POSTER SESSION B

CELL, MOLECULAR, AND TUMOR BIOLOGY: Genetics: Mouse Models

#B202 ***Cdkn2a* and *Prkdc* interact to modify tumor susceptibility in mammary tumor prone *Trp53+/-* mice.** Anneke C. Blackburn, Jennifer S. Brown, S. Christine McLary, Stephen P. Naber, Christopher N. Otis and D. Joseph Jerry. *University of Massachusetts, Amherst, MA; Baystate Medical Center, Springfield, MA.*

Li-Fraumeni syndrome (LFS) is associated with heterozygous germline mutations in the gene encoding p53 (*TP53*). Though breast cancer is the most common tumor type observed among women with LFS, the penetrance varies considerably among individuals. Similarly, the incidence of mammary tumors in mice bearing heterozygous mutations in the p53 gene ($Trp53^{+/-}$) varies among strains (Kuperwasser et al., 2000). To determine the genetic basis for susceptibility and resistance, mammary tumor phenotypes were examined in crosses of C57BL/6 (resistant) and BALB/c (susceptible) strains of mice. Tumor phenotypes were determined in $Trp53^{+/-}$ females from a F1 intercross (C57BL/6 x BALB/c) (n=19) and a N2 backcross [(C57BL/6 x BALB/c) x BALB/c] (n=224). Susceptibility to mammary tumors segregated as a dominant phenotype in F1 females, but recessive-acting modifiers were also evident as mammary tumors occurred with a higher frequency and shorter latency in the N2 backcross population. A high frequency of loss of heterozygosity for *Trp53* was also a dominant phenotype, with 85% of BALB/c tumors (p=0.046) and 93% of F1 tumors (p=0.035) showing LOH compared to 59% of C57BL/6 and 129/Sv tumors. Hypomorphic alleles in BALB/c mice for genes encoding DNA-PKcs (*Prkdc*) and the cyclin-dependent kinase inhibitor $p16^{INK4A}$ (*Cdkn2a*) were previously identified as potential modifiers of tumor susceptibility. Therefore, segregation of these candidate gene alleles ("B" = BALB/c; "+" = wild-type or C57BL/6) was analyzed in the N2 backcross population. Considering both loci, the time to first tumor (all tumor types) was significantly different among the four genotype combinations (p=0.01). This was due mainly to the *Cdkn2a* locus (p=0.008 considering *Cdkn2a* locus only), however, this effect was restricted to mice homozygous for the BALB/c allele (B) of *Prkdc* indicating a strong interaction between the two loci. The median ages to first tumor (weeks) were: $Prkdc^{B/B}Cdkn2a^{B/B}$ 55.5; $Prkdc^{B/B}Cdkn2a^{+/B}$ 64.0; $Prkdc^{+/B}Cdkn2a^{B/B}$ 57.6; $Prkdc^{+/B}Cdkn2a^{+/B}$ 57.7. Differences in mammary tumor occurrence were not statistically significant, however, a decrease in latency of 6-7 weeks was associated with $Cdkn2a^{B/B}$ genotype in mammary tumors occurring after 56 weeks of age, suggesting differences in the mechanism of tumorigenesis between early and late onset mammary tumors in the N2 population. These results identify *Prkdc* and *Cdkn2a* as modifiers of tumor latency in $Trp53^{+/-}$ mice but cannot account for the prevalence of mammary tumors in the BALB/c strain.

POSTER SESSION B 2003690

CELL, MOLECULAR, AND TUMOR BIOLOGY: Genetics: Oncogenomics

#B203 **Exposure response to oxythioquinox in NHMEC: Impact of *p53* polymorphisms.** Maureen R. Gwinn, Diana L. Whipkey, Lora B. Tennant and Ainsley Weston. *NIOSH/CDC, Morgantown, WV.*

Exposure to some pesticides has been linked to adverse health effects, including cancer. One purpose of our study was to determine the role of interindividual variation in response to exposure to a prototypical pesticide from the quinoxaline family. The pesticide oxythioquinox (Morestan™) was first used in 1968 on citrus crops, with active products later confined to nonfood crops, limiting exposure to nursery and greenhouse employees. Recently, this pesticide was classified as a probable human carcinogen (IARC Group B2) and the use of all products containing OTQ was voluntarily canceled. However, as potentially carcinogenic exposures have already occurred, its mechanism of action is of interest. To further understand the mechanism of action of OTQ, gene expression was studied in four strains of primary normal human mammary epithelial cells. The cell strains were derived from tissues discarded at mammoplasty and obtained through the Cooperative Human Tissue Network (NCI/NDRI). Previous work in the laboratory allowed the use of cell strains containing both the major and intermediate haplotype for p53. Variation in response to OTQ by each cell strain at the protein level was detected by indirect immunofluorescence and western blot for cell cycle checkpoint proteins p53 and p21. Transcription in each cell strain was also analyzed with high-density oligonucleotide DNA microarrays (HuGeneFL 6800, Affymetrix). Microarrays were prepared with total RNA collected after OTQ treatment. Gene expression was analyzed over a 2 hr treatment period (0, 15, 60 and 120 min). RNA was harvested from the vehicle control (DMSO) at 2 h. Data Mining Tool software (Affymetrix, Santa Clara, CA) was used to separate genes in clusters based on their expression patterns over time. Interindividual variation in response to OTQ was observed in various clustering patterns for the four cell strains. Approximately 1200 RNA species were clustered in various patterns of expression. Further clustering highlighted >400 species with increased expression after treatment in one or more of the cell strains, including metabolic enzymes and transcription factors. Of these RNA species, only 32 were found to be upregulated for at least one time point in three or more of the cell strains analyzed. Cluster analysis for the >300 RNA species downregulated in one or more cell strains as a result of treatment found only 14 RNA species downregulated in three or more of the cell strains analyzed. Further analysis examined the effects of OTQ on the various genotypes. The two strains expressing the major variant of p53 had only 80 genes altered at one or more time point by 2 fold or more (49 increased, 31 decreased). The intermediate variant strains showed 100 genes altered in both strains (71 increased, 29 decreased). Although the function of these genes varied, these findings provide insight into the effects of OTQ, and emphasize the role of inter-individual variation in gene expression profiles.

