PS 2147 A SINGLE AMINO ACID CONTROLS THE FUNCTIONAL SWITCH OF HUMAN CAR1 TO THE XENOBIOTIC ACTIVATED SPLICING VARIANT CAR3.

T. Chen[1, 2], L. M. Tompkins[2], G. Kim[2], C. J. Omiecinski[1] and H. Wang[2]. *[1]Center for Molecular Toxicology and Carcinogenesis, Pennsylvania State University, University Park, PA and [2]Pharmaceutical Sciences, University of Maryland School of Pharmacy, Batimore, MD.*

The constitutive androstane receptor (CAR) is constitutively activated in immortalized cell lines independent of xenobiotic stimuli. This feature of CAR has limited its use as a sensor for xenobiotic-induced expression of drug-metabolizing enzymes. Recent reports, however, revealed that a splicing variant of human CAR (hCAR3), which contains an insertion of 5 amino acids (APYLT), exhibits low basal but xenobiotic inducible activities in cell-based reporter assays. Nonetheless, the underlying mechanisms of this functional shift are not well understood. We have now generated chimeric constructs containing various residues of the 5 amino acids of hCAR3 and examined their response to typical hCAR activators. Our results showed that the retention of alanine (hCAR1+A) alone is sufficient to confer the constitutively activated hCAR1 to the xenobiotic sensitive hCAR3. Notably, hCAR1+A was significantly activated by a series of known hCAR activators, and displayed activation superior to that of hCAR3. Moreover, intracellular localization assays revealed that hCAR1+A exhibits nuclear accumulation upon CITCO treatment in COS1 cells, which differs from the spontaneous nuclear distribution of hCAR1. Mammalian two-hybrid and GST pull-down assays further demonstrated that hCAR1+A interacts with the co-activator SRC-1 and GRIP-1 at low level prior to activation, while at significantly enhanced level in the presence of CITCO. Thus, the alanine residue in the insertion of hCAR3 largely directs the xenobiotic response of hCAR3 through direct and indirect mechanisms. Activation of hCAR1+A may represent a sensitive methodology for the identification of hCAR activators.

PS 2148 PXR STATUS IS ASSOCIATED WITH CYP INDUCTION, HISTOPATHOLOGICAL EFFECTS, AND REDUCED CLEARANCE OF NONYLPHENOL.

L. C. Mota and W. Baldwin. *Environmental Toxicology, Clemson University, Clemson, SC.*

Nonylphenol, a byproduct of alkylphenol ethoxylates, is a ubiquitous chemical that binds several nuclear receptors. Previous studies in our laboratory have demonstrated that nonylphenol also activates the pregnane X-receptor (PXR), a xenobiotic sensing nuclear receptor. Therefore, we are studying the role of PXR in protecting organisms from nonylphenol. Wild-type, PXR-null, and hPXR (PXR-null mice with the human PXR receptor) mice were treated with nonylphenol at 0, 50 and 75 mg/kg/day for one week, livers excised for histopathology and CYP induction, and serum collected to determine nonylphenol concentrations. Wild-type mice treated with nonylphenol show induction of Cyp2b (males and females), as well as Cyp2c and Cyp3a (only in males). Induction of CYPs in wild-type mice was PXR-dependent as determined by the lack of induction in PXR-null mice. Interestingly, hPXR mice only showed induction of Cyp2b. Liver histopathology indicated significant hepatocyte hypertrophy in nonylphenol-treated wild-type mice, indicating that wild-type mice had an acute response to compensate for nonylphenol exposure. PXR-null and hPXR mice did not show any significant liver histopathological changes; however, these mice show greater hepatocyte hypertrophy in untreated animals. Serum concentrations of nonylphenol were significantly higher in PXR-null mice compared to untreated mice. Taken together the histopathology, lack of CYP induction, and serum nonylphenol concentrations indicate that PXR-null mice are unable to respond to a nonylphenol challenge and eliminate the toxicant as observed by higher nonylphenol serum concentrations, and lack of CYP induction and liver hypertrophy. Overall this data suggest that PXR is important in eliminating nonylphenol and ultimately protecting individuals from its potential adverse effects. Funding for this research is provided by NIEHS.

20036573

PS 2149 CRITICAL ROLE OF NRF2 CYSTEINE RESIDUES IN OXIDANT/ELETROPHILE-SENSING AND SIGNAL TRANSDUCTION.

