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THE UNIQUE N- AND C-TERMINAL DOMAINS OF METALLOTHIONEIN-3 ALTER THE EXPRESSION OF HNRNP A2 IN MCF-7 BREAST CANCER CELLS

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Studies have shown that the MT-3 protein contains 7 additional amino acids that are not present in any other member of the MT gene family, a 6 amino acid C-terminal sequence and a Thr in the N-terminal region. The goal of this study was to characterize the function of the N and C-terminal domains of MT-3 in the breast cancer cell line, MCF-7. For this purpose six different constructs of MTs were prepared which were as follows: wild type (WT) MT-3, MT-3 N-terminal mutation (MT-3ΔNT), MT-3 C-terminal deletion (MT-3ΔCT), WT MT-1E, and MT-1E mutated to contain the N -terminal of MT-3 or the C-terminal or both the N- and the C-terminal of MT-3. Each of these constructs was stably transfected into MCF-7 cells which were then sent out for microarray analysis. Microarray analysis indicated that both glycerophosphodiester phosphodiesterase domain containing 3 (GDPD3) and human nuclear ribonucleoprotein (hnRNP) A2 were repressed. Past research indicates that GDPD3 is up regulated in breast cancer due to the differential expression of phospholipase A2 isoforms, while hnRNPA2 has been known to be over expressed in lung cancer and various other cancers such as breast, pancreas and liver. Validation data obtained in this study indicated that GDPD3 expression was not significantly altered. The expression of hnRNP A2 was significantly repressed in the MT3ΔCT cell line, and expression was significantly induced in the CT-1E cell line. Expression of hnRNP A2 may be increased or decreased depending on the activity of the N- or C-terminal of MT-3. The activity of the N- or C-terminal domains of MT-3 and the alteration in expression of hnRNP A2 may affect the oncogenic activity of the cancer cell.

A PRELIMINARY INVESTIGATION INTO METALLOTHIONEIN-3'S INFLUENCE ON THE EXPRESSION OF GAGE ANTIGENS IN MCF7 CELLS.

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Cancer/testis (CT) antigens are a group of proteins normally expressed in human germline cells and are present in various tumor types. Evidence suggests that GAGE antigens may direct cell proliferation, differentiation, and the survival of germ line cells. Microarray analysis of the MCF7 mutants, performed in our laboratory, indicated that several GAGE antigens were being differentially regulated by the N- or C-terminal of MT-3. Previous research demonstrates that over-expression of MT-3 occurs in the majority of breast cancers and is associated with poor patient outcome. Furthermore, MT-3 has been shown to inhibit the growth of breast cancer and

prostate cancer cell lines. Studies have shown that the MT-3 protein contains 7 additional amino acids that are not present in any other member of the MT gene family, a 6 amino acid C-terminal sequence and a Thr in the N-terminal region. The unique N-terminal sequence is responsible for the growth inhibitory activity of MT-3 in the neuronal system, while the function of C-terminal region remains unknown. Several GAGE family antigens were not present on the microarray but were investigated in this study. These included: GAGE5-1, GAGE6-1, GAGE4, and GAGE2E-2. Real time PCR indicated that GAGE5-1, GAGE6-1, GAGE4, and GAGE2E-2 were overexpressed in the MCF7 cell lines expressing the 1E-NT-CT gene sequence. The GAGE6-1 antigen was significantly overexpressed in all the mutant MCF7 cell lines. In conclusion, this study indicates that a subset of GAGE antigens is overexpressed in the presence or absence of the N- or C-terminal of MT-3.

BIOREMEDIATION OF CONTAMINATED SOILS THROUGH THE USE OF EXTRACELLULAR ENZYMES FROM SAPROPHYTIC FUNGI

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Current methods of soil decontamination from oil spills in the United States involve removing the soil and treating the contaminated soil with chemicals or burning the soil to remove the oil. These processes are expensive and time consuming. Current bioremediation methods include using certain fertilizers to reduce the oil within soils. By researching bioremediation methods used in water-based spills, the idea was formed to alter these methods for use on land-based spills. The method used was to harness the digestive power of enzymes that are secreted by the mycelium of the higher fungi. Saprophytic fungi (or mushrooms) live on dead organic matter. The primary decomposers feed on the lignin of wood. Lignin is a long-chained hydrocarbon that is extremely recalcitrant, yet fungi have developed means to break lignin down into smaller chains through digestive enzyme action. Oil, being a fossil fuel, is made up of long-chained hydrocarbons and hard to decompose. Enter the mushroom. Preliminary results show a decrease in oil contamination of the two samples, leading to a reduction of 36-64% in ppm. This decrease occurred over a period of approximately one month. These results show a possible alternative biological method for bioremediation of oil spills.

