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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. 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. 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: GAGE2A, GAGE10, GAGE13, and GAGE2C. Real time PCR indicated that GAGE2A, GAGE10, GAGE13, and GAGE2C were not expressed in any of the MCF7 cell lines. In conclusion, this study indicates that a subset of GAGE antigens is not expressed in the presence or absence of the N- or C-terminal of MT-3.

THE UNIQUE N-AND C-TERMINAL DOMAINS OF METALLOTHIONEIN-3 INFLUENCE THE EXPRESSION OF GAGE ANTIGENS 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: GAGE12D, GAGE1, GAGE12C, and GAGE12J. Real time PCR indicated that none of those GAGE antigens were expressed in any of the MCF7 cell lines. 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.

SPIRIT LAKE NATION COMPREHENSIVE COMMUNITY ASSESSMENT 2015

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The purpose of the Comprehensive Community Assessment was to identify community health and wellness needs (social, health, mental health, substance use, employment, housing, and education) and to provide support for health, educational, employment, and other program development and implementation. The cross-sectional survey, conducted May through July 2015, was guided by three research questions: 1) What is the status of health (including behavioral health), wellness, and life satisfaction of Spirit Lake Nation tribal members? 2) What factors (education and child care; economic issues; housing; childhood safety; individual behaviors; access to health care, transportation, and communications) influence health (including behavioral health), wellness, and life satisfaction of Spirit Lake Nation tribal members? 3) What are the most important issues at Spirit Lake Nation? The sample included 285 people representing their household. Their average age was 40, ranging from 16 to 89; 70% were female. The level of general health of tribal members was lower than state and national levels; the number of poor health days was higher than state and national rates. Life satisfaction was rated highly. Rates of factors influencing level of health, such as early childhood trauma and levels of smoking, were higher than state and national levels. People completing the survey identified child safety, housing, employment, behavioral health, and chronic disease (especially diabetes) as the most critical health needs.

CROSS-CULTURAL COMMUNICATION TRAINING IN THE EMERGENCY DEPARTMENT

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PURPOSE: American Indian (AI) children have one of the highest rates of emergency department (ED) use and face potential racial discrimination in health care settings. Our goal was to develop and assess a cross-cultural communication training program. **METHODS:** Classroom based and shadowing training modules were implemented for care providers in the ED, but primarily involved nurses. We performed a pre-post survey of parents visiting the ED for their child. Responses were based on the Communication Assessment Tool for Teams (CAT-T) with separate scores for nurses and physicians/advanced care providers (ACP) and the front-desk staff. Multivariate logistic regression was used to assess pre-post differences in high rating scores for nurses and physician/ACP separately. **RESULTS:** A total of 120 and 73 parents responded to the questionnaire, pre and post intervention respectively. After adjusting for child race and age, significant differences were found with an increase in visits with nurses highly rated for communication skills (Odds Ratio = 2.14, 95% Confidence Interval (1.16-3.94), p = 0.02). Subgroup analysis suggested the largest improvement in families with an American Indian child, but this difference was not statistically significant. No pre-post differences were found for physicians/ACP or front desk staff. **CONCLUSIONS:** Comparisons between before and after the intervention showed an increase in highly rated scores for nurses by parents. This training was feasible, well received by care providers, and shows promise of improving communication skills and parental satisfaction with care received.

HOME ASSESSMENT IN MATERNAL-CHILD DYADS FOLLOWING RESIDENTIAL TREATMENT

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While substance abuse is undesirable for all, for women who are pregnant and parenting there is a unique constellation of risk factors that impact the health of both the mother and their child (Niccols et al., 2012). Women in substance abuse treatment programs often report greater prevalence of psychopathology (Whitaker, Orzol, & Kahn, 2006) presenting barriers to providing stable and nurturing environments both for themselves and their children following their time in treatment (Conners-Burrow et al., 2013). Assessing mothers and their children post-treatment allows us to understand the needs of this population and provide programming focused on effective strategies to prepare mothers and their children for success. Examination of maternal-child outcomes following residential treatment, however, has often been overlooked. The current study examined the mother-child relationship and home environment of mothers and children post-treatment and further examined differences between post-treatment and non-treatment mother-child dyads. Nineteen mother-child dyads (60% American Indian (AI); 35% European American; 5% Asian American) participated. Mothers successfully completed a substance abuse residential treatment program that primarily serves American Indian (AI) pregnant and parenting mothers. Mothers and their child (youngest between 0-7 years) who successfully completed a residential treatment program completed a one-hour in-home assessment (HOME; Bradley & Caldwell, 20000) examining the mother-child relationship and the learning environment. Significant differences were found for HOME scores based on maternal education. Specifically, findings indicated that mother-child pairs with at least a high school degree scored significantly higher than mother-child pairs with mothers with less than a high school degree. This was found for the overall HOME score (t (19) = 2.66, p < .05), parental warmth (t (19) = 2.85, p <.05), and learning and literacy (t (19) = 2.61, p<.01) subscales. Preliminary findings support the need for treatment programs that provide mothers with educational opportunities (i.e., GED or HS degree) as maternal education was a significant factor in determining HOME assessment scores post-treatment. Further analyses will examine covariates including race, SES, and maternal age. Overall, in examining the mother-child dyad we can provide an opportunity to break an intergenerational cycle, working to better maternal outcomes and subsequently provide their children with the best possibility for success.

INNOVATIVE TOOLS AND APPROACHES FOR STREAMLINING THE RESEARCH REVIEW PROCESS FOR AMERICAN INDIAN TRIBAL IRBS

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Tribal Nations are increasingly establishing their own ethics review boards, which enable tribes to exercise their inherent sovereignty and have an active voice in research. These entities provide direct oversight for research conducted on tribal lands and ensure community protections, protection of tribal individuals, cultural practices and Tribal resources involved in research. The emergence of tribal nation's ethics review boards and IRBs has created the need for the development of tools and resources that ensure inclusion of tribal law and values in the research review process and educate tribal IRB members on

federal human subjects' protections regulations and IRB operations. This poster will describe the efforts of the Regulatory Knowledge Core within the Collaborative Research Center for American Indian Health and its' tribal partners to develop and evaluate tools for Tribal IRB member education, IRB administration, and review and management of protocols.

Results demonstrate that these innovative tools have been successful in streamlining the review process for tribal IRBs.

IMPLEMENTING DRAW THE LINE/RESPECT THE LINE IN GREAT PLAINS TRIBAL COMMUNITIES: COLLABORATING FOR PROGRAM SUSTAINABILITY

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Despite the progress made in teen pregnancy and STI prevention, there are still disparities in minority teen pregnancy and sexually transmitted infection (STI) rates throughout the United States. Vital records in the Great Plains area reveal teen birth rates are three times higher in counties that fall within the districts of tribal nations compared to overall state rates. In South Dakota, rates of chlamydia among American Indian populations are nearly 8 times higher, and rates of gonorrhea are 21 times higher, than the general population**.** To address these disparities, the "Draw the Line/Respect the Line" curriculum was implemented in school health classes through joint partnerships. These schools were located in three different tribal nations in South Dakota, Nebraska, and Iowa. A process and outcome evaluation has been conducted for more than three years to assess short- and long-term goals. Pre- and post-test assessments were positive overall. For example, student knowledge of STIs and contraception increased ($t=-5.41$ to -3.74, $p<.001$), as did their understanding [8th grade only] of "Draw the Line/Respect the Line" ($t=2.54$, $p<.05$). Students increased their knowledge about how to set and maintain their sexual limits and respect the limits of others. Evaluation data are particularly important for underserved communities to assure program quality, validity, and effectiveness for culturally specific community needs. This project underscores how strong partnerships can result in long-term program sustainability.

A RESEARCH EXPERIENCE FOR NATIVE AMERICAN UNDERGRADUATE STUDENTS: AN APPLICATION OF THE CIRCLE OF COURAGE MODEL

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The present study examines the application of the Circle of Courage model to a Summer Undergraduate Research Experience (SURE) for Native American students. SURE is a 10-week program which provides mentored research training to Native undergraduate students interested in pursuing careers in the health sciences and health disparities professions. SURE utilizes the Circle of Courage model of positive youth development which is based on the universal needs for belonging, mastery, independence, and generosity. Data was collected by means of focus groups, which were conducted in the summers of 2012–2014 with the interns on the last day of their participation in the program. The study sample included 20 participants (15 females, 5 males; mean age of 24.15 years). Focus groups were tape-recorded and transcribed verbatim and analyzed via content analysis in which themes emerged directly from the text. Findings

indicate that the program was successful in building mentee's capacities to conduct independent research while preparing them with greater skills and knowledge to develop their future career paths within a supportive, trusting environment. This model, rooted in indigenous child socialization philosophies and theories of human development, served as a fitting theory for conceptualizing optimal outcomes for Native mentees.

