CEEH Pilot Projects Program Abstracts from Year 9

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Molecular Mechanisms and Functional Significance of As3+-Induced Regulation of the Glutathione Antioxidant Defense System • Christopher C. Franklin, PhD, Research Assistant Professor, Department of Pathology, UW

Trivalent arsenite (As3+) is a known human carcinogen associated with cancers of the skin, lung, liver, and bladder. While the molecular events mediating Az3+-induced cell transformation remain unclear, increased production of reactive oxygen species (ROS) and aberrant gene expression have been implicated in As3+-induced genotoxicity and cytotoxicity. Mammalian cells possess a number of antioxidant defense mechanisms to counteract the deleterious effects of oxidative stress. The tripeptide glutathione (GSH) is the most abundant non-protein thiol antioxidant within the cell and plays a central role in maintaining cellular redox homeostasis and protecting against oxidative injury. Glutamate cysteine ligase (GCL) is the first and rate-limiting step in GSH biosynthesis. GCL is a heterodimeric holoenzyme consisting of a catalytic subunit (GCLC) and a modifier subunit (GCLM). Transcriptional control mechanisms play important roles in regulating GCL activity and intracellular GSH homeostasis and the expression of both GCL subunits are transcriptionally induced in response to most xenobiotics that induce Phase II detoxication enzymes, including heavy metals, oxidants, phenolic antioxidants, and GSH-pepleting agents. Interesting, our preliminary studies suggest that while high concentrations of As3+ (>5 uM) induce apoptotic cell death in hepatocytes, subtoxic doses of As3+ (>5 uM) enhance cellular GSH biosynthethic capacity by inducing GCL expression and activity. However, while GSH regulates cellular sensitivity to As3+-induced apoptosis, it is not clear whether up-regulation of the GSH antioxidant defense system per se alters cellular sensitivity to As3+-induced apoptotic cell death. The goals of this study are to examine the molecular mechanisms mediating As3+-induced GSH biosynthesis and determine whether this response regulates cellular sensitivity to As3+-induced cytotoxicity. The specific aims of this study are: (1) To identify the cis-acting promoter elements and transription factors that mediate As3+-inducible expression of gclc and gclm. (2) To determine whether increase GSH biosynthetic capacity regulates cellular sensitivity to As3+-induced cytotoxicity.

Chemical-Viral Interaction in a Mouse Model for Liver Cancer • Warren C. Ladiges, DVM, Professor, Department of Comparative Medicine, UW

Primary hepatocellular carcinoma is one of the most common malignancies worldwide. It is the most frequent primary malignancy of the liver and is rising in incidence at an alarming rate in the United States and other developed western countries. Clinically, HCC is usually diagnosed late in the disease process, so the prognosis is extremely poor with medium survivals of less than one year. The main risk factors of HCC are the hepatitis B and hepatitis C viruses, which together account for three fourths of all cases worldwide. Other risk factors include aflatoxin B1 ingestion, alcohol consumption, and several hereditary diseases. Recently, diabetes has been implicated as an additional risk factor. Even though these risk factors have been associated with HCC, the viral-chemical etiology, as well as molecular mechanisms, of HCC pathogenesis remains largely unknown. The objective of this proposal is to determine if chemical carcinogenesis in the liver is enhanced by hepatic expression of hepatitis C NS5A viral protein in a transgenic mouse model. The specific aims are to 1) evaluate the liver tumor phenotype NS5A transgenic mice treated with the chemical carcinogen diethyl-nitrosamine (DEN), and 2) investigate the molecular mechanisms in the liver associated with DEN-NS5A interaction. Further characterization of the investigators' NS5A transgenic mouse model would help establish its usefulness in identifying important chemical risk factors. This pilot project proposes to use the well-established hepatic carcinogen DEN as a prototype chemical to investigate the pathological effect of exposure to a carcinogen in the presence of NS5A viral protein.

Development of an in situ Assay for Detection of Random Single Nucleotide Mutations • Lawrence A. Loeb, MD, PhD, Professor, and John P. Anderson, PhD, Department of Pathology, UW

The goal of this study is to measure and quantitate random mutations in normal human tissue following exposure to exogenous carcinogens. Detection of random nucleotide mutations in DNA obtained from human tissue samples is hampered by the exceedingly low rate of mutation for these events. For random base substitutions estimates of 1 x 10-7 – 10-10 nucleotides/cell/generation have been measured at the HPRT (hypoxanthine guanine phosphoribosyl transferase) locus. The HPRT reversion assay utilized the ability of HPRT mutants to grow in 6-thioguanine selective media while cells with functional HPRT genes cannot. This assay is limited to cells that can grow from single clones and has been applied to peripheral lymphocytes and fibroblasts obtained from human and other species, as well as to cells grown in tissue culture. The investigators are developing a method to measure single nucleotide changes within the repetitive rRNA genes that relies on detection and amplification of a single base change and subsequent visualization in intact nuclei. In addition, the assay should be applicable to any organism that has multiple identical copies of rRNA genes and will be useful for a

variety of studies including endogenous and environmental mutagenic exposures, mutagenicity of chronic disease and population studies. Preliminary results have shown that the assay can detect a single nucleotide change in nuclei obtained from mouse cells grown in tissue culture, with a sensitivity of approximately 1x10-6/nt/cell.

