and identification of aneugens. Besides the genotoxicity markers, these assays also provide information about cell survival, membrane integrity and cell cycle information.

3118 Comparative Study of DNA Damage Response in Human Lung Cells Exposed to Genotoxic Compounds

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Background and Purpose: DNA damage is a crucial factor that disrupts normal cellular functions. It plays a significant role in the development of various pathophysiological processes. The DNA damage response (DDR) is a critical cellular mechanism that responds to DNA damage and decides the fate of the cell–whether it will repair the damage, enter senescence, or undergo apoptosis. The primary goal of this study was to explore how different genotoxic agents affect the DNA damage response and cell fate in human lung cells. The tested compounds were selected to represent different mechanisms of the genotoxic activity. Two polycyclic aromatic hydrocarbons (PAHs): Benzo[a]pyrene (B[a]P) forming highly reactive intermediates upon metabolic activation that give rise to diverse DNA damage, such as oxidized DNA lesions and DNA adducts; and 1-nitropyrene (1-NP) that requires metabolic activation by nitro reduction or aromatic ring oxidation to form DNA adducts or elicit oxidative DNA damage; and two anticancer drugs: Doxorubicin hydrochloride (DOX) has been suggested to cause topoisomerase II poisoning, DNA adduct formation or oxidative stress leading to senescence or cell death; and 5-Bromo-2'-deoxyuridine (BrdU), a thymidine analog, whose incorporation into newly synthesized DNA results in various DNA lesions, such as mutations, fragile sites, chromatid breaks and sister chromatid exchanges. **Methods:** A series of *in vitro* assays was employed to comprehensively evaluate the effects of the selected genotoxic substances in immortalized human bronchial epithelial cells BEAS-2B. To determine sub-cytotoxic doses, WST-1 and LDH cytotoxicity assays were carried out 24, 48, and 72h after exposure. The alkaline version of the comet assay with and without restriction enzyme (Fpg) was employed to detect DNA strand breaks and oxidative DNA lesions, respectively, after 4 and 24h exposure. Double strand break formation was evaluated by γH2AX foci labeling and ELISA based HT gamma-H2AX Pharmacodynamic Assay 4, 24, and 48h after exposure. The effects on the cell cycle were assessed using Propidium Iodide staining and analyzed by flow cytometry after 24, 48, and 48+72h (cells exposed for 48h to the tested substances, followed by incubation in a compound-free medium for another 72h). Flow cytometry was further used to detect the proportion of apoptotic and necrotic cells using Annexin V conjugated with Alexa 647 and Hoechst 33258 staining, respectively. Senescence-associated β-Galactosidase Assay was employed to determine β-Galactosidase as a senescence marker. The expression of selected senescence-associated genes (CDKN1A, CDKN1B, *CDKN2A*, CCND1) was evaluated using qRT-PCR after 48+72h exposure. Western blotting was used to detect expression levels of selected DNA damage response proteins (p53, γH2AX, p21) 4, 24, 48, and 48+72h after exposure. **Results:** Based on the cytotoxicity assay results, 10 μM was determined as the highest dose for further investigation for both B[a]P and 1-NP, and the doses of 100 nM and 100 μM were selected for DOX and BrdU, respectively. Comet assay revealed strong induction of oxidative DNA damage in B[a]P (10 μM), 1-NP (1 and 10 μM), and a slight yet significant increase in DOX (10 and 100 nM). Senescence was detected in DOX (1, 10, 100 nM) and BrdU (10 and 100 μM), corresponding with an increased expression of CDKN1A (encoding p21–a direct target of p53 that mediates cell cycle arrest, apoptosis, DNA repair or senescence) and CCND1, an established marker of senescence. DOX and BrdU also elicited changes in the cell cycle, with the highest dose of DOX strongly increasing G2 arrest (more than 80% of cells after all exposure times) and a mild increase of G1 arrest by the highest dose of BrdU. Only 100 nM DOX significantly increased apoptosis after 48+72h exposure. While p53 activation (Phosphorylation of p53 at Ser-15) was detected in all the tested substances, p21 expression was only increased in DOX. **Conclusions:** In summary, the tested PAHs (B[a]P and 1-NP) caused oxidative DNA damage without altering cell cycle progression or inducing senescence. However, prolonged B[a]P exposure resulted in a minor accumulation of cells G2 phase and increased cell mortality. The anticancer agents DOX and BrdU demonstrated low genotoxic potential. However, DOX induced the most pronounced p53-mediated response, leading to cell cycle arrest in G2 phase, triggering apoptosis and senescence. BrdU, while activating p53, predominantly led to G1 phase accumulation and a significant increase in cellular senescence.Our findings offer a comprehensive perspective on the cell-specific outcomes triggered by distinct DNA lesions and suggest that the cell fate decision upon genotoxic insult is driven by a complex interplay of various factors, including the types of DNA lesions and the persistence of p53 signaling with possible involvement of DNA repair and DNA damage tolerance.

3119 4,4′-Methylene Diphenyl Diisocyanate Exposure Downregulates Endogenous hsa-miR-206-3p Through Induction of hsa_circ_0008726 in Macrophages

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Background and Purpose: Exposure to 4,4′-methylene diphenyl diisocyanate (MDI), the most widely used monomeric diisocyanate, in the occupational setting may lead to the development of occupational asthma (OA). Currently, the underlying molecular mechanism(s) by which MDI induces OA have yet to be elucidated. Alveolar macrophage (MØ) dysfunction plays an important role in asthma pathogenesis. Previously, our laboratory revealed that MDI exposure downregulates endogenous *microRNA(miR)-206-3p,* activating *miR-206-3p*-regulated signaling, including PPP3CA/calcineurin/NFAT signaling activation in MØs, to increase chemokine production and promote chemotaxis activities of immune cells. Competitive endogenous RNA (ceRNA) species including circular RNAs (circRNAs) and long non-coding RNAs (lncRNAs) have been shown to regulate the levels of endogenous miRs through miR responding elements (MRE) binding to endogenous miRs in different cell types. The circRNA species are highly stable, circular structure RNA species that play important roles in many different biological processes and their expression can be regulated via exposures to outside stimuli; however, whether MDI-exposure can influence the expression of endogenous circRNA species is unknown. Several endogenous circRNAs have been reported to regulate endogenous *hsa-miR-206-3p* levels through potential ceRNA mechanisms. We hypothesize that MDI-exposure induces endogenous circRNA(s) to regulate *hsa-miR-206-3p* in MØs. **Methods:** We determined the expression of candidate *hsa-miR-206-3p* binding cricRNAs including *hsa_circ_0000199, hsa_circ_0001264, hsa_circ_0001982, hsa_circ_0004662, hsa_circ_0007428, hsa_circ_0008726, hsa_circ_0056618, hsa_circ_0057558, hsa_circ_0058141,* and *hsa_circ_0072088* from MDI-glutathione (GSH) conjugate-treated differentiated THP-1 macrophages using RT-qPCR. **Results:** *In vitro* MDI-GSH exposures result in the upregulation of endogenous *hsa_circ_0008726* and its host gene transcript *DNAJB6*; whereas other circRNA(s) examined were neither detected nor changed in MDI-GSH exposed MØs. RNA-induced silencing complex-immunoprecipitation (RISC-IP) experiments indicated that *hsa-miR-206-3p* can bind to *hsa_circ_0008726* in MØs. The expression of endogenous *hsa-miR-206-3p* was either up- or down-regulated by transfection of either *hsa_circ_0008726* siRNAs or overexpression plasmids of *hsa_circ_0008726* in MØs, respectively. **Conclusions:** These results suggest MDI-exposure may downregulate endogenous *hsa-miR-206-3p* through induction of *hsa_circ_0008726/DNAJB6* thus contributing to the upregulation of *hsa-miR-206-3p*-mediated regulations in MØs.

3120 Characterizing Lead Exposure Impact on piRNA Pathways in the Cardiovascular System

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Background and Purpose: Cardiovascular disease (CVD) is the leading cause of death worldwide, and developmental exposure to the metal lead (Pb) may contribute to its incidence and progression. One potential mechanism underlying Pb-induced CVD is modification of DNA methylation. DNA methylation is the addition of a methyl group to cytosine bases in DNA, resulting in modulation of gene transcription without changing the underlying DNA sequence. Alterations in DNA methylation have been implicated in the development of coronary heart disease, heart failure, hypertension, and other CVDs. We have previously reported that developmental Pb exposure in mice alters DNA methylation in the heart in a sex-specific manner, but the underlying regulatory mechanisms are unclear. One potential mechanism by which Pb may modulate DNA methylation is dysregulation of PIWI-interacting RNA (piRNA) pathways. piRNA are a class of small non-coding RNA that canonically contribute to genomic stability by controlling DNA methylation of transposable elements in the germline. piRNA associate with PIWIL machinery, which are critical for their biogenesis and function (PIWILs 1-4 for humans and PIWILs 1, 2, and 4 for mice). While piRNA were long thought to be exclusively expressed in the germline, work from our group and others has overturned this with measurement of somatic piRNA expression in mice and humans. Altered piRNA expression has been reported in the context of CVD, but no studies thus far have linked Pb exposure and piRNA expression in the heart. The purpose of this project is to investigate Pb exposure on DNA methylation and piRNA expression in the heart during early development, using human induced pluripotent stem cell (hiPSC) derived cardiomyocytes and an *in vivo* mouse model. We investigate how Pb exposure may dysregulate the DNA methyltransferases (DMNTs) and TET dioxygenases (TETs), which catalyze the methylation and demethylation of DNA, respectively, as well as piRNA and their PIWIL machinery. **Methods:** For the *in vitro* studies, one cell line of female-derived hiPSCs was differentiated into immature ventricular cardiomyocytes using established small molecule methods that temporally modulate Wnt signaling. Cells were exposed to human physiologically relevant doses of Pb (0.5 μM and 5 μM) concurrently during the first eight days

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