OFF THE ELECTRICAL GRID

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Some Americans have already chosen to go off the grid and there are many more considering it. These project details calculations used to determine the amount of renewable energy that a 1000 sq. ft. home in Jamestown, ND would need to be off the grid. Initial electrical load calculations were done for the summer and winter months and it was determined that more than one energy source was needed. A combination of wind and solar was selected. The average wind speeds for the region were calculated for the summer and winter months and an appropriate wind turbine was selected. The formula used to determine a preliminary estimate of the turbine that is as

follows: AEO = Annual energy output, kilowatts per hour (kWh)/year or kWh month. D = rotor diameter, feet. V = annual wind speed for particular month. AEO = 0.01328 D^2 V^3. Then a PVWatts calculator was used along with the data from the solar panel selected to determine the energy output of the panel. These calculations were utilized to estimate the number of solar panels and number of turbines needed. After adding up the kWh per month that each of the systems (wind and sun) would produce, it was determined that this house would in fact be able to stay off the grid.

SOLAR AND WIND TURBINE POWER USE TO OVERCOME ENERGY NEEDS

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This project was completed to determine if the use of solar power in conjunction with power derived from wind turbines is a valid choice to a power a 1000 sq. ft. home of a family of four located in Devils Lake, North Dakota. Occupancy has a proportional effect on the amount of energy that the house will need. The aforementioned statement is based on the fact that calculated loads of the building are multiplied by the number hours each load is turned on resulting in Kilowatt Hours (KWH) of power needed. Our calculated deficiencies in energy were calculated at 4359 KWh for July and 3423 KWh for December which is equivalent $418.46 and $328.72 respectively; based on current cost per KWh in Devils Lake, ND. It was then determined how much energy could be derived from solar panels and wind turbines. Wind turbine performance was calculated using a formula in which the energy output is proportional to the rotor diameter squared multiplied by the average monthly wind speed cubed. A solar panel was selected and the array DC rating at STC was entered into the web-based PVWatts calculator, along with the proposed location of installation, to determine the energy output for all twelve months. The deficient energy needed due to the loads on the house with values gained through the use of our selected solar panels and wind turbines and were able to calculate that by using 20 solar panels and 4 wind mills the house would have sufficient energy available.

THE UNIQUE N- AND C-TERMINAL DOMAINS OF METALLOTHIONEIN-3 INFLUENCE THE EXPRESSION OF GAGE ANTIGENS 12H, 12G, 12C, and 2C IN MCF7 CELLS

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Toxic insult from the heavy metal cadmium is known to induce the expression of metallothioneins (MT) which are cysteine-rich heavy metal binding proteins six to seven kilodaltons in size. Previous research demonstrates that over-expression of MT-3 occurs in the majority of breast cancers and is associated with poor patient outcome. Furthermore, MT-3 has been shown to inhibit the growth of breast cancer and prostate cancer cell lines. Studies have shown that the MT-3 protein contains 7 additional amino acids that are not present in any other member of the MT gene family, a 6 amino acid C-terminal sequence and a Thr in the N-terminal region. The unique N-terminal sequence is responsible for the growth inhibitory activity of MT-3 in the neuronal system, while the function of C-terminal region remains unknown. Previous

microarray analysis of the MCF7 mutants, performed in our laboratory, indicated that several GAGE antigens were being differentially regulated by the N- or C-terminal of MT-3. Several GAGE family antigens were not present on the microarray but were investigated in this study. These included: GAGE12H, GAGE12G, GAGE12J, and GAGE12I. Real time PCR indicated that the expression of the GAGE antigens was upregulated in the absence of the N-terminal of MT-3 and downregulated if the N-terminal was present. In conclusion, this study further characterizes the unique properties of the N- and C-terminal domain of MT-3 and the potential role that it may play in the differentiation of certain breast cancers.

INCREASING TRIBAL COLLEGE STUDENT SUCCESS THROUGH INNOVATIVE MATH COURSE DELIVERY

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The purpose of the evaluation of the ICE-TI project is to learn whether the project increases the overall capacity of mathematics instruction and learning through newly structured course delivery to improve STEM capacity and increase the number of students participating. To learn the impact of this project and the modifications to the mathematics curriculum, students in all Cankdeska Cikana Community College Math classes completed pre and post surveys. The study is a staggered prospective multiple-cohort design (Fienberg and Mason, 1985), with new students entering the program each semester. Cross sectional surveys were taken at the beginning and end of each semester cross and employ a mixed method design, including 1) electronic pre and post-experience surveys with quantitative and qualitative components, 2) student and faculty interviews (individual and group), 3) review of course progress, and 4) student academic progress and success. Student characteristics include: 1) Students' overall motivation to participate in science, technology, engineering, and mathematics (STEM) coursework, measured by the Motivated Strategies for Learning Questionnaire (MSLQ), and its subscales (Task Value, Control Beliefs about Learning, and Self-Efficacy for Learning and Performance), 2) Openness to new ideas (FIRNI), and the Career Decision Self-Efficacy Scale (CDSE). Student demographic and contact information were collected to enable longitudinal tracking. The ICE-TI pre and post survey collected both qualitative and quantitative data to measure student, family, and community factors identified through the literature and our previous research that influence academic and career choices.

INVESTIGATING POLLINATORS ON THE FORT BERTHOLD INDIAN RESERVATION

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For Native Americans, the Juneberry plant has played a significant role due to its nutritional value and medicinal purposes. Its cultural importance, along with nutrition and medical purposes, has made the Juneberry plant the main study organism at the Nueta HiAdatsa Sahnish College under Dr. Kerry Hartman. This culturally significant plant is slowly disappearing. To further understand the native pollinators role in the vitality of the Juneberry plant population,

five sampling sites on the Fort Berthold Indian Reservation were used to collect multiple species of bees. Various species of bees were collected at the sites by using blue and vanes, and blue, yellow, and white bowls. The study is attempting to understand and learn more about local native pollinators and establishing baseline data. Results of the study have shown at least five species of Bumbus being the major pollinator that is pollinating in western North Dakota.

COMPARISON OF YIELD OF VARIOUS CULTIVARS OF *AMELANCHIER* (JUNEBERRIES) ON FORT BERTHOLD INDIAN RESERVATION

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For centuries tribal people of the Northern Great Plains have utilized plants for cultural, medicinal, structural, and nutritional uses. Juneberry (*Amelanchier*) historically played an important part in the diet and culture of the Mandan, Hidatsa, and Arikara Tribal Nations. Juneberries have polyphenol antioxidants and there is recent scientific validation to the historical use of the berries, twigs and roots of the Amelanchier genus trees by Native Americans for treatment of a diverse assortment of medical conditions (Conti 2006). Restoration of the Juneberry plant on the Fort Berthold Indian Reservation will lead to rejuvenation of cultural, nutritional, and economic uses of the plant. One important aspect of the restoration will be the understanding the different variables that impact yield. For my study I collected samples from both wild Juneberry plots and cultivated orchards to compare the yields. My results showed significant difference between the wild Juneberry yield and the cultivated Juneberry yield. One possible hypothesis for the difference in yields is the impact of numerous variables related to fruit yield.

AN INVESTIGATION INTO THE PRESENCE OF MERCURY IN LAKE SAKAKAWEA

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Methyl mercury is a powerful neurotoxin and people exposed to high levels may experience adverse effects. Humans are usually exposed to it 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 collected fish samples from two different sights (4 Bears Bay vs. Indian Hills Bay) in Lake Sakakawea to test their methyl 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 (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 Lake Sakakawea has abnormally high levels of mercury in walleye meat. These results will be used by Three Affiliated Tribes Game and Fish for further study and possible walleye consumption guidelines.

PARTICULATE MATTER EXPOSURE ALONG DESIGNATED TRAFFIC ROUTES ON THE FORT BERTHOLD RESERVATION, NORTH DAKOTA

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Increased road traffic related to oil development on the Fort Berthold Reservation in western North Dakota has resulted in increased road dust generated along the many unpaved roads. There is a concern among residents regarding whether fine particulate matter (PM) in this "fugitive dust" results in adverse health effects. The goal of this research is to quantify the amount, particle-size distribution, and mineral composition of dust being generated along highly traveled gravel roads in the Reservation that are impacted by petroleum development. MiniVol™ TAS portable samplers (Air metrics, Springfield, OR, USA) have been deployed to collect dust samples. Dust will be quantified as particulate concentration in terms of $\mu g/m^3$ of air, for particles of size less than 2.5 µm and 10 µm ($PM_{2.5}$ and PM_{10}, respectively). Scanning electron microscopy (SEM), X-ray Diffraction (XRD) and chemical analysis will be used to characterize and identify the mineralogical, biological and chemical components of the dust samples.

UNDERSTANDING THE SYNTHESIS PROCEDURE FOR A MODEL COMPOUND POLYMER WITH PHOTO-DEGRADATION ABILITY MADE FROM FDCA AND A NITRO-PHOTOTRIGGER

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Polymers are chemicals that define our modern society. They are macromolecules built from smaller units called monomers. Polymers can be classified as natural or synthetic, and have a broad range of desirable properties. Bio-based polymers derived from sustainable agricultural plant materials are being tested as replacements for synthetic polymers. They have the advantage 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. Bio-based polymers can undergo degradation facilitated by the input of radiation energy. This programmed photo-degradation process requires the use of a suitable photo-trigger to induce the breakdown. We designed, synthesized, and tested the photo-degradation ability of a model polymer made from plant based material. The model compound polymer was made from *2,5-Furandicarboxylic acid* (FDCA), a fructose derived biopolymer, and *2-nitro-1,3-Benzenedimethanol 6*, a unique nitro-phototrigger. FDCA was successfully bonded with the nitro-phototrigger molecule to form the model polymer and its ability to photodegrade was tested. Model compound was placed in tetrahydrofuran (THF) -H_2O solution in a 4:1 ratio. It was then irradiated and an NMR study confirmed degradation of the compound to form the original monomers. The results of the test show the polymer was successfully broken to it monomer components and confirms polymer degradation using radiation source. This has

important implications for the ability of polymers such as plastics to degrade and reduce their pollution potential and impact in the environment.