Q. Ma[1, 2] and X. He[1]. *[1]Receptor Biology Lab/TMBB/HELD, National Institute for Occupational Safety and Health, Morgantown, WV and [2]Department of Biochemistry, West Virginia University School of Medicine, Morgantown, WV.*

Cells respond to oxidants and electrophiles by activating receptor/transcription factor Nrf2 to coordinate induction of cytoprotective genes critical for defense against oxidative and other stresses. Activation involves blocking the ubiquitination-proteasomal degradation of Nrf2. Modification of cysteine thiol groups by inducers in the linker region of Keap1, which congregates Nrf2 into the Keap1/Cul-3-based E3 complex for ubiquitination, is important but not sufficient for activation of Nrf2. Here we show that evolutionally conserved cysteine residues of Nrf2 are critical for Nrf2 regulation. FlAsH (an arsenic-based fluorophore) and phenylarsine oxide (PAO) potently induce Nrf2 target genes and bind to Nrf2 in vitro and in vivo. Binding is inhibited by prototypical inducers arsenic and tBHQ. PAO affinity pulldown and mutation of individual cysteine to alanine reveal that C235, C311, C316, C414 and C506 are critical for binding and binding is modulated by intra-molecular interactions. To corroborate the functions of cysteine residues, Nrf2 wild-type or mutants are expressed in Nrf2 knockout cells to reconstitute Nrf2 regulation. Nrf2 mutants have reduced t1/2 values that inversely correlate with increased binding to Keap1 and polyubiquitination of mutant proteins. Remarkably, the mutants fail to respond to arsenic for Nrf2 activation and gene induction. Furthermore, mutations at C119, C235, and C506 impede binding of Nrf2 to endogenous antioxidant response element and to coactivator CBP/p300. The findings demonstrate for the first time that Nrf2 cysteine residues critically regulate oxidant/eletrophile sensing, repress Keap1-dependent ubiquitination-proteasomal degradation, and promote recruitment of co-activators, such that chemical sensing, receptor activation, and transcription activation are integrated at the receptor molecule.

PS 2150 SEARCHING FOR THE SPECIFIC INTRACELLULAR TARGET OF BORIC ACID THAT LEADS TO THE INHIBITION OF CALCIUM RELEASE IN PROSTATE CANCER CELL LINES.

S. E. Kobylewski[1], K. A. Henderson[1] and C. D. Eckhert[2]. *[1]Molecular Toxicology, University of California Los Angeles, Los Angeles, CA and [2]Environmental Health Sciences, University of California Los Angeles, Los Angeles, CA.*

Boric acid (BA) is a dietary component found naturally in the environment. Low levels reduce the incidence and mortality of prostate cancer. Our current studies have shown that low doses of BA can inhibit calcium release from the ryanodine receptor (RyR) in the endoplasmic reticulum in response to RyR agonists in DU-145 prostate cancer cells. The dose of BA required to inhibit RyR-dependent release of stored calcium is 10 to 100 fold higher in non-tumor PWR-1E prostate cells than DU-145 cells, indicating that there may be differences in RyR isoform expression between cell lines. In this project our objective is to identify the specific RyR isoforms and/or accessory proteins that potentially interact with BA and cause the inhibition of calcium release. The identification of each cell line's specific RyR isoforms was assessed with reverse-transcription PCR and restriction enzyme digestion. Both DU-145 and PWR-1E cells express RyR isoform 1 (RyR1) but LNCaP prostate cancer cells do not. All three cell lines express RyR2 and lack RyR3. Real-time PCR showed that RyR2 expression is lower in DU-145 cells than it is in the other two cell lines. Overall RyR mRNA expression in DU-145 cells treated with varying doses of BA was measured using real-time PCR. There was no significant change in mRNA expression indicating that BA exerts its effects at the receptor and not the transcription level. These results indicate that the BA-sensitivity between tumorgenic and non-tumorgenic cell lines is probably not due to a difference in RyR isoform expression. The cytoplasmic surface of all three RyR isoforms serves as a surface for binding of several accessory proteins that modulate opening and closing of the calcium channel. Future studies are needed to determine if differences in calcium release inhibition by BA can be explained by the interaction of BA with one or more of these accessory proteins or their RyR binding sites.

PS 2151 TRPV1 MEDIATES LUNG TOXICITIES OF SPECIFIC PARTICULATE MATERIALS.

C. A. Reilly, M. Johansen, K. Thomas, J. Veranth and G. Yost. *Pharmacology and Toxicology, University of Utah, Salt Lake City, UT.*

Particulate materials (PM) are ubiquitous air contaminants with established effects on human respiratory and cardiovascular health. Multiple features of PM have been correlated with adverse outcomes. However, the molecular sensors that detect, differentiate, and initiate responses to PM as well as the contributions of PM:PM sensor interactions to PM toxicity have not been fully elucidated. We hypothesized that specific calcium channels expressed by lung cells can both differentially detect PM and initiate responses that ultimately manifest as lung damage. Transient Receptor Potential Vanilloid-1 (TRPV1) over-expressing lung cells engineered to express high levels of cell surface TRPV1, were treated with multiple prototype PM. Calcium flux initiated by 3 of the PM, coal fly ash medium (CFAm), crystalline silica (Xtal), and synthetic residual oil fly ash (ROFA) was inhibited by the TRPV1