regards to SPARC playing a role in multiple cell biological processes. SPARC may promote cell spreading once a cell has attached in a distant site ultimately supporting cell survival and tumor metastasis. SPARC may also play a role in cellular communication between the tumor and stroma promoting tumor progression.

Cadmium Induces Noncompaction of Ventricular Myocardium in Mouse Embryo

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Cadmium (Cd) is a toxic heavy metal that can be highly concentrated into environment by industry activities. Parental exposure of cadmium causes a wide range of abnormalities in embryo development, including neural, gastrointestinal and limb defects. However, the impact of cadmium exposure on heart development was under-studied. In this study, we investigated the cardiovascular development in mouse embryos with prenatal exposure of CdCl₂ at various doses, 50 mg/L, 10 mg/L, and 2 mg/L. Though cadmium was quickly cleaned from the mother's blood, it has high concentration in the placenta and crossed the placental barrier to accumulate in the embryo's bladder, kidney and heart. Histological examination of E14.5 and newborn hearts

found high degree of noncompaction of ventricular myocardium in 50 mg/L and 10 mg/L of CdCl₂ treated mice. And the newborn mice could not survive until P2. The low-dose Cd-treated mice did not show ventricular hypertrophy until adulthood. In E14.5 embryos of high-dose Cd treatment, the expression of *Gata4*, an important cardiogenic transcription factor, was up-regulated. When *Gata4* was knockdown by heterozygous alleles, the embryos exposed to 10 mg/L of CdCl₂ were rescued from ventricular noncompaction phenotype. Therefore, we conclude that cadmium exposure induced intrauterine growth retardation and fetal noncompaction of ventricular myocardium. Our data suggested that *Gata4* overexpression played an important role in Cd-induced ventricular noncompaction.

The α_{1A} -adrenergic receptor is important for preventing seizure vulnerability and crucial for α_1 -adrenergic receptor antiepileptic effects

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This study aimed to further our understanding of the antiepileptic effects of the neurotransmitter norepinephrine. Our experiments used knockout mice as a novel method for assessing subtype-specific effects of adrenergic receptor activation. Specifically, we were interested in the role of the α_{1A} -Adrenergic Receptor (α_{1A} -AR) subtype, which has shown significant promise for positively affecting cognition and mood. We hypothesized that the α_{1A} -AR is crucial for maintaining seizure threshold and that α_{1A} -AR activation would reduce epileptiform event frequency. Wild-type control or knockout mice lacking either α_{1A} -ARs or α_{1B} -ARs were placed in an open field for observation of spontaneous seizures. Then, we used electrophysiological recordings to measure epileptiform events in hippocampal slices, which were induced by magnesium deprivation. Hippocampal slices were used to generate dose-response curves using the selective α_1 -AR agonist phenylephrine; fitted curves were used to predict efficacy and potency. Behavioral observations showed that α_{1A} -AR knockout mice were vulnerable to spontaneous seizures, while neither wild-type control or α_{1B} -AR knockout mice exhibited seizures. Electrophysiological recordings showed that phenylephrine is capable of reducing epileptiform event frequency, but α_{1A} -AR expression is requisite for this effect. Additionally, we found that α_{1A} -AR knockout mice exhibited higher baseline epileptiform frequency than either wild-type controls or α_{1B} -AR knockout mice. We can conclude that the α_{1A} -AR is important for maintaining seizure threshold and that this subtype is essential for α_1 -AR mediated antiepileptic effects. This research may lead to the development of novel epilepsy therapies, where only two-thirds of epilepsy patients have effective treatment and all current therapies have significant side-effects.

Integrative analysis of transcriptomes, chromatin landscapes and DNA methylation reveal essential regulatory elements during early heart development

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Cardiac cell lineage specification is mediated by transcription factors as well as other epigenetic cues. *Osr1*-expressing cells have been shown to contribute to atrial septum progenitors between E8.0 and E11.0. In this study, we investigate the downstream targets of *Osr1* by examining gene expression profiles, *Osr1* binding events, and epigenetic marks on the chromatin. We select pure populations of *Osr1*-expressing cells in the second heart field at E9.5 and E10.5 using fluorescent-activated cell sorting and by combining RNA-seq and ChIP-seq, we identify downstream genes and pathways of *Osr1* with high accuracy. We also investigate the context of these regulatory regions by examining the chromatin landscape and DNA accessibility using ATAC-seq and whole-genome bisulfite sequencing. This is a comprehensive study that provides a detailed view of the dynamic events in cardiac progenitor cells.