POSTER SESSION B

CARCINOGENESIS: DNA Damage and Repair Mechanisms

#B204 **Prediction of deleterious single nucleotide polymorphisms (SNPs) in DNA repair genes for breast cancer association studies.** Sevtap Savas, Julia A. Knight, Laurent Briollais and Hilmi Ozcelik. *Fred A. Litwin Centre for Cancer Genetics, Mount Sinai Hospital Samuel Lunenfeld Research Institute, Toronto, ON, Canada; Epidemiology and Biostatistics, Samuel Lunenfeld Research Institute, Toronto, ON, Canada; Department of Public Health Sciences, University of Toronto, Toronto, ON, Canada; Departments of Pathology and Laboratory Medicine, (5) Mount Sinai Hospital, and (6) Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada.*

Single Nucleotide Polymorphisms are considered useful genetic markers for genetic association studies of multigenic disorders. A fraction of SNPs are located within the genes, which may affect the quantity and quality of the encoded protein. In order to study the possible association of variants of a group of DNA repair proteins with breast cancer risk, we applied a bioinformatics strategy to predict SNPs with a possible effect on protein function. Five different international SNP databases were utilized to extract SNPs in the coding regions of 19 DNA repair genes. A total of 99 validated SNPs were identified, 64 of which resulted in amino acid change (non-synonymous (ns) SNPs). NCBI conserved domain search tool was used to predict protein domains, and as a result, 26 nsSNPs were found to be located on a functional domain of the proteins analyzed. Application of phylogenic analysis using SIFT program has also suggested that a total of 26 SNPs occurred in evolutionary conserved codons. Ten nsSNPs were predicted to be both located in a protein domain and evolutionary conserved codon, suggesting that they are more likely to have effect on protein function. The frequencies of six of these nsSNPs were reported to be equal or less than 1%, indicating that they are not fixed in the populations analyzed yet. This result suggests that they are likely to be either mutations or recently occurred variations. We are currently performing further computational analyses to determine the effect of these nsSNPs on the secondary and tertiary structure of proteins. The approaches used in this study have a potential to identify important breast cancer predisposition alleles. Using pooled DNA samples from population based breast cancer and control cases, our lab is currently establishing a real-time amplification system using fluorescent probes to quantify allelic frequencies of SNPs in DNA repair genes to evaluate their possible association with breast cancer.

#B205 **Evidence of variation in poly(ADP-ribose) reactions in benign and malignant prostate cell lines.** Tamara L. McNealy, Manfred Frey, Peter Alken and M. S. Michel. *University Hospital, Mannheim, Germany; Steinbeis-Transferzentrum für Angewandte Biologische Chemie, Mannheim, Germany.*

Poly(ADP-ribosyl)ation plays important roles in cellular DNA repair mechanisms as well as in cellular proliferation and genomic stability. It has been theorized that individual differences in repair ability and poly(ADP-ribose) polymerase (PARP) activity could be used as biomarkers for cancer susceptibility. Two carcinoma cell lines, LNCaP and MatLu, and one prostate epithelial cell line, PNT1A, were tested using the H10 antibody to poly(ADP-ribose) by Western activity blotting and FACS analysis for basal and activated (10 mM H_2O_2) PARP activity. In Western blotting and FACS analysis, higher levels of basal polymer were present in the LNCaP cell line as in the prostate epithelial cell line or the MatLu cell line. LNCaP cells demonstrated greater than 90% positive analyzed

Journal Title: CANCER EPIDEMIOLOGY BIOMARKERS & PREVENTION
Volume: 11
Issue: 10 Part 2
Month: Oct
Year: 2002
Inclusive Pages: 1178S
Article Author: Gwinn MR, Whipkey DL, Tennant LB, Weston A
Article Title: Exposure response to oxythioquinox in NHMEC: Impact of p53 polymorphisms
Item Author:
Item Place:
Item Publisher:
Item Edition:
ISSN/ISBN: 1055-9965
Cited In:
Cited Title:
Cited Date:
Cited Volume:
Cited Pages:
Not Wanted After: 08/15/2008
Accept Non English: Yes
Accept Alternate Edition: No
Due Date: 9/24/2007
Renewals Allowed?: Yes
Max Cost: