PS 1181 ELUCIDATION OF FACTORS DETERMINING CARBON NANOTUBES' ABILITY TO PENETRATE ALVEOLAR EPITHELIAL BARRIER AND INTERACT WITH LUNG FIBROBLASTS *IN VITRO.*

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Nanomaterials possess unique physicochemical and biological properties but can also exhibit different adverse reactions if inhaled. Our previous *in vivo* study showed upon alveolar deposition, dispersed single-walled carbon nanotubes (DSWCNT) rapidly enter interstitial area (1 day post-exposure) and induce interstitial fibrotic response as early as 1 week post-exposure. Direct stimulation of cultured lung fibroblasts, a major interstitial cell, by DSWCNT was shown to enhance proliferation and collagen production, a hallmark of lung fibrosis. Furthermore, penetration of DSWCNT through lung epithelial barrier into interstitium could be a key event of DSWCNT-induced interstitial fibrosis. To investigate this alveolar epithelial barrier, an experimental model was developed using immortalized human lung epithelial cell line (ATCC, Manassas, VA). Epithelial cells were cultured on the apical surface of Transwell® microporous membrane and exposed to non-dispersed SWCNT and DSWCNT. Samples from the apical compartment, cell monolayer, and basolateral compartment were collected at various times and analyzed for CNT penetrability. Electron microscopy and CytoViva hyperspectral imaging were used to aid characterization of the penetration pathway (paracellular vs. transcellular) of nanoparticles across alveolar epithelial membrane. The effect of CNT dispersion status on penetration rate was also assessed. Our data suggest CNT penetrated through epithelial cells on apical side and translocated to the other side of the Transwell membrane and the amount of CNT transferred, measured by hyperspectral imaging, was sufficient to induce fibroblast proliferation and collagen production based on previous data. The Transwell system is a suitable model for studying translocation of CNT across epithelial layer and aids in mechanistic studies of CNT- induced interstitial lung fibrosis.

PS 1182 *IN VITRO* ASSESSMENT OF POTENTIAL TUMORGENICITY OF CHRONIC SWCNT AND MWCNT EXPOSURE TO LUNG EPITHELIUM.

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Carbon nanotube use in multiple consumer and industrial products has resulted in increased concern for long-term risks to human health. Recent *in vivo* studies suggest that inhaled carbon nanotubes induce interstitial fibrosis and persist in exposed lung tissues. No clear evidence or experimental model exists to assess whether long-term pulmonary exposures of CNT to lung tissue results in transformed cells expressing a tumorigenic phenotype. This study tested the hypothesis that chronic exposure to dispersed carbon nanotubes induces neoplastic transformation in lung epithelial cells *in vitro.* Small airway lung epithelial cells were exposed for 25 weeks to either dispersed single wall (D-SWCNT) or multi-wall (D-MWCNT) carbon nanotubes at non-cytotoxic levels (0.02 μg/cm^2). Dispersed ultra fine carbon black (D-CB) and asbestsos (ABS) served as negative and positive controls. Following exposure each treatment was evaluated for tumorigenic phenotypes using cell proliferation, cell invasion and colony formation assays in untreated media. D-SWCNT exhibited a significant 1.5-fold increase in cell proliferation compared to passage control cells while D-MWCNT and D-CB showed a modest increase. In addition, D-SWCNT cells displayed significantly greater invasive potential than D-MWCNT, ABS and passage control. Lastly, a tumor formation assay resulted in D-SWCNT cells possessing a significant 5-fold increase in the number of colonies formed above controls while D-MWCNT and D-CB exhibited only a 1.5-fold increase. Our chronic, low dose *in vitro* exposure model suggested that D-SWCNT exposure caused neoplastic transformation resulting in a tumorigenic phenotype while D-MWCNT exposure displayed less malignant potential. In conclusion, use of *in vitro* screening methods, along with comparable *in vivo* data, can assist in investigation of tumorigenic risks associated with carbon nanotube and other nanomaterial exposures.

PS 1183 ASSESSMENT OF FIBROGENIC BIOMARKERS INDUCED BY MULTI WALL CARBON NANOTUBES.