BISON HEALTH: INFECTIOUS DISEASE STATUS AND MANAGEMENT IMPLICATIONS ON A PASTURE ON STANDING ROCK SIOUX RESERVATION

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Bison are sacred animals to the Lakota/Dakota of North and South Dakota. They are keystone species on the Great Plains of North America. Their habitat role makes an understanding of their health ecology essential. Livestock diseases have a measurable impact that can inhibit production. Diseases impact livestock productivity by causing infertility, abortion and death. Endemic viral and bacterial pathogens will cause reproductive tract infections leading to infertility and abortions in bison. The goal was to characterize prevailing herd health parameters that influence herd production. Samples were collected over a 2 year period and the following parameters were determined: disease statuses for Bovine viral diarrhea virus, Leptospirosis, and bovine herpes virus-1. Calving percentage, nutrition, age, and calf crop were determined and correlated with disease status. A microscopic agglutination test to measure antibodies along with PCR assay as a definitive diagnosis were used to determine the status of these diseases. The calf crop was 39% and herd conception rate was 38%. The herd was found to be free of infections of bovine herpesvirus-1 as well as bovine viral diarrhea virus 1 and 2, since none of the animals tested had antibodies against these viral pathogens. The herd has been exposed to Leptospirosis Bratislava with a high significant antibody titer found in a two year-old heifer. Overall, herd health as related to disease status were very good and control measures against Leptospirosis need to be implemented. Identifying sound health and reproduction programs in combination with other programs are paramount to improving herd productivity.

THE USE OF A CULTURALLY RELEVANT WELLNESS CURRICULUM WITH NATIVE AMERICAN CHILDREN: A FEASIBILITY PILOT

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Effective mechanisms for creating culturally relevant wellness education for Native American (NA) youth are poorly understood in the literature. At the same time, researchers have suggested that NA youth engage deeper in school curriculum that is relevant to their culture; and NA youth are known to be at high risk for obesity and diabetes, suggesting that improving health knowledge could be valuable in this population. This investigation sought to create and pilot a culturally relevant, school-based nutrition and physical activity curriculum for NA elementary school children in South Dakota's Siouan tribes, which would have the potential to improve knowledge of these topics in this population. The inquiry began with a qualitative study to gain insight as to Siouan elder perspectives, and gather stories used to teach children about nutrition and physical activity. Elders are highly respected in NA communities and their participation was sought to lend credibility to the project. The elder insights and stories were then used to create a culturally relevant curriculum with wellness messaging embedded in lesson materials that were created to be appropriate to the Siouan tribal communities of South Dakota (SD). The curriculum development was a collaborative effort with SD State University Extension and utilized feedback from NA tribal members in the process. The resulting curriculum was piloted for feasibility in elementary

schools on two tribal reservations in SD. Outcome data from the feasibility study demonstrated that the curriculum is practical, culturally acceptable and was well received by both teachers and the elementary school children.

THE RELATIONSHIP BETWEEN SIX GENETIC VARIANTS AND RISK OF ASTHMA AMONG AMERICAN INDIAN CHILDREN

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PURPOSE: Asthma is recognized as a complex, multifactorial condition with a genetic component that is well recognized. While certain genetic variants have been found associated with asthma in a number of populations,[2] it is not known if these associations are seen in American Indian children with asthma. **METHODS**: The electronic medical records of a northern plains Indian Health Service facility identified all children between ages 6 and 17 with a clinical diagnosis of asthma (N=108). Detailed medical records were reviewed for case defining criteria. Control children (N=216), matched for age, were identified. Real-time polymerase chain reaction, TaqMan (Life Technologies) assays were used to genotype 6 single nucleotide polymorphisms (SNPs). Mean values were compared between cases and controls using Student's t test; and genotypic distributions by case/control status were evaluated by chi-square and logistic regression methods. **RESULTS**: Of three possibly modifying covariates, age, gender and body-mass index (BMI), only age and BMI were found to be independently associated with asthma (OR=0.92, $p<0.049$, OR=1.05, $p<0.006$). One SNP shows marginally significant association with asthma (OR=1.46, $p=0.057$), while an additional 58 samples remain to be genotyped. One other variant (rs4795405) also shows a trend toward association, although only about half of samples have been genotyped. **CONCLUSION**: As found in other populations[3], American Indian children appear to show an increased risk of asthma associated with obesity. Although many participants remain to be genotyped, we have been unable to replicate associations in this American Indian population between these six genetic variants and asthma.

 Wu H, Romieu I, Shi M, et al. (2010) J Allergy Clin Immunol. 125:321-327.
[2] Bonnelykke K, Sleiman P, et al. (2014) Nature Genetics. 46:51-55.
[3] Permaul P, Kanchongkittiphon W, Phipatanakul W. (2014) Ann Allergy Asthma Immunol. 113:244-6.

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

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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 103 cases of PE and 143 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 5.60 (95% CI 2.82-11.13) p<0.001 and OR 1.06 (95% CI 1.02-1.10) p<0.002 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.

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

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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 107 cases of PE and 146 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 5.60 (95% CI 2.82-11.13) p<0.001 and OR 1.06 (95% CI 1.02-1.10) p<0.002 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.

PRE-ECLAMPSIA AND RISK OF SUBSEQUENT HYPERTENSION: IN AN AMERICAN INDIAN POPULATION

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BACKGROUND AND OBJECTIVES: Preeclampsia (PE) is a pregnancy-specific disorder of unknown etiology. A number of proposed pathophysiologic mechanisms relate to similar factors implicated in cardiovascular disease. Pre-eclampsia has been associated with subsequent hypertension, cardiovascular disease and related mortality in later life. **METHODS:** Recent blood pressures, body mass index (BMI) and use of hypertensive medications were recorded from clinic visits of 130 PE cases and 289 normal pregnancies. Student's t test, chi-square testing, multivariate linear and logistic regression were used to evaluate differences between cases and controls, as well as the effects of risk factors on systolic and

diastolic blood pressure (SBP, DBP respectively) and the diagnosis of subsequent hypertension.

RESULTS: Results: Follow-up measurements occurred a mean of 13.11 years (range 3.6 to 36.7 years) post PE pregnancy. Multivariate linear regression showed significant and independent association between BMI (SBP β=0.47, DBP β=0.29), age (SBP β=0.19, DBP β=0.14), previous history of PE (SBP β=4.47, DBP β=2.71) and current systolic and diastolic blood pressure (all p values <0.002). Analysis of the quartile with follow-up of less than 7.19 years also shows independent association of prior PE with both SBP (B=5.72, p<0.033) and DBP (B=4.73, p<0.006). Multivariate logistic regression analysis found an odds ratio of 3.71, 95% CI 2.04 - 6.72, p=0.001 for subsequent hypertension.

DISCUSSION AND CONCLUSIONS: PE appears to confer risk of subsequent hypertension on American Indian women after a period of about 7 years. This risk is independent of additional risk factors such as increased BMI and age.

A GENETIC VARIANT (rs928413) IS NOT ASSOCIATED WITH ASTHMA AMONG AMERICAN INDIAN 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 rs928413, 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 rs928413 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.

EXPRESSION OF C-REACTIVE PROTEIN IS INCREASED IN PLACENTAL TISSUE OF WOMEN WITH PRE-ECLAMPSIA

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PURPOSE: The etiology of the pregnancy complication, pre-eclampsia (PE), is unknown; but multiple lines of evidence implicate immunologic factors as important contributors. C-reactive protein (CRP) is a prominent component of the innate immune system; and we previously reported inherited genetic variants that increase the risk of PE. Very recently the placenta has been found to express CRP and infusion of CRP into pregnant mice has recapitulated various features of PE. We sought to replicate some of these latter findings. **METHODS:** Placental tissue from 6 women with PE and 8 with normal pregnancies was obtained. RNA was extracted and cDNA produced using standard methods. Quantitative, real-time PCR using BioRad primers for human CRP and a standard "housekeeping" gene (GAPDH) was used to estimate placental expression of CRP (the Cq value) as a ratio of GAPDH expression (CRP/GAPDH). Each expression run measured SYBR fluorescence in triplicate and Cq values were determined against a standard curve derived from BioRad template standards of known concentration. Student's t test examined possible differences of mean CRP/GAPDH ratios. **RESULTS:** There were a total of 22 triplicate runs of CRP/GAPDH expression. Intra-run CV's averaged 1.51%. The mean (SD) CRP/GAPDH ratio was 1.03 (0.024) and 1.14 (0.060) for cases and controls respectively, $p < 0.001$. **CONCLUSION:** We show that CRP is expressed in placental tissue and that expression is greater relative to a standard reference gene among those with PE compared with normal pregnancies. Our findings are consistent with the single previous investigation of this question.