Transcriptional and Behavioral Changes Associated with Milk Allergy-Induced Peripheral Inflammation

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Peripheral immune reactions due to allergic hypersensitivity are often found comorbid with neuropsychiatric disorders. In particular, cow's milk allergy has been suspected to elicit or exacerbate behavioral symptoms in patients with attention deficit hyperactivity and autism spectrum disorders. To define the underlying biological mechanism of milk-allergy-mediated behavioral manifestations, we investigated the behavior and gut and brain pathology in mice sensitized to a milk allergen, β -lactoglobulin (BLG). BLG-sensitized animals presented with increased repetitive and anxiety-like behaviors that were associated with increased BLG-specific IgE levels in the serum. Furthermore, differential protein and RNA expressions were found in the gut and brain of BLG mice, indicating potential dysregulation of intestinal and blood-brain-barrier permeability, inflammatory response, iron transport, neurological cell fate, and neurovascular remodeling. These results suggest that food allergy triggers a variety of changes in the gut and brain in susceptible individuals, ultimately influencing their behavior.

Effect of cadmium on the stem/progenitor cell populations in cultures of renal proximal tubular cells.

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The proximal tubules of the kidney are common site of toxic insult, cell death and regeneration as well as a major site for the development of renal tubular diseases. Toxic insult can result from heavy metals, pharmaceuticals, diabetic induced nephropathy or from ischemia. Recent studies suggest that tubular regeneration after toxic insult may involve progenitor/ stem like cells that are residing among the renal tubular cells. Our previous study have shown that renal tubular cells cultures contain 25-30% of the CD24+ cells whereas 70-75% of the CD133+CD24+ cells. The CD133+CD24+ cells are considered as stem cells whereas the function of the CD24+ cells is not known. The goal of this study was to determine the response of these cell population after exposure to cadmium. These cells population were sorted from the RPTEC/TERT1 cells following which they were cultured and treated with 4.5 μ M and 9 μ M Cd²⁺ for an approximately 30 days. The results demonstrate that the CD133+24+ cells are more resistant to cadmium exposure as there was no change in the number of double positive cells population post-treatment, whereas the number of CD24+ cells significantly decreased. Both of the cell population form domes in culture indicative of vectorial active transport. In addition, the double positive CD133+CD24+ cells grow a lot faster when compared to the CD24+ cells. In conclusion, our data suggests that the CD133+CD24+ cells show characteristics of progenitor cells that may be involved in tubular regeneration whereas the role of CD24+ cells is still unclear.

Designation of As³⁺ and Cd²⁺ Malignantly Transformed Cell Lines, Urospheres, and Tumors into Bladder Cancer Subtypes

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Cadmium (Cd²⁺) and arsenic (As³⁺) are environmental carcinogens associated with the development of bladder cancer. This cancer can be described as either non-muscle invasive or muscle invasive, with the latter being much more life threatening. Recent studies have shown that muscle invasive bladder cancer (MIBC) can be characterized into two subtypes, basal or luminal, based on the expression of specific biomarkers. Our laboratory has developed and advanced the UROtsa cell line as a model system to study environmentally induced human urothelial cancer (UC). Our previous studies demonstrate that the UROtsa cell line is malignantly transformed by both Cd²⁺ and As³⁺, as evidenced by tumor formation in immune compromised mice. The histology of the tumors resemble UC with squamous differentiation. The present study has several goals. The first is to determine if the UROtsa cell culture model reflects the basal subtype of UC. The second is to determine the heterogeneity in the expression of squamous differentiation, at both the gene and protein levels, among the independently generated UROtsa cell lines and associated tumor heterotransplants. The final goal is to determine if cancer-initiating cells (CIC) from the independently generated UROtsa cell lines also reflect the basal

subtype. The results obtained from our study demonstrate that the transformed cell lines express high levels of basal markers such as KRT1, KRT5, KRT6, KRT14, KRT16, TP63 and CD44. The expression of luminal markers such as KRT20, FOXA1, GATA3 and PPAR γ were reduced or absent in these cell lines, however, the following luminal markers, KRT8, KRT18, KRT19 and CD24 were expressed in the cell lines. This expression pattern was similar in the CICs as well as the tumor heterotransplants. Immuno-histochemical analysis of the tumors confirmed predominant staining for basal markers in the tumor heterotransplants. In conclusion, our data shows that the Cd²⁺ and As³⁺-transformed cells express gene patterns similar to the basal subtype of MIBC, and that this is a good model system to study the molecular changes that occur during the development of MIBC.