GENETIC VARIANTS (RS7216389, RS1558641, RS7216389) ARE NOT ASSOCIATEDWITH 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 American Indian children. This variant in the RAD50 gene was associated with asthma in a case/control study of asthma among the Han Chinese. **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=216), matched for age, were identified. Salivary DNA was genotyped for rs6871536 rs1558641, rs7216389, a single nucleotide polymorphism (SNP) by TaqMan (ThermoFisher Scientific) assay. Appropriate Student's t test, chi-square statistics and logistic regression methods were employed for analysis. Additive, dominant and recessive genetic models were considered. **RESULTS**: Hardy-Weinberg equilibrium was satisfied for both case and control groups. No significant difference in allelic frequency was found between cases and controls. Similarly, no significant effect of rs6871536, rs1558641, rs7216389 on risk of pre-eclampsia was detected for any genetic model, using multivariate logistic regression modeling, with simultaneous adjustment for age and body mass index (BMI). BMI shows a positive, independent and significant association with asthma in this cohort. **CONCLUSION**: As found in other populations, BMI is associated with asthma American Indian children; but this genetic variant does not seem to be associated with asthma in this community.

RISK OF PRE-ECLAMPSIA IN AN AMERICAN INDIAN POPULATION IS NOT ASSOCIATED WITH A VARIANT OF THE C-REACTIVE PROTEIN GENE (RS2808628)

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Objective: The cause of pre-eclampsia (PE) is unknown; but it is known that normal pregnancy represents a challenge to the maternal immune system. Genetic changes coding for a component of the innate immune system, C-reactive protein (CRP), are associated with preeclampsia. Our goal was to investigate the effects of additional CRP variants. **Methods:** There were 132 cases of PE and 253 matched controls from an American Indian population that participated in the study. An allele specific, real-time PCR method (Applied Biosystems "Taqman" assay) was used to genotype the CRP gene. Conditional logistic regression was used to analyze the potential association of CRP rs2808628 with preeclampsia. **Results:** The minor allele frequency was 44.7 % (95% CI 40.3 – 49.1%); and there was no significant deviation from Hardy-Weinberg equilibrium. We found no significant association between CRP rs2808628 and PE, using either

univariate or multivariate analysis of dominant, recessive or additive genetic models. There was a significant association between preeclampsia and nulliparity and body mass index (BMI) with an Odds Ratio (OR) of 2.91 (95% CI 1.86-4.52) p<0.001 and OR 1.05 (95% CI 1.02-1.09) p<0.001 respectively. **Conclusion:** The CRP SNP rs2808628, is in the 3' flanking region, approximately 6 Kb from the CRP gene and does not appear to be associated with PE in this American Indian cohort. This variant is associated with functional effects on CRP concentration and cortisol production in humans.

RISK OF PRE-ECLAMPSIA IN AN AMERICAN INDIAN POPULATION IS NOT ASSOCIATED WITH A VARIANTOF THE C-REACTIVE PROTEIN GENE (RS2794520)

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Objective: The cause of pre-eclampsia (PE) is unknown; but it is known that normal pregnancy represents a challenge to the maternal immune system. Genetic changes coding for a component of the innate immune system, C-reactive protein (CRP), are associated with preeclampsia. Our goal was to investigate the effects of additional CRP variants. **Methods:** There were 136 cases of PE and 256 matched controls from an American Indian population that participated in the study. An allele specific, real-time PCR method (Applied Biosystems "Taqman" assay) was used to genotype the *CRP gene*. Conditional logistic regression was used to analyze the potential association of *CRP* rs2794520 with preeclampsia. **Results:** The minor allele frequency was 44.1 % (95% CI 39.7 – 48.4%); and there was no significant deviation from Hardy-Weinberg equilibrium. We found no significant association between *CRP* rs2794520 and PE, using either univariate or multivariate analysis of dominant, recessive or additive genetic models. There was a significant association between preeclampsia and nulliparity and body mass index (BMI) with an Odds Ratio (OR) of 2.91 (95% CI 1.88-4.5) p<0.001 and OR 1.05 (95% CI 1.02-1.08) p<0.001 respectively. **Conclusion:** The *CRP* SNP rs2794520, is in the 3' flanking region, approximately 3 Kb from the *CRP* gene and does not appear to be associated with PE in this American Indian cohort. This variant is associated with functional effects on CRP concentration and recurrent pregnancy loss in humans.