FIBRONECTIN AND ITS ALTERNATIVE SPLICE VARIANT EXPRESSION IN A CELL CULTURE MODEL OF BLADDER CANCER

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This study focuses on the extracellular matrix protein fibronectin and its expression in a bladder cancer cell culture system. Our system models bladder cancer due to environmental exposure of the heavy metals arsenic and cadmium. Previous results from our laboratory have shown that fibronectin expression is upregulated in several of the transformed cell lines compared to the control cell line. This study focuses on the levels of fibronectin and its splice variants in the tumor initiating cells, called urospheres, which are a subpopulation of each transformed cell line capable of generating a tumor. Results showed that fibronectin and its splice variants were significantly repressed in the urosphere sub-population of most of the malignantly transformed cell lines. We also found that all 13 transformed cell lines show similar expression of fibronectin splice variants. Fibronectin and its alternative splice variant expression levels were determined using real time RT-PCR. Fibronectin could potentially be targeted when a bladder tumor begins to focally express fibronectin to prevent tumor formation.

A$_{1A}$-ADRENERGIC RECEPTOR ACTIVITY AND EPILEPTIFORM EVENTS IN THE CA3 HIPPOCAMPAL REGION

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This study aims to increase our understanding of the antiepileptic effects of norepinephrine observed *in vivo*. We hypothesize that α_{1A}-AR activation results in a decrease in event frequency, while receptor blockade restores basal event frequencies. Here, we use the kainate-induced model of seizure-generation in mouse hippocampal slices to identify the effects of α_{1A}-adrenergic receptors (α_{1A}-ARs) on epileptiform activity. Electrophysiological field recordings of basal event frequency were measured in the *stratum pyramidale* of hippocampal area CA3, whereupon slices were challenged with a non-selective agonist (cirazoline) with or without a selective antagonist (5-methylurapidil) present to isolate α_{1A}-AR effects on epileptiform activity. Initial results suggest that α_{1A}-AR activation leads to a significant decrease in event frequency, while receptor blockade restores event occurrence to basal frequencies.

A GENETIC VARIANT (RS6871536) IS NOT ASSOCIATED WITH ASTHMA AMONG AMERICAN INDIAN 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, 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 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.

INVOLVEMENT OF HYPERGLYCEMIA OR CADMIUM EXPOSURE IN THE INDUCTION OF THE POLYOL PATHWAY AND MORPHOLOGICAL CHANGES IN HUMAN PROXIMAL TUBULE CELLS.

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Diabetic nephropathy (DN) is a major cause of end-stage renal disease (ESRD), where prolonged 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. Development of this disease also is likely to occur in the context of exposure to other renal toxins, and the heavy metal cadmium (Cd^{2+}) may be the most relevant due to the accumulation of this metal in the major cell type involved in glucose reabsorption: PT cells. Preliminary microarray analysis has shown human proximal tubule (HPT) cells exposed acutely and chronically to Cd^{2+} have an increased expression of an aldose reductase (AR) isoform, AKR1B10. This isoform along with AKR1B1 and sorbitol dehydrogenase (SORD) are involved in glucose metabolism under hyperglycemic conditions via the polyol pathway. The goal of this study was to verify and extend these observations in culture of HPT cells. For this purpose, HPT cells were exposed to one of the three following treatments; 5.5 (control), 7.5, 11, or 16 mM glucose concentrations for 8 days, 9, 27, 45 μM cadmium for 24 hours (acute), or 4.5, 9, 27 μM Cd^{2+} for 13 days (chronic). Real-time PCR was used to measure the expression level of these enzymes. Exposures to either hyperglycemia or Cd^{2+} stimulated a significant induction of AKR1B10 in HPT cells; however, exposure to these renal toxins had no effect on AKR1B1 or SORD expression. We also observed glucose-induced loss of epithelial morphology that correlated to an induction of N- Cadherin (CDH2), a mesenchymal marker. These results are suggestive of potential synergistic effects of Cd^{2+} and hyperglycemia in the toxic responses of the PT during the development of DN.

EXPRESSION OF VIMENTIN IN UROtsa PARENT, ARSENITE, AND CADMIUM TRANSFORMED CELL LINES AND UROSPHERES

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BACKGROUND: The heavy metals arsenite (As^{+3}) and cadmium (Cd^{+2}) are known carcinogens that cause bladder cancer. Our laboratory has shown an in vitro model of bladder cancer by exposing the immortalized bladder epithelial cell line UROtsa and urospheres to the heavy metals As^{+3} and Cd^{+2}. **PURPOSE:** The goal of this study is to determine the expression level of Vimentin (VIM) in the UROtsa parent cell line, the As^{+3} and the Cd^{+2} - transformed cell lines and the urospheres derived from the parent as well as the transformed cell lines. **METHODS:** Real time (RT-PCR) was used to determine the expression levels of VIM in the UROtsa parent cell line, the As^{+3} and Cd^{+2} - transformed UROtsa cell line and urospheres. **RESULTS:** Our results show that the expression of VIM was increased in some of the As^{+3} and Cd^{+2} transformed cell lines when compared to the UROtsa parent cell line. The expression level of VIM in the urospheres derived from the transformed cell lines was similar or decreased when compared to the urospheres derived from the UROtsa parent cell lines with the exception of a couple of isolates that had an increase in expression. When the level of expression was compared between the cell lines and the urospheres, the results showed that the urospheres had decreased expression of VIM when compared to the corresponding cell line. **CONCLUSIONS:** Our data shows that the urospheres that are

putative stem cells derived from the cell lines and are a marker of mesenchymal cells, have decreased expression of VIM when compared to the cell lines.

EXPLORING THE ROLES OF MT-3, GELSOLIN, AND DPYSL3 IN THE RENAL EPITHELIAL-TO-MESENCHYMAL TRANSITION

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Metallothioneins (MT) are essential proteins that sequester heavy metals such as cadmium, which is an environmental toxin. Chronic exposure to cadmium can result in renal fibrosis, which may occur by a process known as epithelial to mesenchymal transition (EMT). In the human kidney, the 3rd isoform of MT (MT-3) is expressed in the proximal tubules. The role of MT-3 in the kidney is not well defined. Previous studies form this laboratory have shown that the immortal human proximal tubular cell line HK-2 has a more mesenchymal phenotype, does not express MT-3, and cannot form domes when cultured in-vitro. Transfection of MT-3 into the HK-2 cells restores dome formation in these cells. This suggests that MT-3 can cause the reversal of a mesenchymal cell into an epithelial one. Global gene expression analysis of the HK-2 cell and the HK-2 cells transfected with the MT-3 gene (HK-2 MT-3) showed several genes including Gelsolin (GSN), and dihydropyrimidinase-like 3 (DPYSL3) to be differentially expressed between these cell types. The goal of the present study is to confirm the results of the microarray and to elucidate the binding partners of MT-3 that may play a role in the MET process in the HK-2 cells. In order to do this, real-time PCR was performed to the expression of GSN and DPYSL3 genes, MT-3 pull down experiments were done to determine the binding partners of MT-3, and confocal microscopy was performed to validate MT-3 binding partners. RT-PCR analysis showed that the expression of these genes in the MT-3 transfected HK-2 cell line was significantly different for GSN, implicating some role for MT-3 in this variation. Confocal studies confirmed that MT-3 showed co-localization with enolase and aldolase at cell and dome peripheries, as well as some co-localization with myosin. These studies demonstrate that MT-3 interacting proteins include not only those responsible for cytoskeleton dynamics, but also other metalloproteins which function in other processes.

EXPRESSION OF ITGAV AND ITGB3 IN ARSENIC AND CADMIUM TRANSFORMED UROTSA CELLS

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Integrin αv (ITGAV) and Integrin $\beta 3$ (ITGB3) genes have shown heightened expression in bladder cancer cells compared to normal cells. Both genes are involved in processes like angiogenesis and cell proliferation. While not many studies have been done on these genes, it is thought that they play a key role in bladder cancer development. Using transformed cell lines and a healthy parent line we were able to study gene expression deviations between healthy and cancerous cells. Heavy metals have been shown to be involved in causing different types of cancers throughout the human body. There are six heavy metals known to be involved in cancer formation: Aluminium, Arsenic, Cadmium, Lead, Mercury, and Uranium. Specifically Arsenic (As) and Cadmium (Cd) have been attributed as factors involved in human bladder cancer. All of the heavy metals listed are immunosuppressants, or agents that can cripple the immune system. These cancer-inducing agents can be found in many places including: soil, drinking water, certain foods, and even the air we breathe. UROtsa cell lines are the model system we use to study heavy metals and bladder cancer. UROtsa cells are immortalized cells from a human urothelium cell line.

