PS 1261 SITE-SPECIFIC DNA METHYLATION AND OBESITY IN MEXICAN-AMERICAN CHILDREN.

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In the last 30 years there has been a sharp increase in obesity among children, and minority populations are particularly vulnerable. Although etiology of obesity is thought to be multifactorial with causes stemming from diet, environment, genetics and their interaction, no clear molecular pathways have been identified. However, there is increasing evidence that epigenetic changes, specifically differential CpG methylation, play important roles in determining body weight and metabolic status. The goals of this study are to (1) characterize associations between site-specific methylation and obesity in newborns and 9-year old children and (2) determine whether methylation of the key adipogenic gene peroxisome proliferator-activated receptor gamma (PPARγ) is associated with child obesity and adiponectin / leptin levels. Site-specific DNA methylation, interrogating 485,577 CpG sites, was assessed in 138 newborns and 9-year old CHAMACOS boys and girls by Infinium Illumina 450K BeadChips. Adiponectin and leptin levels were measured for a subset (n=105) of these 9-year old children. Preliminary results showed negative associations between child adiponectin and triglycerides (β=-0.43, P=0.01), very low-density lipoproteins (β=-0.08, P=0.01) and systolic blood pressure (β=-0.06, P=0.02), adjusting for child BMI z-score. Child leptin levels were positively associated with systolic and diastolic blood pressure (P<0.01; P=0.07). Further, we found that higher PPARγ methylation was directly associated with higher adiponectin (β=15.4, p=0.01, n=53) and negatively associated with lower leptin (β=-0.16, p=0.08, n=53), in girls. This research takes advantage of the samples and data from the ongoing longitudinal study of Mexican-American mothers and children from Salinas Valley, CA (CHAMACOS). This population is amenable to studying pathways of obesity as 53% of children are overweight or obese, improving power to examine effects of methylation on obesity and related metabolic parameters and biomarkers. Supported by NIEHS and EPA grants.

PS 1262 EPIGENETIC ALTERATION IN THE TESTICULAR GENE EXPRESSION FOLLOWING *IN UTERO* TRICLOSAN EXPOSURE.

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Transgenerational epigenetic modification is playing an important role in regulation of testicular development and spermatogenesis. Previous study has been indicated that several genes in the testes are regulated by epigenetic mechanisms on the process of spermatogenesis. The current study investigates the direct effects of *in utero* triclosan exposure on the testis of F1 mice. Pregnant BL6 mice were treated with triclosan (0, 10 or 50 mg/kg/day) from 8 days before mating to gestation day (GD) 17 and male pups were sacrificed at PND 42. In F1 mice, dose-dependent increase in sex ratio was observed at dose of 10 mg/kg triclosan compared to control. However, and the ratio of anogenital distance (AGD) was significantly decreased in both male and female F1 mice. Down-regulation of acetylated H3 and H4 and the up-regulation of HDACs were observed in the testis and caudal epididymis of mice treated with triclosan. Microarray analyses were performed to compare control and triclosan treated testis transcriptomes. A total of 250 differentially expressed genes (DEGs) were identified and the major cellular functions and pathways associated with these altered transcripts were examined. The sets of regulated genes at the testis were found to be protein metabolism, intracellular protein traffic, signal transduction and cell cycle. We measured the DNA methylation status in several genes and Rrh and Cyp4f40 were hypermethylated in the promoter region. Triclosan may affect fertility via epigenetic modifications, but specific cellular pathway involved in spermatogenesis is needed to understanding molecular mechanism of triclosan-induced male infertility.

PS 1263 MOLECULAR MECHANISMS OF SILENCING AND REACTIVATION OF LINE-1 RETROELEMENT BY BENZO(A)PYRENE.

