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Correlation between *CYP1A1* RNA Transcript, Protein Level, Enzyme Activity, and DNA Adducts in Primary Normal Human Mammary Epithelial Cells Exposed to Benzo[a]pyrene. Divi RL[1], Lindeman TE[1], Shockley ME[1], Keshava C[2], Weston A[3], Poirier MC[1]. [1]National Cancer Institute, Bethesda, MD, United States, [2]National Center for Environmental Assessment, EPA, Research Triangle Park, NC, United States, [3]Division of Respiratory Disease Studies, NIOSH, CDC, Morgantown, WV, United States.

Benzo(*a*)pyrene (BP) undergoes metabolic activation and forms DNA adducts. The goal of this study is to identify the key players that contribute to BP-DNA adduct formation in mammary epithelial cells. We quantified RNA copies/ng cDNA (RNA cpn) of *Cytochrome P450 1A1 (CYP1A1) and CYP1B1,* genes which code for metabolic enzymes that form r7, t8, t9-trihydroxy-c-10-(*N*^{2}-deoxyguanosyl)-7,8,9,10-tetrahydro-benzo[*a*]-pyrene (BPdG), the major BP-DNA adduct, and *NAD(P)H:Quinone Oxidoreductase 1 (NQO1),* which codes for NQO1 that converts BP quinones to less toxic hydroquinones. Primary normal human mammary epithelial cell (NHMEC) strains from 16 healthy women and MCF-7 breast cancer cells were used for comparison. We found 56-836 and 251-13234 *CYP1A1*, 336-5587 and 4133-57077 *CYP1B1*, and 5943-40112 and 4456-55887 *NQO1* RNA cpn in unexposed and BP exposed (4 µM, 12h) NHMECs, respectively. NHMECs had 7.47 (median; range: 0.85-15.8) BPdG adducts/10^8 nucleotides while MCF-7 cells had 790 adducts. In the NHMECs, a linear association (p=0.0015) was observed between BPdG adducts and BP-induced *CYP1A1*, and no correlation with other genes examined. Western blots of 4 NHMEC strains, chosen for different levels of BPdG adducts, showed a linear correlation (p=0.013) between BPdG and CYP1A1, but none between BPdG and CYP1B1 or NQO1. Ethoxyresorufin-O-deethylase (EROD) activity, which measures CYP1A1/1B1 together, correlated (p=0.038) with BPdG in NHMECs, and was highly induced by BP in MCF-7 cells. Overall, the data suggest that CYP1A1 is critical for BPdG adduct formation in NHMECs. The 10-fold higher adduct level found in BP-exposed MCF-7 cells, compared to NHMECs, appears likely due to high EROD activity.

P69

Use of Directed Evolution to Study Substrate Discrimination by ALKBH2. Lepore AL, Troll C, Alexander DL, Camps M. University of California, Santa Cruz, Santa Cruz, CA, United States.

The human DNA repair enzyme ALKBH2 is a direct repair enzyme that acts to remove cytotoxic methyl damage and mutagenic etheno adducts from DNA. While previous research has uncovered many important structural features of ALKBH2, it is still unknown how the enzyme discriminates between these two types of DNA damage given that once they are flipped into the active site of the enzyme, these two lesions occupy nearly identical positions in the active site. In order to identify key residues involved in the discrimination of methyl and etheno lesions, we are screening a collection of ALKBH2 mutants previously selected for increased protection to exposure to the methylating agent MNNG for differential methyl versus etheno repair. As a screening method, we use lysogenic infection of a phage treated with the SN2 methylating agent methyl methane sulfonate (methylation repair) or chloroacetaldehyde (etheno repair). We identified mutants in this library that selectively reduce methyl repair while leaving etheno repair, and plan to confirm these results genetically by looking at differences in mutation expression and biochemically by mass spectrometry. The identification of mechanisms of substrate discrimination for ALKBH2 should facilitate the production of adjuvant ALKBH2 inhibitors specific for methyl repair. These inhibitors would enhance the therapeutic effect of methylating agents while minimizing the potential carcinogenic effects of etheno-induced mutagenesis.

P70

Low-Dose H_2O_2 Induced Clustered DNA Lesions and Mutagenesis: Contribution of Error-Prone NHEJ Repair Pathway. Sharma V, Collins LB, Swenberg JA, Nakamura J. University of North Carolina, Chapel Hill, NC, United States.

Although the induction of oxidatively induced clustered DNA lesions (OCDLs) has been believed as a finger print of radiation-induced DNA damage, few studies have also associated elevated levels of OCDLs with chronic inflammation and human malignancies. There is a knowledge gap regarding formation of OCDLs/DSBs as a result of low levels of endogenous/exogenous oxidative stress (OS) and their role in mutagenesis. Therefore, in the present study, we sought to understand the generation of OCDLs and OS induced mutagenesis caused by low levels of H_2O_2 and identify DNA repair pathways that may affect OS induced susceptibility to mutagenesis. Low concentrations of H_2O_2 actually found in cells during inflammatory processes were taken. Interestingly, DNA Damage Response analyses in DT40 cells, using reverse genetic approach, revealed hypersensitivity of Rad54, Rad51c, XRCC2, Ku70 and Lig IV deficient cells to H_2O_2, indicating the potential role of DSBs in H_2O_2 toxicity. High levels of 8-oxo-dG lesions and OCDLs measured with a modified PFGE version were also found in H_2O_2 exposed cells. The induction of OCDLs and DSBs directed us to investigate the role of error-prone NHEJ in mutagenesis. Ku70, DNA PKcs and Lig IV (NHEJ proteins) deficient cells revealed a drastic decrease in mutation frequency despite the presence of equivalent levels of 8-oxo-dG as in the wild-type DT40 cells. Our results indicate that OS, even at low levels, can cause clustered DNA damage that leads to DSBs with complex DNA ends and repairing such complex DSBs with NHEJ increases the likelihood that mutations will result.