Cell culturing techniques were used to grow these cells. Arsenic and cadmium were used to malignantly transform these cell lines. The protocol utilizing TRI-reagent was used for RNA isolation. cDNA was made from the isolated RNA using reverse transcriptase. Real-Time PCR was performed and an iCycler real-time detection system was used to determine the expression of ITGAV and ITGB3. Amplification was determined by SYBR green fluorescence. Gel electrophoresis on a 2% agarose gel was conducted to verify that the desired PCR product was amplified. Our results revealed that ITGAV is highly expressed in arsenic cell lines as well as most cadmium cell lines. ITGB3 did not appear to be as big of a player, but was still up-regulated in a few arsenic cell lines. ITGB3 had no significant increase in gene expression in any of the cadmium transformed cell lines when compared to the normal UROtsa parent cell line. It can be stated that there is a strong relationship between bladder cancer and ITGAV. ITGB3 cannot be written off and could still be a key indirect player in bladder cancer development, even though there is not a significant jump in gene expression.

PUT A LITTLE "METAL" IN IT: CD44 AND ITGB6 EXPRESSION IN CADMIUM (CD^{+2}) AND ARSENIC (AS^{+3}) TRANSFORMED UROTSA CELLS

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Heavy metals, arsenic (As^{+3}) and cadmium (Cd^{+2}), are known carcinogens that aid in the development of bladder cancer. This laboratory has shown that the exposure of UROtsa cells to either As^{+3} or Cd^{+2} can cause malignant transformation of these cells. Microarray analysis of the transformed cell lines showed increased expression of several mesenchymal markers, one being fibronectin. The goal of the present study was determine if CD44 Molecule (Indian Blood Group) and Integrin, beta 6 (ITGB6) both receptors for fibronectin, could be developed as a potential biomarker for diagnosis, or prognosis, of bladder cancer. For this purpose, Real Time-PCR was performed on RNA samples isolated from the UROtsa parental cell line, the As^{+3}, and the Cd^{+2} transformed cell lines. The data obtained indicates that the expression of the CD44 and ITGB6 are increased in the transformed cell lines when compared to the UROtsa parent cells. These results suggest that overexpression of CD44 and ITGB6 may be associated with tumor progression and could be used as a biomarker in the diagnosis and prognosis of bladder cancer.

COMPARISON OF THE EXPRESSION OF STEM CELL MARKERS IN MONOLAYER AND SPHEROID CELL CULTURES OF HUMAN RENAL EPITHELIAL CELLS WITH THAT EXPRESSED IN THE IMMORTALIZED HUMAN PROXIMAL TUBULE CELL LINE, HK-2

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Proximal tubules of the kidney are a 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 either chemicals such as heavy metals, pharmaceuticals such as aminoglycoside antibiotics, or from ischemia. While many studies have focused on surviving proximal tubule cells as the source of regenerative cells for the proximal tubular portion of the nephron, recent studies are now focusing on stem-cell-like cells that repopulate the nephon in vivo. The current study is to characterize stem cell populations within mortal cultures of renal epithelial cells compared to the immortalized proximal tubule cell line, HK-2, both commonly utilized in toxicological studies in vitro. In both cell systems, spheroids were prepared by culturing each cell type in non-attachable culture flasks. In general, spheroids are often considered to be enriched in stem cells as demonstrated in other in vitro cell culture systems and serve in the current study as a useful comparison to each monolayer culture. Four stem cell markers were chosen, two of which have been shown to be

expressed in renal stem cells in vivo, CD24 and CD133, as well as CD44 and ALDH1A1 which are commonly expressed in many other stem cell niches. Stem cell marker expression was assessed with real-time PCR. Human proximal tubule cells and spheroids derived from these mortal cultures of proximal tubule cells expressed high levels of ALDH1A1, CD24, CD44 and CD133 whereas HK-2 cells expressed attenuated expression of all markers with the exception of CD133 which showed similar expression to that of proximal tubule cells. Our results show that the proximal tubule cells isolated from the human renal cortex as well as the spheroids derived from them contain a population of cells that exhibit many stem-like markers and that the expression of these markers are considerably attenuated in the immortalized HK-2 monolayer and the spheroids.

CADHERIN EXPRESSION, VECTORIAL ACTIVE TRANSPORT, AND METALLOTHIONEIN ISOFORM 3 MEDIATED EMT/MET RESPONSES IN CULTURED PRIMARY AND IMMORTALIZED HUMAN PROXIMAL TUBULE CELLS

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The proximal tubule of the kidney is particularly susceptible to toxicant-induced damage and cell cultures of human proximal tubule cells are widely utilized to study the role of epithelial-mesenchymal transition (EMT) in renal disease. Cadmium is a heavy metal that is known to produce renal tubular necrosis, and accumulates in the proximal tubule. This metal binds to a family of cysteine rich metal binding proteins known as metallothioneins (MT) that are found in abundance in the kidney. Previous studies from our laboratory have shown that the third isoform of metallothionein (MT-3) is expressed in the epithelial cells of the human kidney, including the cells of the proximal tubule. An immortalized proximal tubule cell line does not express MT-3 and does not demonstrate vectorial active transport. Transfection of the MT-3 gene into the HK-2 cells restores vectorial active transport as evidenced by dome formation. This suggests that MT-3 is involved in mesenchymal to epithelial transition (MET). The goal of this study was to define the role of growth media composition on classic EMT responses, define the expression of E- and N-cadherin, and define the functional epitope of MT-3 that mediates MET in HK-2 cells. It was shown that both E- and N-cadherin mRNA and protein are expressed in the human renal proximal tubule. Based on the pattern of cadherin expression, vectorial active transport, and transepithelial resistance, it seems that the HK-2 cell line has already undergone many of the early features associated with EMT. Our data also shows that the unique, six amino acid C-terminal sequence of MT-3 is required to induce MET in HK-2 cells. Thus the HK-2 cell line can be an effective model to study later stages in the conversion of the renal epithelial cell to a mesenchymal cell and when transfected with MT-3 it may be an effective model to study the process of MET.

A ROLE FOR ANTERIOR GRADIENT 2 IN A MCF-10A CELL MODEL OF ARSENIC INDUCED BREAST CANCERS

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Arsenic and cadmium are environmental toxicants that have been implicated in the development of various cancers including breast cancer. Previous studies from our laboratory have shown that both arsenite and cadmium can malignantly transform the breast epithelial cell line, MCF-10A. Global gene expression analysis of the transformed cells showed the over-expression of proto-oncogene Anterior Gradient 2 (AGR2) in the arsenite transformed cell line when compared to the parent and the cadmium

transformed cells. The goal of this study was to determine the role of AGR2 in the development of arsenite and cadmium induced breast cancers. Our study shows that the expression of AGR2 is significantly increased in MCF-10A cells transformed with arsenite when compared to cadmium transformed cells. Exposure of the parent MCF-10A cells to arsenite for 48 hours resulted in a significant increase in AGR2 expression suggesting that arsenite has the potential to induce AGR2 in MCF-10A cells. In order to further investigate the effects of AGR2 expression on breast epithelial cells, the parent MCF-10A cells and cadmium transformed MCF-10A cells were stably transfected with the AGR2 gene. Over-expression of AGR2 in the MCF-10A cells increased the ability of the cells to migrate faster in the wound scratch assay. However transfection of the cadmium transformed MCF-10 cells with AGR2 resulted in slowing the ability of the cells to migrate in the assay. Thus, it seems that the effect of AGR2 expression on cell migration is different in the transformed versus the non-transformed cells.

ASSISTING WITH BUILDING RESEARCH CAPACITY BY ENGAGING LOCAL TRIBAL COLLEGE STUDENTS IN RESEARCH THROUGH NDSU INBRE PROJECTS

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The goal of NDSU INBRE is to engage local tribal college students in research through three main projects: Research Training Modules (RTMs), Summer Undergraduate Research Program (SURP), and the Tribal Air Quality Pilot Study. NDSU Faculty develop RTMs for tribal college instructors to use in the desired capacity to engage students in various forms of research. The SURP program is a two week research program to introduce tribal college students into various forms of research. Throughout the program, students explore research methods, career opportunities, professional development skills, and meet potential mentors. The final project on air quality is a partnership with Nueta-Hidatsa-Sahnish College (NHSC) to promote student research. NHSC students work collaboratively with NDSU researchers and laboratories to analyze their particulate matter (PM) samples. This will be the first year the training modules are implemented at local tribal colleges. Evaluations have been developed for the pilot round of modules to serve as a means of identifying research topics and areas that will assist tribal colleges in building research capacity. The SURP program utilized pre and post evaluations. Results showed an increased interest in research by including American Indian researchers as presenters. Another outcome of SURP was an increased interest in STEM and public health research by 25%. The air quality project has led NHSC students to begin the analysis of their PM samples using the scanning electron microscope. NDSU INBRE will continue to engage and receive feedback from tribal colleges in the development of these projects.