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The mammalian genome is largely constituted by repetitive elements that play important roles in global control of chromatin structure and function. Long Interspersed Nuclear Element-1 (LINE 1 or L1) is a retroelement that mobilizes within the mammalian genome via a copy and paste mechanism involving RNA intermediates and self-encoded reverse transcriptase activity. We recently reported that the retinoblastoma (Rb) family of proteins regulates the epigenetic landscape around the L1 promoter by stabilizing histone methylation marks and recruiting protein complexes with histone modifying activities. Although the complex transcriptional control mechanisms of L1 are not yet well understood, L1 reactivation has been described in several human cancers and following exposure of human or murine cells to benzo(a)pyrene (BaP), an environmental carcinogen. Here we investigated the epigenetic mechanisms involved in L1 silencing and reactivation of mouse and human cells. Using real time PCR we found that BaP challenge of Rb family-null mouse embryo fibroblasts (TKO MEFs) exhibited a markedly exacerbated reactivation of L1 message compared to wild type counterparts. Chromatin immunoprecipitation in MCF-7 and HeLa cells using antisera against the pRB-interacting protein complexes showed recruitment of proteins involved in the formation of multiple corepressor complexes to the human L1 promoter. Live cell cycle sorting coupled to L1 mRNA quantification showed that retroelement expression varies as a function of cell division cycle. On the basis of these data we conclude that the presence of Rb proteins is essential for maintaining L1 epigenetic silencing, and likely this process requires assembly of repressor complexes. These modifications ultimately establish the long-term silencing effect that is lost during the course of environmentally-mediated human and animal disease.

PS 1264 AEROSOLIZATION, FATE, AND PULMONARY TOXICITY OF MESOPOROUS SILICA NANOCAGES.

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Background: Lung cancer is the leading cause of cancer-related deaths worldwide. Many toxic cancer therapeutic drugs are non-specific and damage healthy cells. Nano carriers offer the potential for targeted drug delivery. Functionalized mesoporous silica nanocages (F-MSiN) have internal pores that can store drugs and possess surface modifications to assist in unloading drugs at specific sites. Inhalation allows for direct delivery to the lungs without encountering liver metabolism, systemic dilution, or gastrointestinal proteolytic cleavage experienced in intravenous and oral delivery. However, the effectiveness and safety of F-MSiN in an inhalation model has not been extensively studied. Objective: To use aerosolization of F-MSiN in suspension to deliver, detect, and assess toxicity of F-MSiN to the respiratory system. Methods: F-MSiN (50 nm, polyethylene glycol—polyethyleneimine copolymer, fluorescently tagged) were dispersed in nanopure water. Aerosolization was achieved through a micro-droplet nebulizer administered through a nose-only port system to mice for 5 hours. Fluorescent microscopy, SEM, EDS, and TEM analysis of cascade impactors and electrostatic precipitator samples characterized aerosol size distribution. Animals were necropsied 1-day and 8-days post exposure; bronchoalveolar lavage fluid (BALF) was collected for fluorescent imaging, cell counts and cell differentials to assess pulmonary inflammation. Results: Aerosolized F-MSiN sizes ranged from 50 nm to 2 μm, appropriate for lung deposition. F-MSiN was found in BALF alveolar macrophages (500-900 nm) at 1 and 8 days. BALF demonstrated no influx of neutrophils or eosinophils. Conclusions: Not only can F-MSiN be effectively aerosolized using a standard nebulizer system, but the process also creates completely respirable particles that reach the entire respiratory tract with no detected toxicity. These findings suggest that inhalation delivery of F-MSiN has the potential to be used as drug carriers to directly deposit hydrophobic and even toxic drugs to their targeted sites for respiratory diseases.

PS 1265 PHENOTYPIC ANCHORING OF SUBCHRONIC CARBON NANOTUBE AND ASBESTOS EXPOSURE TO SMALL AIRWAY EPITHELIAL CELLS: LINKING TOXICOGENOMIC AND NEOPLASTIC TRANSFORMATION RESPONSES.

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Recent studies reported that inhaled carbon nanotube (CNT) exposure results in elevated risk for rapid interstitial fibrosis and persistence within exposed tissues. To our knowledge, no study has yet evaluated long-term human health risks associated with chronic pulmonary CNT exposures compared to asbestos, a known lung carcinogen with similar shape. To address this knowledge gap, we conducted subchronic *in vitro* exposures of dispersed single-walled CNT (D-SWCNT), multi-walled CNT (D-MWCNT) and crocidolite asbestos (ASB) to human small airway epithelial cells (SAEC). Ultrafine carbon black (D-UFCB) and dispersant-only exposed cells (DISP) served as negative controls. SAEC were exposed for 25 weeks to 0.02 μg/cm^2 and evaluated for cancer cell phenotype. Next, mRNA samples were