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Multi Wall Carbon Nanotubes (MWCNT), a graphene based nanoparticle, possess unique physiochemical properties. Considered as a technological breakthrough, production of MWCNT is rapidly increasing worldwide but toxicity profile of the nanomaterials is not clearly understood. Among the adverse effects, CNT have been shown to induce (the development of unusual) interstitial lung fibrosis at physiologically relevant exposure (10μg/mouse); however, the underlying mechanism is not fully known. In this present study, we investigated important MWCNT-induced fibrogenic mediators using cultured lung cell systems. Human bronchial epithelial (BEAS-2B) cells, alveolar epithelial (A549) cells, and lung fibroblast (CRL1490) cells were treated with MWCNT. Viability of MWCNT-exposed cells was determined by cell counting and WST-1 assay. Fibrogenic mediators, including Fibroblast Growth Factor-2, Vascular Growth Factor, Transforming Growth Factor β1 (TGF-β1), and Platelet Derived Growth Factor-A, were analyzed using Western Blots and end point ELISA. Our results show that 1) MWCNT decreased cell viability of epithelial cells in a dose and time dependent manner, 2) MWCNT exposure induced secretion of fibrogenic mediators from lung epithelial cells and fibroblasts at physiologically relevant concentrations of 0.02 to 0.6 μg/cm^2 and 3) MWCNT directly induced collagen production from lung fibroblasts. In conclusion, MWCNT induced fibrogenic mediators in cultured human lung epithelial cells and stimulated collagen production from fibroblasts. These data are consistent with *in vivo* observations. Therefore, the *in-vitro* cell culture systems can be used for mechanistic studies and screening tests for MWCNT and similar fibrogenic nanoparticles.

PS 1184 DIFFERENTIAL EFFECTS OF SINGLE-WALLED CARBON NANOTUBES ON HUMAN HEPATIC, RENAL, AND COLORECTAL CARCINOMA CELL LINES.

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Carbon nanotubes (CNTs) are currently one of the promising materials for the development of nano-technologies. However, CNT toxicity is a major concern. Many in vitro studies have assessed the cytotoxicity of CNTs, but the results differ according to the cell lines and catalysts used for synthesizing CNTs. We have previously reported the differential effects of single-walled CNTs (SWCNTs) on human lung carcinoma A549 and human head and neck carcinoma FaDu cells (SOT 2010 annual meeting). The present study aimed to clarify the cytotoxic effects of SWCNTs on HepG2, ACHN, and Caco-2 cells derived from the human liver, kidney, and colon, respectively, because SWCNTs are expected to be used as drug and gene carriers in medical fields. The SWCNTs used in this study were manufactured using 2 types of arc electrical discharge method, with Ni and Y (SO-SWCNTs) and Fe (FH-P-SWCNTs) as catalysts. Cell viability was evaluated on the basis of the ATP content and metabolic capacity of the cell. SWCNTs were exposed to the cells at concentrations of up to 1.0 mg/ml. On 24-h exposure of 1.0 mg/ml SO-SWCNTs to HepG2, ACHN, and Caco-2 cells, the ATP content of these cells decreased to 91%, 87%, and 90% of the ATP content of untreated cells, respectively. Under identical conditions, HepG2, ACHN, and Caco-2 cells exposed to FH-P-SWCNTs showed similar results (decrease in ATP content to 91%, 97%, and 93%, respectively). However, the metabolic capacity of SO-SWCNT-exposed cells was slightly higher than that of FH-P-SWCNT-exposed cells, that is, 63%, 88%, and 69% vs. 50%, 93%, and 48% for HepG2, ACHN, and Caco-2 cells, respectively. These results suggest that exposure to SWCNTs has a greater effect on metabolic activity than on ATP content of these cells and that ACHN cells are most resistant to SWCNT exposure. The present study clarified that the effects of SWCNTs on cell viability differed depending on the type of cell, SWCNT, and analytical method used to assess cell viability.

PS 1185 MULTI-WALL CARBON NANOTUBE (MWCNT)-INDUCED GENE EXPRESSION IN THE MOUSE LUNG: IMPLICATION OF CARCINOGENESIS RISK.