THE EFFECTS AND ROLE OF SLC12A1 AND CASR ON THE KIDNEY

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Introduction: We measured the mRNA expression levels of SLC12A1 and CaSR in human kidney isolated and cell lines, confirming microarray results. These genes serve as important transporters that help to regulate electrolyte absorption and secretion in kidney filtrate therefore playing a vital role in the physiological properties of the kidney. When these genes are repressed, detrimental effects occur in the kidney which will serve as the main focus of this project. **Methods**: Gene expression was assessed by real time RT-qPCR. Real-time PCR was performed utilizing SYBR Green (Bio-Rad) technology with 2 μL (10 ng) of cDNA and 2 μL (0.2 μM) of

gene specific primers in a total volume of 20 μL. **Results**: In my results both genes were highly expressed in TERT & HPT1-6. SLC12A1 expression was also induced in HK2(MT3) cells. CaSR expression was repressed in HK2 and HK2(MT3) cells. **Conclusion**: This study suggests that HK-2 cells will not serve as a good model system to study solute transport whereas the TERT cell line would be the ideal model. The differences seen in the various kidney cell lines and primary cultures could be explained by the cell's ability to maintain in vivo properties in vitro studies. The variation in expression levels could serve as a reflection of the variation in expression level in the human population.

SPARC IN A CELL CULTURE MODEL OF HEAVY METAL INDUCED BLADDER TRANSITIONAL CELL CARCINOMA

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This study focuses on the matrix associated protein SPARC in a cell culture system that models bladder cancer due to environmental exposure of the heavy metals arsenic and cadmium. Previous results from our laboratory have shown that SPARC expression is significantly downregulated in all of the heavy metal malignantly transformed cell lines compared to the control cell line. Previous studies have shown that SPARC interacts with collagen affecting downstream cellular signaling pathways including cell adhesion, proliferation, and survival. Therefore, SPARC's role in cellular attachment and spreading of the transformed cell lines compared to the control cell line in our model system was assessed. Cells were seeded on a collagen matrix and cell attachment and spreading was observed at specified time points. The cells were methanol fixed and imaged using phase-contrast microscopy and images were analyzed using Fiji and LASX software from NIH and Leica, respectively. Results show that cellular attachment is inhibited or deterred in cells expressing SPARC compared to malignantly transformed cells that do not express SPARC. Subsequently, results show increased spreading of cells that express SPARC when compared to malignantly transformed cells that do not express SPARC. These results suggest that SPARC is binding to collagen effectively inhibiting or deterring cells from attaching to the collagen. Ultimately, SPARC expression could hinder bladder tumor cells from initial seeding into the connective tissue and/or metastasizing to a distant location by impeding cellular interactions with collagen.

A_{1A}-ADRENERGIC RECEPTOR ACTIVITY DECREASES SEIZURE LIKE EVENT FREQUENCY IN THE HIPPOCAMPAL CA3 REGION

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This study aims to increase our understanding of the antiepileptic effects of norepinephrine. We hypothesized that α_{1A}-Adrenergic Receptor (α_{1A}-AR) activation would result in decreased

seizure-like event (SLE) frequency. Here, we used the magnesium deprivation seizure model in mouse hippocampal slices to investigate the effects that α_{1A}-ARs have on epileptiform activity. Electrophysiological local field potential recordings of spontaneous SLE frequency were measured in the *stratum pyramidale* of hippocampal CA3 region, induced by magnesium deprivation. Slices were then challenged with α_1-AR (cirazoline) agonists, with or without an α_{1A}-AR selective antagonist (5-methyl urapidil). Then, we performed dose-response curves using multiple classes of selective α_1-AR agonists to investigate the physiological mechanism(s) underlying the α_{1A}-AR antiepileptic effect. Our results suggest that α_{1A}-AR activation leads to a significant decrease in SLE frequency (~30-50 %), while receptor blockade abolishes reductions in SLE frequency. Also, dose-response curves suggest that catecholamines, phenethylamines, and imidazolines may have similar efficacies for α_{1A}-AR-mediated SLE reduction, but large differences in potency (epinephrine > phenylephrine >> cirazoline). These experiments may result in new therapies for epilepsy, as well as increase our fundamental understanding of brain norepinephrine.

PROLONG HYPERGLYCEMIC EXPOSURES INDUCE PATHOLOGICAL CHANGES IN HUMAN PROXIMAL TUBULE CELLS

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Renal fibrosis is a major consequence of the diabetic nephropathy (DN) disease progression to end-stage renal disease (ESRD), where prolong exposure to hyperglycemia induces damage to proximal tubule (PT) cells of the kidney. Since progression to ESRD correlates to pathological changes in the tubular segments of the kidney, the effects of hyperglycemia in the PT portion of the nephron may be particularly relevant to the progression of DN. Epithelial to mesenchymal transition (EMT) of PT cells may play a role in the disease manifestation and progression. The goal of this study was to characterize the pathological changes that occur in human proximal tubule (HPT) cells exposed to hyperglycemia. For this purpose, cells were exposed to one of the following treatments; 5.5 (control), 7.5, 11, or 16 mM glucose concentrations for 8 days then consecutively subcultured for two more passages. Real-time PCR and western blot analysis was used to measure the expression levels of CDH2 and CDH1 at the mRNA and protein levels, respectively. Exposures to hyperglycemia induced morphological changes and stimulated a significant induction of CDH2 in HPT cells; however, exposure had no effect on CDH1 expression. Hyperglycemia also induced the expression of SNAI1 at the mRNA level. The data from the current study suggests hyperglycemic induced damage of the PT in the kidney during the development of DN occurs through an EMT phenomenon.