subjected to whole genome microarray and rtPCR analyses for toxicogenomic evaluation. Differentially expressed genes were uploaded to Ingenuity Pathway Analysis to identify novel mechanisms promoting neoplastic transformation. Both D-SWCNT and D-MWCNT-treated cells exhibited increased proliferation, invasion, anchorage-independent cell growth and angiogenesis compared to other treatments. Hierarchical cluster analysis revealed that D-SWCNT and D-MWCNT cells shared the most similar genome expression profile while ASB, D-UFCB and DISP cells expressed dissimilar genome profiles. Both D-SWCNT and D-MWCNT cells expressed significant changes in genes associated with cell death, movement, proliferation and cancer. Top ranked pathway along with western blot analyses identified several altered signaling pathways and transcription factors associated with oncogenesis. Phenotypic anchoring of toxicogenomic response to neoplastic cell transformation following *in vitro* subchronic nanomaterial exposure can potentially serve to identify novel mechanisms of action and provide human health risk assessment data.

PS 1266 MAGNETITE NANOPARTICLES FUNCTIONALIZED WITH ALPHA TOCOPHERYL SUCCINATE: CYTOTOXICITY AND ANTITUMOR EFFECT IN BREAST CANCER CELLS.

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BACKGROUND: Magnetite nanoparticles can be used to enhance and improve the efficiency in anticancer drug delivery. Herein we report the effect of magnetite nanoparticles functionalized with Alpha tocopheryl succinate (alpha-TOS), the most effective form of vitamin E in inducing apoptosis in cancer cells. PURPOSES: To investigate the cytotoxicity and antitumor effect magnetite nanoparticles functionalized with Alpha -TOS. MATERIALS AND METHOS: Magnetite nanoparticles were prepared by a coprecipitation method and functionalized with alpha-TOS. The particle size was analyzed by SEM. Then two different human breast cancer cell lines (MDA MB231 and T47D) were treated with the various concentrations. Its effects on cytotoxicity, cell proliferation, and apoptosis were evaluated using confocal microscopy. RESULTS: We found magnetite nanoparticles coupled to alpha-TOS is more cytotoxic and effective that alpha- TOS alone and inhibits the growth of breast cancer cell at lower doses. We also observed dramatic changes in morphology in treated cells associated to apoptosis and more cellular uptake of the nanoparticles functionalized with alpha-TOS. Conclusion: In this study we found that magnetite nanoparticles when is functionalized with alpha-TOS enhances the anti-tumor effect in breast cancer cells. We propose that addition of magnetite nanoparticles to alpha-TOS may be considered for cancer therapy.

PS 1267 DIFFERENTIAL GENE EXPRESSION CHANGES IN TWO HUMAN HEPATOCYTE MODEL SYSTEMS TO QUANTUM DOT EXPOSURE.

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Quantum dots (Qdots) are a class of engineered nanoparticle (ENP) that hold promise in advancing cancer diagnostic and therapeutic modalities, and thus require preclinical safety assessment. The core structure of semiconductor Qdots is composed of potentially toxic heavy metals (Hg, Pb, Cd). A variety of surface coatings have been employed to improve biocompatibility. We have tested the response of Qdots coated with poly(maleic anhydride-alt-1-tetradecene), tri-n-octylphosphineoxide (PMAT-TOPO) in two culture systems, primary human hepatocytes and human hepatoma-derived HepG2 cells, utilized to represent the human liver. We have previously shown that this coating protects hepatocytes from acute toxicity, however, the effects of Qdots on these *in vitro* models have not been fully characterized. Primary hepatocyte and HepG2 cultures were treated with 2.5 to 40nM PMAT-TOPO Qdots for 24 hrs. Gene expression changes from 4 candidate genes of cellular stress and pro-inflammatory signaling were measured by qRT-PCR in both cells types. HepG2 cells show minimal response in gene expression changes with *MT1A* (15-fold) being the only change. On the contrary, PHH cultures show a much more robust response with a 3-fold increase in *HMOX1*, 15-fold increase in *MT1A*. Pro-inflammatory signaling genes also had a robust response with a 5-fold increase of *CXCL8* and *CCL3*, and 20-fold increase in *IL1β* with only minimal changes in *TNFα*. These results illustrate that PMAT-TOPO-coated Qdots elicit a *TNFα*-independent pro-inflammatory response in PHH cultures but no response in HepG2 cells, suggesting primary human hepatocytes may be a more appropriate model than immortalized cell lines for nanoparticle exposure studies.