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MWCNT's fibrous-like shape and durability suggest they may pose a similar carcinogenic effect on humans as asbestos. Therefore, this study sought to investigate how previously identified lung cancer prognostic biomarkers and the related cancer signaling pathways are affected in the mouse lung following in vivo pharyngeal aspiration of MWCNT. A total of 63 identified lung cancer prognostic biomarker genes were analyzed using quantitative PCR assays in the mouse lung exposed by aspiration to 0, 10, 20, 40, 80 μg of MWCNT at 7-days and 56-days post-exposure. At 7- and 56-days post-exposures, a set of 7 genes and a set of 11 genes showed significant differential expression in the mouse lungs exposed to MWCNT vs. unexposed control groups, respectively. These significant genes could clearly separate

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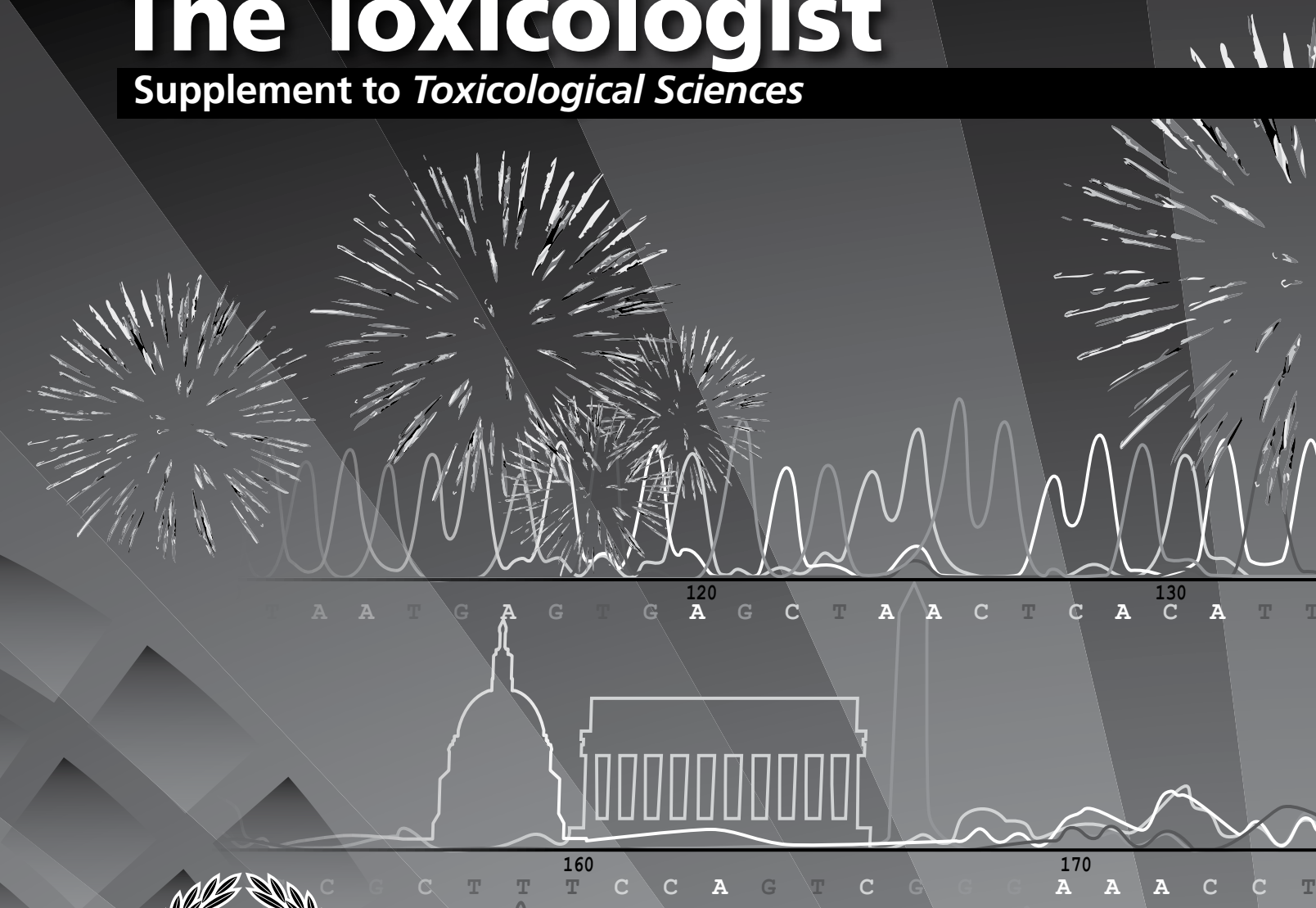

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Preface

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An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 578.

The issue also contains a Key Word Index (by subject or chemical) of all the presentations, beginning on page 606.

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