PS 1244 Dose- and Time-Dependent Assessment of Human Mesothelial Cell Neoplastic Transformation Potential after Functionalized MWCNT Exposure

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MWCNTs are characterized by asbestos-like fiber morphology, large surface area and surface chemistries. Exposure results in pulmonary fibrosis, biopersistence, extrapulmonary transport and promotion of adenocarcinoma and sarcomatous mesothelioma. As MWCNTs become widely used, elevated cancer risk in pleural mesothelium following inhalation exposure is a concern. Long-term exposure risks resulting from surface functionalization of MWCNT on pleural mesothelioma potential is largely unknown, but critically needed. We hypothesized that the effect of MWCNT surface functionalization on human mesothelial cell neoplastic transformation potential depends on dose and duration of exposure. Human immortalized mesothelial cells were continuously exposed to fully characterized prepared (pMWCNT), carboxylated (MW-COOH) and aminated (MW-NH_2) for 6 months (M) at 0.002 and 0.02 μg/cm^2 which are relevant to animal exposure doses. Saline (SAL), dispersant (DISP) and crocidolite asbestos (ASB) exposed cells served as controls. At regular intervals during exposure, each treatment group was assessed for several cancer hallmarks. Results indicated that 1) low dose MW-COOH and MW-NH_2 exposure caused significant increases in cell proliferation compared to controls starting at 1 M and persisted over 6 M exposure. High dose exposure alleviated this effect. 2) pMWCNT, MW-NH_2 and ASB cells exhibited significantly greater numbers of soft agar colonies at both doses compared to controls starting at 4 M. Low dose fMWCNT treatment resulted in a more potent colony-forming effect than high dose, while both doses of asbestos possessed an equipotent effect. 3) Only MW-COOH cells at 6 M exhibited a significant increase in invasion ability compared to all treatments. Lastly, ASB cells displayed the largest transformation frequency while all fMWNCT exposures caused moderate effect. In summary, exposure dose, duration and type of surface functionalization determine MWCNT neoplastic transformation potential in pleural mesothelial cells.

PS 1245 Lessening Genotoxicity Using Nitrogen-Doping of Multiwalled Carbon Nanotubes

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Multi-walled carbon nanotubes (MWCNT) have many industrial applications. However, the low density and small size of MWCNT makes respiratory exposures in workers likely. Nitrogen-doped MWCNT (ND-MWCNT) has been shown to be less inflammatory *in vivo* than pristine MWCNT (P-MWCNT) of the same dimensions. In order to investigate the potential for lessened *in vitro* toxicity of ND-MWCNT we exposed immortalized and primary respiratory epithelial cells to 0.024, 0.24, 2.4, 24, and 48 ug/cm2 MWCNT. Three-dimensional reconstruction of Raman confocal optical images determined that both ND-MWCNT and P-MWCNT were taken up by the cell and localized in the nucleus. Further analysis by enhanced darkfield microscopy (Cytoviva) demonstrated that 4% of the cells had P-MWCNT in the nucleus while only 0.8% contained ND-MWCNT. ND-MWCNT caused significantly less cellular necrosis than P-MWCNT after 72 hours of exposure. Cell cycle analysis of both cell types showed that exposure to P-MWCNT caused a significant G1/S block 24 hours after exposure indicating genotoxicity. By contrast, ND-MWCNT did not induce a similar block in the cell cycle. In the primary cells, P-MWCNT induced a dramatic G1/S block after 72 hours while ND-MWCNT caused a moderate G1/S block. Preliminary studies indicate mitotic spindle aberrations in both P-MWCNT and ND-MWCNT, though a higher dose is required for ND-MWCNT. Further investigation of mitotic spindle disruption and chromosome errors following exposure to P-MWCNT and ND-MWCNT at occupationally-relevant doses is needed. However, these data indicate nitrogen-doping of MWCNT may have ramifications for lessening the toxicity of pristine MWCNT and protecting worker health.

PS 1246 Carbon Nanotubes Promote Lung Tumor Progression through the Induction of Cancer-Associated Fibroblasts and Cancer Stem-Like Cells

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Pulmonary exposure to carbon nanotubes (CNTs) has recently been shown to promote the growth and neoplastic progression of lung adenocarcinoma in animals, but the underlying mechanisms are unknown. Accumulating evidence suggests that cancer-associated fibroblasts (CAFs) may promote tumor growth by providing appropriate tumor microenvironment. In the present study we investigated whether CNTs can induce CAFs and whether these cells can promote tumor formation in a xenograft mouse model. We show that exposure of normal human lung fibroblasts (NHLFs) to physiological relevant concentrations of single-walled (SW) CNTs (0.08-0.15 μg/cm^2) upregulated the expression of podoplanin, a known CAF marker of human lung adenocarcinoma, suggesting that SWCNTs can trigger NHLFs to initiate CAFs. Subcutaneous injection of the SWCNT-exposed NHLFs along with human lung carcinoma H460 cells in NSG mice resulted in a high rate of tumor formation compared with the coinjection of vehicle-exposed NHLFs and H460 cells, indicating the tumor-promoting effect of SWCNT-exposed NHLFs. The mechanism by which SWCNT-exposed fibroblasts promote tumor growth was shown to involve cancer stem cell (CSC) induction, as determined by tumor sphere formation and side population assays. Together, our study unveils a novel mechanism of CNT-promoted lung carcinoma through the acquisition of CAFs that regulate CSC formation.

PS 1247 Differential Sensitivity of Healthy and Asthmatic Human Bronchial Epithelia to Multiwalled Carbon Nanotubes

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Inhalation of multi-walled carbon nanotubes (MWCNT) can induce significant pulmonary pathology owing to their nanoscale, fibrous morphology and/or biopersistance. MWCNT were shown to aggravate asthmatic responses using various animal models. However, there is need of validating these findings in more translational relevant models. We sought to elucidate 1) the differential sensitivity of human bronchial epithelial cells from healthy and asthmatic subjects to low dose MWCNT exposures and 2) to elaborate molecular mechanisms through which MWCNT induce toxic effects. HBE cells were collected from healthy (BEC) and asthmatic (ABEC) human volunteers through fiber optic bronchoscopy, exposed to fully characterized MWCNT suspensions (1.5 or 12 μg/mL) for 24 or 48 hours and analyzed for toxic or inflammatory responses. Cells cultured in the presence or absence of IL-13 were exposed to MWCNT to elaborate the effect of asthmatic lung environment. Global gene expression profiling was done using Agilent Whole Human Genome oligo arrays and results were verified using RT-qPCR. ABECs show higher sensitivity to MWCNT and significant toxicity is observed after 24 hours exposure at lower doses as compared to BECs. Interestingly, BECS recover from toxic effects at 48 hours while significant toxicity persists in ABECs. MWCNT are internalized by both types of HBE cells but were seen to localize preferentially in cytoplasm in asthmatic BECs. Microarray revealed significant changes in inflammatory, immunological, cell survival and proliferation pathways. No amplification of toxicity was observed when cells were exposed to MWCNT in the presence of IL-13. IL-13 induced a significant increase in the endoplasmic reticulum stress signaling which was impaired by MWCNT exposures. Our results demonstrate that ABECs are more sensitive to the toxicity of MWCNTs and show differences in cellular signaling as compared to BECs. These findings suggest that asthmatic individuals are at greater risk to the toxic effects of MWCNTs.

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