PS 1268 DISTRIBUTION, BIOPERSISTENCE, AND EFFECTS OF A SYSTEMICALLY-INTRODUCED LOW-ASPECT RATIO CERIA ENGINEERED NANOMATERIAL IN RATS.

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Background: Nanoscale ceria is used as a diesel fuel additive and in chemico-mechanical planarization and is being developed as an antioxidant therapeutic agent. Objectives: To determine the influence of shape, specifically a low-aspect ratio (rod-like) form, of an inert metal oxide engineered nanomaterial (ENM) on its distribution, biopersistence, and effects after a single systemic administration to rats, compared to polyhedral 5 to 55 nm ceria. Methods: An aqueous dispersion of citrate-stabilized ceria ENM rods (~ 10 nm diameter, 40 to 650 nm long), synthesized and characterized in-house, was infused intravenously into rats (50 mg/kg), terminated 1 hour or 30 days later. Control rats received vehicle. At termination, 6 organs were weighed and samples collected from multiple sites and blood for cerium determination by ICP-MS, oxidative stress endpoints, and histology. Results: The low-aspect-ratio ceria ENM was not acutely toxic and did not produce mortality. Its initial distribution was similar to 15 to 30 nm polyhedral ceria, whereas at 30 days less was in the liver, skeletal system, and bone marrow. Less of the dose could be accounted for, suggesting more clearance than polyhedral ceria, which are not significantly cleared up to 90 days. Spleen weight was significantly increased at 30 days. Hepatic granulomas were fewer and smaller than after 15 and 30 nm polyhedral ceria. Protein carbonyls in the hippocampus were significantly decreased at 1 h and significantly increased at 30 days. Conclusions: Other than its more limited distribution and apparent more rapid clearance from the rat, this low-aspect-ratio ceria ENM produced effects qualitatively similar to those seen with 5 to 55 nm polyhedral ceria ENM. Support: US EPA STAR Grant RD-833772.

PS 1269 SUBTOXIC TIO2-NP PREDISPOSES TO DECREASE THE MITOCHONDRIAL FUNCTION IN LUNG TISSUE AND CYTOSKELETON DISRUPTION IN ALVEOLAR EPITHELIAL CELLS.

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Titanium dioxide nanoparticles (TiO2-NPs) are used in an increasing number of human products such as cosmetics, sunscreen, toothpaste and paints. The susceptibility to develop further damage after TiO2 NPs exposure has been less investigated the exposure. However, the harmful effects associated with TiO2-NPs exposure are not completely described, but it has been demonstrated that reactive oxygen species derived from mitochondria are involved in cytotoxic effects.
The aim of this work was to test if a sub-toxic TiO2-NPs (5 μg/mg protein) concentration was able to enhance further mitochondrial damage induced by hydrogen peroxide (H2O2, 5 μM), which is a common molecule released during inflammation, in mitochondria isolated from lung tissue. In addition, we hypothesized that mitochondrial dysfunction induced by sub-toxic TiO2 NPs exposure, could impact in cytoskeleton organization and to test this hypothesis, alveolar epithelial cells were exposed to sub-toxic TiO2-NPs (5μg/cm2) and challenged with sub-toxic H2O2 exposure. Our results showed the following parameters for TiO2 NPs characterization zeta potential=-10.6±2.4, hydrodynamic diameter=783±37 nm and polydispersity index =0.317. The isolated mitochondria exposed to non-toxic TiO2 NPs and then exposed to H2O2, developed higher susceptibility to mitochondrial dysfunction showed as the decrease in respiratory control index (decrease of 50%), mitochondrial membrane potential (70% of decrease), P/O ratio (50% of decrease).In addition, alveolar epithelial cells exposed to H2O2 but previously exposed to TiO2, showed higher cytoskeleton disruption. In conclusion, the sub-toxic TiO2-NPs exposure enhances the susceptibility to cause mitochondrial dysfunction and cytoskeleton disruption induced by H2O2.

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