CHARACTERIZATION OF SUBCUTANEOUS HETEROTRANSPLANT TUMORS AND SPHEROIDS GENERATED FROM MALIGNANTLY TRANSFORMED UROtsa BLADDER CANCER CELLS

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Introduction: Arsenite (As^{+3}) and cadmium (Cd^{+2}) are known human carcinogens that have been linked to the development of bladder cancer. Human exposure to these heavy metals occurs primarily through cigarette smoking, agricultural products, and contaminated water sources. Our laboratory has shown that the exposure of UROtsa cells to either As^{+3} or Cd^{+2} can cause malignant transformation of UROtsa bladder cancer cells grown in culture. Recent studies have shown that muscle-invasive bladder cancer can be characterized into two subtypes - basal and luminal - based on the expression specific biomarkers. The goal of this study was to characterize tumors and urospheres generated from As^{+3}- and Cd^{+2}- transformed cell lines as basal or luminal subtypes. **Methodology:** Performed real time PCR to determine the expression of 8 luminal bladder cancers markers CYP2J2, ERBB3, FGFR3, FOXA1, GATA3, GPX2, KRT8, KRT19, and PPARG in subcutaneous heterotransplant tumors and spheroids derived from As^{+3}- and Cd^{+2}- transformed UROtsa cells. **Results:** The study shows that the majority of transformed UROtsa isolates showed a preponderance of expressed basal markers albeit with the expression of some luminal markers. **Conclusions:** Preliminary data shows that the subcutaneous transplant tumors and urospheres derived from As^{+3}- and Cd^{+2}- transformed UROtsa cells exhibit more basal-like characteristic features based on gene expression patterns of known basal and luminal biomarkers. **Significance:** The characterization of the UROtsa transformed isolates into basal-like and luminal-like bladder cancer isolates will enhance the applicability of this *in-vitro*, toxicant-specific carcinogenesis model to specific types of human bladder cancers.

FOOD ALLERGEN-INDUCED BEHAVIORAL ABNORMALITY IS CORRELATED WITH MAST CELL ACCUMULATIONAND GLIAL CELL ACTIVATION IN THE MURINE CENTRAL NERVOUS SYSTEM

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Despite growing scientific inquiry, the mechanisms by which food allergy triggers behavioral disorders such as depression, anxiety, attention deficit disorder, and autism, are not well understood. We hypothesized that mast cells (MCs) would serve as peripheral inflammatory mediators in response to food allergens by migrating to, and causing dysregulation of the central nervous system. A mouse model of milk allergy was generated by sensitizing mice to whey protein (WP). One-month and Ten-month-old male and female C57BL/6 mice were subjected to five-week WP sensitization followed by a WP challenge. Changes in digging behavior, brain and ileum MC numbers, and glia cell morphology in WP-sensitized mice were compared with age and gender-matched sham control mice. WP-sensitized male mice showed significantly lower digging activity compared to male sham. Additionally, WP-sensitization increased the number of

metachromatically identified MCs, particularly in the subarachnoid space between the midbrain and hippocampus in young male mice. Ileum MC numbers were increased in old WP-sensitized male mice compared to male sham, and no change in metachromatically stained MCs was seen in either gender of young mice. Additionally, phenotypic changes in astrocytes were observed in select regions of the WP-sensitized brain in old mice. Food hypersensitivity altered behavior in male mice in both age groups. Changes in brain and gut MC numbers and glial morphology that are age and gender-dependent were observed. Our results confirmed that food allergy induced behavioral abnormality and cellular alterations in the brain, suggesting that undetected food allergy may be an underlying cause of psychosocial disorders.

BLADDER CANCER SUBTYPE CHARACTERIZATION IN SPHEROIDS AND TUMORS GENERATED FROM AS+3AND CD+2 MALIGNANTLY TRANSFORMED UROTSA CELL ISOLATES

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Bladder cancer can be described as being 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 and luminal – based on the expression of specific biomarkers. With arsenic (As+3) and cadmium (Cd+2) being known carcinogens involved in the development of bladder cancer, the goal of this study was to characterize spheroids and subcutaneous heterotransplant tumors generated from (As+3) and (Cd+2) malignantly transformed UROtsa cell isolates into these subtypes.