Genomic Response After Exposure to Particulate Matter • Daniel Luchtel, PhD, Department of Environmental and Occupational Health Sciences, University of Washington

The proposed research is a parallel physiological and genetic study of the mechanism of toxicity caused by exposure to ambient particulate matter (PM). The physiological study is supported by an existing grant while funding is being requested to carry out the genetic (microarray) studies. Responses of a normal C57BL/6J mouse strain will be compared with two transgenic single knockout strains–apolipoprotein E null (ApoE-/-) and glutamate cysteine ligase modifier subunit null (GCLM-/-)–and one double knockout strain (ApoE-/-, GCLM-/-), all on the same background. Two types of exposure will be done: 1) a fine fraction of Seattle ambient PM collected on filters will be administered to animals via oro-laryngeal instillation and 2) diesel exhaust inhalation studies. Cardiovascular function will be monitored continuously during and after exposure to determine the temporal response to PM exposure. This data will then be used to determine time points when genetic responses will be studied using microarrays. The aim is not to determine the response of individual genes, but to identify genetic and metabolic pathways that are upregulated or downregulated.

Functional Polymorphisms of BCRP • Qingcheng Mao, PhD, Assistant Professor, Department of Pharmaceutics, UW

The human breast cancer resistance protein (BCRP/ABCG2), the recently discovered ABC transporter, has substantial substrate and tissue localization overlap with P-glycoprotein. Therefore, like P-glycoprotein, BCRP may play a significant role in drug disposition of a wide variety of drugs. Recent clinical studies demonstrated a considerable degree of interindividual variability in the oral bioavailability and clearance of drugs that are BCRP substrates such as topotecan. This interpatient variation is likely a consequence of genetic variations within the BCRP gene that might alter BCRP expression or activity. Indeed, common allelic variants of BCRP have recently been identified in DNA isolated from different ethnic groups with marked variatiion in genotypes and allele frequencies among different populations. At present, these naturally occurring mutations have not been fully characterized with respect to their effect on BCRP function and expression. Changes in BCRP activity associated with drug-selected mutations have been reported. Selection of cells in mitoxantrone or adriamycin plus verapamil resulted in changes in amino acid at position 482 in BCRP and the

mutants displayed altered substrate specificity. This suggests that genetic variations leading to differences in function and expression are possible for BCRP. Furthermore, BCRP has been implicated in protection from toxicity of environmental factors such as normal food constituents. Thus, humans with genetic variations in BCRP gene leading to reduced transport activity may be at increased risk exposure to environmental toxins that are BCRP substrates. To investigate genetic basis for the apparent interindividual variability in the pharmacokinetics and toxicity of BCRP substrates, the investigators propose to characterize naturally occurring BCRP variants with respect to their expression and transport activity. A genetic basis of changes in BCRP function and expression that the investigators expect to observe can help explain and predict alterations in pharmacokinetics and toxicity of drugs or environmental toxins that are BCRP substrates in patients.

Loss of Imprinting in Chemically Induced Mammary Carcinogenesis • Helmut Zarbl, PhD, Cancer Biology, Fred Hutchinson Cancer Research Center; Affiliate Professor, Dept. of Environmental and Occupational Health Sciences, UW

The CTCF gene encodes a multivalent transcription factor with tumor suppressor activity. A comparison of CTCF cDNA sequences in rat strains that are resistant (Cop) or susceptible (F344) to mammary carcinogenesis failed to detect constitutional differences within the CTCF coding sequences. CTCF protein levels were also comparable in normal mammary cells of F344 and Cop rats. However, a comparison of CTCF protein levels in normal mammary tissues to those in tumors, demonstrated significantly reduced expression of CTCF protein levels in about 70% of mammary carcinomas, and in general lower CTCF expression was correlated with more malignant histopathologies. When the investigators evaluated the level of CTCF protein expressed in mammary tumors arising in ((F344 X Cop) F1 X F344) N2 backcross progeny, there was a lack of concordance between reduced CTCF expression levels and susceptibility to mammary carcinogenesis. Thus genetic susceptibility to mammary carcinogenesis was not due to mutations in CTCF or regulations of its expression. However, our data suggested that somatic alterations in CTCF protein expression do contribute to chemically induced mammary carcinogenesis. In addition to its function as a transcription factor, CTCF plays a central role in gene imprinting. More recent studies have identified BORIS (Brother of the Regulator of Imprinted Sites), a paralog of CTCF with a high degree of structural similarity. However, unlike the ubiquitously expressed CTCF gene, BORIS is only expressed in testis during spermatogenesis, and is associated with genome wide demethylation (loss of imprinting) and concomitant silencing of CTCF expression. Co-expression of CTCF and BORIS has only been detected in tumors, where BORIS may direct epigenetic reprogramming of CTCF target sites, thereby interfering with the expression and tumor-suppressing function of CTCF. The overall goal of the proposed studies is to define the role of the altered DNA methylation, repression of CTCF, and reactivation of BORIS expression in mammary carcinogenesis.