technique has been applied to models of metal carcinogenesis. Most recently, immunofluorescence and immunochemistry have been used to determine the distribution of free radicals in cells and tissues. In summary, the advances the immunospin trapping technique affords in accurate and sensitive determination of biological free radicals is having a major impact on our understanding of the role of such radicals in toxic response and mechanism.

S 635 MOLECULAR AND GENOMIC INSIGHTS INTO THE NRF2-REGULATED OXIDATIVE STRESS RESPONSE: IMPACT ON CARCINOGENESIS.

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The transcription factor Nrf2 plays a significant role in protecting cells from endogenous and exogenous stresses. Mice lacking Nrf2 are more sensitive to the hepatic, pulmonary, ovarian, and neurotoxic consequences of acute exposures to environmental agents and drugs as well as chronic exposures to cigarette smoke and other carcinogens. Under quiescent conditions, Nrf2 interacts with the actin-anchored protein Keap1, leading to low basal expression of Nrf2-regulated genes. However, upon recognition of chemical signals imparted by oxidative and electrophilic molecules, Nrf2 is released from Keap1, escapes proteasomal degradation, translocates to the nucleus, and trans-activates the expression of several dozen cytoprotective genes that enhance cell survival. This symposium will highlight recent exciting findings in the field including different mechanisms by which chemical exposure can activate Nrf2, the role of Nrf2 in modifying chemical carcinogenesis and how cancer cells can highjack this protective system to increase survival.

S 636 FUNCTION AND SIGNAL TRANSDUCTION OF NRF2 FROM A METAL'S PERSPECTIVE.

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Metals comprise a large group of environmental and occupational toxicants; many are human carcinogens. Although the mechanism by which metals cause cancer and toxicity remains unclear, oxidative damage is known to be a critical component of metal action. In recent years, Nrf2 has emerged as a prooxidant/antioxidant-activated receptor/transcription factor that integrates a variety of oxidative stimuli from both exogenous and endogenous sources and coordinately regulates the antioxidant response element-dependent transcription of a battery of cytoprotective genes, thereby protecting cells from oxidative stress and damage. The function and signal transduction of Nrf2 in response to carcinogenic metals were analyzed in this study, with focus on transcriptional protective mechanisms against metal-induced oxidative stress by Nrf2 and the molecular events of Nrf2 activation by toxic metals. Exposure to carcinogenic metals such as As, Cr, and Cd induces apoptosis and increased production of ROS. Loss of Nrf2 by gene knockout and SiRNA knockdown markedly increases the ROS production and cell death in the presence of the metals. Metals activate Nrf2 by stabilizing the Nrf2 protein via a signaling mechanism distinctively different from that of phenolic antioxidants in that metals disrupt Nrf2/Keap1 association in the nucleus whereas phenolic inducers do not. Both Nrf2 and Keap1 are ubiquitinated in the cytoplasm; upon activation, the two proteins translocate into the nucleus in a complex and are deubiquitinated in the nucleus. Chromatin immunoprecipitation revealed binding of Nrf2 and Maf G/K to the ARE located in the enhancers of Nqo1 and Ho1 for gene transcription. The findings suggest a unique molecular model of metal action in metal sensing and transcriptional gene regulation in mammalian cells.

S 637 REDOX REGULATION OF NRF2: *IN VITRO* SIGNALING CIRCUITRY AND *IN VIVO* CARCINOGENESIS/ANTI-CARCINOGENESIS FUNCTIONS.

T. Kong. *The State University of New Jersey, Piscataway, NJ.* Sponsor: C. Corton.

The bZIP transcription factor Nrf2 plays an important role in antioxidant defense mechanisms against oxidative and electrophilic stresses involved in many disease processes including cancer. Many dietary cancer preventive compounds and exogenous/endogenous oxidants can activate cellular Nrf2 and enhance its nuclear transcriptional activities through redox signaling of a combination of signaling proteins including Keap1, PKC, MAPK, PI3K, GSK, FYN depending on the types of the cells and stimuli. Activation of Nrf2 induces phase II detoxifying/antioxidant enzymes GST, QR, HO-1 and GCS as well as genes involved in ubiquitination, electron transport, transporters, cell growth and apoptosis, cell adhesion, kinase and phosphatases, transcription factors and co-regulators. The overall biological functions would appear to afford cellular protection against oxidative and carcinogenic damages, at least in normal tissues such as the liver and intestine. Consistent with this, Nrf2-/- mice are more prone to carcinogen-induced cancers including skin, colon and other cancers, and Nrf2 is required for the protective effects of many cancer preventive compounds. Additionally, Nrf2-/- mice are more susceptible to dextran sulfate-induced colon inflammation in part due to decreased expression of antioxidant enzymes coupled with enhanced expression of inflammatory cytokines and mediators. The regulation of antioxidant and inflammatory (whether directly or indirectly) signaling by Nrf2 would dictate the carcinogenesis versus the anti-carcinogenesis effects in response to external environmental cues. (Supported by NIH grant R01-CA094828).

S 638 NRF2-MEDIATED SIGNALING: ROLE IN LUNG INFLAMMATION AND TUMORIGENESIS.

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NRF2-mediated signaling is critical to protection of the lung against oxidative stress-induced injury and inflammation. Chronic lung inflammation is also an important determinant of tumorigenesis. Recent evidence has suggested that NRF2 protects against the pathogenesis of lung injury and inflammation in response to oxidant stimuli, but may contribute to the development of tumors. However, the mechanisms of NRF2 signaling in pulmonary diseases are not completely understood. To begin to address this question, we have developed in silico computational tools for discovering functional single nucleotide polymorphisms (SNPs) in transcription factor binding sites that may impact target gene expression. In this presentation, we discuss the integrated application of computational tools and knockout mouse models to investigate NRF2 effector mechanisms in models of lung injury, inflammation, and tumorigenesis.