METALLOTHIONEIN-3 PROTEIN INTERACTIONS PROMOTE VECTORIAL ACTIVE TRANSPORT IN HUMAN PROXIMAL TUBULE CELLS

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Metallothionein 3 (MT-3) is a small, cysteine-rich protein that binds to essential metal ions required for homeostasis, as well as to heavy metals that have the potential to exert toxic effects on cells. MT-3 is expressed by epithelial cells of the human kidney, including the cells of the proximal tubule. This laboratory has previously shown that mortal cultures of human proximal tubule (HPT) cells express MT-3 and form domes in the cell monolayer, a morphological feature indicative of vectorial active transport, an essential function of the proximal tubule. However, an immortalized proximal tubular cell line HK-2 lacks the expression of MT-3 and fails to form domes in the monolayer. Transfection of HK-2 cells with the MT-3 gene restores dome formation in these cells suggesting that MT-3 is required for vectorial active transport. In order to determine how MT-3 imparts this essential feature to the proximal tubule we sought to identify proteins that interact either directly or indirectly with MT-3 *in vitro*. Using a combination of pulldowns, co-

immunoprecipitations, and mass spectrometry analysis putative protein interactants were identified and subsequently confirmed by western blotting and confocal microscopy. Here we show that MT-3 interacts with myosin, aldolase a, enolase-1, beta-actin, and tropomyosin and that these interactions occur at the periphery of the apical membrane of doming proximal tubule cells. Together these observations reveal that MT-3 interacts with proteins that are involved in cytoskeletal organization, and that these interactions at the apical membrane promote vectorial active transport and cell differentiation in proximal tubule cultures.

AS^{+3}- AND CD^{+2}- 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 (As^{+3}) and cadmium (Cd^{+2}) 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 As^{+3}- and Cd^{+2}- 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 As^{+3}- and Cd^{+2}- transformed isolates. Initial gene expression data of all the basal and luminal markers shows that the subcutaneous transplant tumors and spheroids derived from As+3- and Cd+2- 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.

AN *IN-VITRO* HUMAN PROXIMAL TUBULE MODEL TO STUDY TOXIC EFFECTS OF CADMIUM

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The proximal tubules of the kidney are target sites of injury by various toxicants. Cadmium (Cd^{2+}), an environmental nephrotoxicant can cause adverse effects and overt renal damage. To decipher the mechanism involved in nephrotoxicity, an *in vitro* model system is required. Mortal cultures of human proximal tubule (HPT) cells have served, as models but are difficult to acquire and do

not lend themselves to stable transfection. The immortalized human proximal tubule cell line HK-2, has served as a model but it lacks vectorial active transport and shows signs of lost epithelial features. Recently a new proximal tubule cell line was developed, the RPTEC/TERT1, and the goal of this study was to determine if this cell line could serve as a model to study nephrotoxicity. Global gene expression analysis of this cell line in comparison to the HK-2 and HPT cells showed that the RPTEC/TERT1 cells had gene expression patterns similar to HPT cells when compared to the HK-2 cells. The HPT and the RPTEC/TERT1 cell line had an increased population of stem/progenitor cells co-expressing CD24 and CD133 when compared to the HK-2 cells. The level of expression of cadherins, claudins and occludin molecules was also similar between the RPTEC/TERT1 and the HPT cells. Acute exposure to Cd2+ resulted in necrosis of the RPTEC/TERT1 cells when compared to the HK-2 cells which died by apoptosis. Thus, the RPTEC/TERT1 cells are similar to HPT cells and can serve as a good model system to study mechanisms involved in toxicant induced renal damage.

CHARACTERIZATION OF BRAIN TRANSCRIPTOMIC AND EPIGENOMIC PROFILES IN THE MOUSE MODEL OF MILK ALLERGY

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Allergies have been demonstrated in human studies to be comorbid with neuropsychiatric disorders such as ADHD, and anxiety. Although peripheral immune responses are implicated in the behavioral outcome, other factors that are associated with neurotransmissions and synaptic restructuring may also contribute to dysregulation of neuronal functions and therefore need to be assessed for their involvement. Our study aims to profile the effects of cow's milk allergy on the transcriptome and epigenome of the mouse brain. Male and female 4-week-old C57BL/6J were orally sensitized with either vehicle (sham) or β-Lactoglobulin (BLG) for 5 weeks using cholera toxin as an adjuvant. At week 6, the mice were challenged with a higher dose of BLG and sacrificed the following day to harvest the brain and serum. BLG-specific immunoglobulin E (IgE) concentration in the serum was evaluated with ELISA. The brain was dissected into the striatum, hippocampus, thalamus, and mid brain regions, and RNA and DNA were isolated from each of the 4 regions for the sequencing of mRNA and immunoprecipitation-enriched methylated DNA, respectively. BLG-sensitized mice demonstrated an increase in serum IgE. We are in the process of generating data to establish transcriptomic and epigenomic differences between sham and BLG-sensitized mice. The increased BLG-specific serum IgE levels in the BLG-sensitized mice confirmed their allergic response to BLG, validating the experimental model. Profiling food-allergen induced transcriptomic and epigenomic modification in the brain will facilitate better understanding of the multi-system interactions that are triggered by food allergies, and may provide novel therapeutic targets and/or diagnostic markers.