P71

BPA Modulates Repair of Oxidative DNA Damage by Base Excision Repair Pathway. Gassman NR, Stefanick DF, Horton JK, Wilson SH. Laboratory of Structural Biology, National Institute of Environmental Health Sciences, National Institute of Health, Research Triangle Park, NC, United States.

Bisphenol A (BPA) has become a target of intense public scrutiny since concerns about its association with human diseases such as obesity, diabetes, reproductive disorders, and cancer have emerged. Several recent studies have linked genotoxicity of BPA with the generation of oxidative DNA damage. Reactive oxygen species (ROS) that damage DNA are generated by metabolism of BPA and can generate DNA strand breaks and damaged DNA bases. Base excision repair (BER) is responsible for removing oxidative base lesions, such as 8-oxo-2'-deoxyguanosine (8-oxoG), and repairing single strand breaks (SSBs), yet the relationship between BPA and BER has yet to be examined. Further, the ubiquitous nature of BPA causes continual exposure of the human genome concurrent with the normal endogenous and exogenous insults to the genome, and this co-exposure may impact DNA damage response and repair. To determine the effect of BPA exposure on repair of oxidative DNA damage, DNA repair proficient and deficient cells lines were co-exposed with BPA and the oxidizing agent, potassium bromate. Repair-deficient cell lines were found to be more sensitive to the generation of oxidative damage; however, in the presence of BPA an enhanced cell survival was observed coupled with increases in the 8-oxoG content of the DNA. This protective effect and increased DNA lesion load resembles a DNA glycosylase-deficient cell phenotype, suggesting that initiation of BER is suppressed by BPA. The role of BPA in suppression of DNA repair and reduction of cell death will be discussed.

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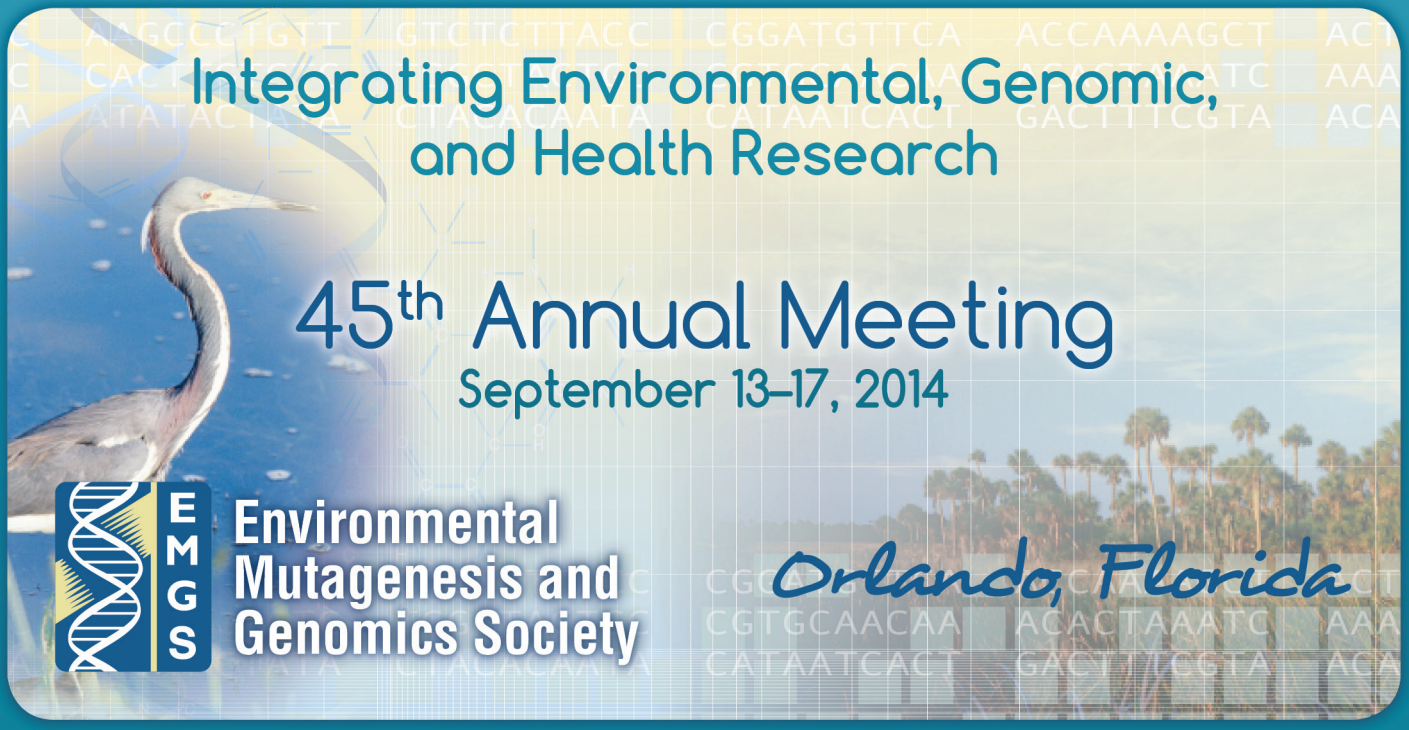

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Program Chair: Suzanne M. Morris | New Investigator Co-Chair: Michelle C. DeSimone

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