S 639 DYSFUNCTIONAL KEAP1-NRF2 INTERACTIONS IN LUNG CANCER AND IMPLICATIONS FOR OTHER CANCERS.

S. Biswal. *Johns Hopkins University, Baltimore, MD.* Sponsor: C. Corton.

Nuclear factor erythroid-2 related factor 2 (NRF2) is a redox-sensitive transcription factor that positively regulates the expression of genes encoding antioxidants, xenobiotic detoxification enzymes, and drug efflux pumps, and confers cytoprotection against oxidative stress and xenobiotics in normal cells. Kelch-like ECH-associated protein 1 (KEAP1) negatively regulates NRF2 activity by targeting it to proteasomal degradation. Increased expression of cellular antioxidants and xenobiotic detoxification enzymes has been implicated in resistance of tumor cells against chemotherapeutic drugs. Systematic analysis of the KEAP1 genomic locus in lung cancer tumors from patient and cell lines revealed deletion, insertion, and missense mutations in functionally important domains of KEAP1 and a very high percentage of loss of heterozygosity at 19p13.2, suggesting that biallelic inactivation of KEAP1 in lung cancer is a common event. Sequencing of KEAP1 in lung cancer cell lines and non-small-cell lung cancer (NSCLC) samples revealed somatic mutations in KEAP1 gene. All the mutations were within highly conserved amino acid residues located in the Kelch or intervening region domain of the KEAP1 protein, suggesting that these mutations would likely abolish KEAP1 repressor activity. Evaluation of loss of heterozygosity at 19p13.2 revealed allelic losses in 61% of the NSCLC cell lines and 41% of the tumor samples. Decreased KEAP1 activity in cancer cells induced greater nuclear accumulation of NRF2, causing enhanced transcriptional induction of antioxidants, xenobiotic metabolism enzymes, and drug efflux pumps. Biallelic inactivation of KEAP1 is a frequent genetic alteration in NSCLC. More recently, we and others have found mutations in KEAP1 gene in prostate and breast cancer. This presentation will focus on presenting evidence that loss of KEAP1 function leading to gain of NRF2-mediated gene expression in cancer helps tumor cells to manipulate the NRF2 pathway for their survival against chemotherapeutic agents and radiotherapy.

S 640 KEAP1-NRF2 SIGNALING AS A TARGET FOR CANCER CHEMOPREVENTION: PRE-CLINICAL AND CLINICAL PERSPECTIVES.

T. W. Kensler. *Environmental Health Sciences, Johns Hopkins University, Baltimore, MD.*

The development of Nrf2 knockout mice provided the first key insights into the toxicological importance of this transcription factor signaling pathway. As examples, Nrf2 knockout mice are more sensitive to the carcinogenicity of benzo[a]pyrene and N-nitrosobutyl-(4-hydroxybutyl)-amine in the forestomach and bladder, respectively. Nrf2-regulated genes govern a broad-based adaptive response to oxidant and electrophilic stresses. Nrf2-mediated transcription of these genes is activated by a number of cellular stresses (endoplasmic reticulum stress, ox-

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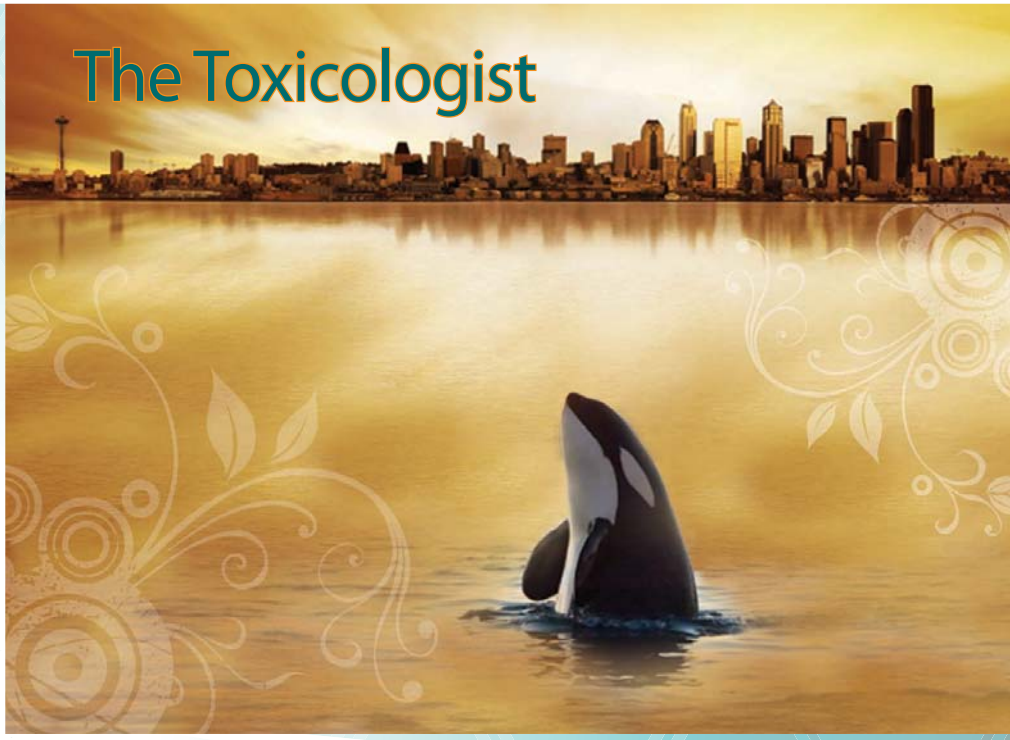

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