ANTIGEN CHALLENGE ALTERS INNATE BEHAVIOR AND EXPRESSION OF BRAIN HISTAMINERGIC AUTORECEPTOR IN MOUSE MODEL OF MILK ALLERGY

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A growing number of studies have validated a causative role of food allergies in aggravation of neuropsychiatric symptoms, although the underlying mechanism has yet to be elucidated. Based on their "first responder" role in allergic and other inflammatory events occurring peripherally and centrally, migratory and/or resident mast cells and their secretory mediators, particularly histamine, are likely to be involved. Since histamine is also a major neurotransmitter, we postulated that mast cells could, upon allergen challenge, increase brain histamine levels resulting in central histaminergic dysregulation and ultimately behavioral manifestations. We therefore hypothesized that increased histamine levels would augment the expression of its autoreceptor, histamine H3 receptor (H3R), via a negative feedback mechanism. To test this hypothesis, we utilized a mouse model of whey protein (WP) allergy and assessed behavior and H3R expression following an oral challenge with WP. Digging and object burying behaviors intrinsic to mice were significantly decreased in WP-sensitized male mice but not in females. These behavioral changes were accompanied by apparent increases in H3R-immunoreactivity, particularly in brain regions important for limbic and cognitive functions. Transcriptional analyses with quantitative reverse-transcriptase polymerase chain reactions (RT-qPCR) further revealed that transcription of histamine receptor subtypes were differentially regulated in brain regions that receive histaminergic input. These results indicated that allergic reaction, and possibly increased histamine, altered innate behavior and H3R expression in mice. With more concrete evidence, avoidance of offensive food in susceptible individuals could provide a preventative approach to the treatment of behavioral disorders rather than palliative therapy with behavior-modifying medications.

TRANSCRIPTION FACTOR OSR1 IS AN ESSENTIAL REGULATOR OF CARDIAC PROGENITOR DIFFERENTIATION

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Cardiac cell lineage specification is mediated by transcription factors (TFs). Null mutation of Osr1 causes cardiac defects in the mouse embryo, as demonstrated by absence of septum primum, venous valve, and dilated atria at embryonic day (E) 11.5 and death at E12.0. Previous studies show that Osr1-expressing cells contribute to atrial septum progenitors between E8.0 and E11.0 and that Osr1 interacts with TF Tbx5 to regulate posterior second heart field (SHF) cell cycle progression. In this study, we investigate the downstream targets of Osr1 by examining its occupancy in the genome and the transcriptomic profiles of sorted Osr1-expressing cells. RNA-

seq was performed on E9.5 $Osr1^{-/-}$ and wildtype embryos to investigate Osr1-dependent gene expression in the first heart field, anterior and posterior SHF. Candidate target genes were validated using qPCR and promoter occupancy was investigated using Osr1 ChIP-qPCR. Osr1-expressing cells in the posterior SHF were detected by flow cytometry and ready to be isolated using fluorescence-activated cell sorting for downstream analysis. Smo and Disp1, receptors in the Sonic Hedgehog pathway, were found with decreased expression in the posterior SHF of $Osr1^{-/-}$ embryos. OSR1 binds to the promoter of Smo and Disp1, with binding strength inversely correlated to the distance between binding region and transcription start site. In conclusion, Osr1 is a regulator of Shh pathway for posterior SHF differentiation. This study provides information on the mechanism of heart development. The findings are of great value to the prognosis and prevention of congenital heart defects.

A COMPARISON OF ANTIMICROBIAL ACTIVITIES OF CULTIVATED VERSUS WILD *ECHINACEA ANGUSTIFOLIA* (PURPLE CONEFLOWER)

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Infectious diseases caused by bacteria, fungi, viruses and parasites are a major threat to public health, despite the tremendous progress in human medicine. Their impact is particularly large in developing countries due to the relative unavailability of medicines and the emergence of widespread drug resistance. Medicinal plants have been used for centuries to treat various diseases all over the world, but only a small percent of traditionally prescribed plant species on the earth have been studied for their therapeutic value. *Echinacea angustifolia* preparations have become the bestselling herbal immune-stimulant in Europe and North America. Wild Echinacea was collected and cultivated Echinacea was purchased from a commercial supplier for this experiment. A well diffusion assay test was used to compare the antimicrobial activities of the types of plants against five bio level one bacteria. The hypothesis that cultivated plants would show more antimicrobial activity than wild plants was not supported. There was not a significant difference between wild and cultivated *E. angustifolia* antimicrobial activity (P-value = 0.2746). Harvesting the plant using either process of collecting wild or processing your own cultivated plants would produce similar antimicrobial benefits.