CAD MAPK site reversed the response of effectors resulting in a decreased *de novo*

n DNA damage induced G1 cell cycle

i, Zhiyuan Shen. Molecular Genetics and New Mexico School of Medicine, 915 erque, NM 87131

identified as a BRCA2 and Cip1/p21 to a highly conserved domain of BRCA2 omain of the CDK-inhibitor p21/Cip1. t the BCCIPα enhances the inhibitory e H1 kinase activity of CDK2, and that of certain tumor cells. An alternatively so expressed in various human tissues.

hat overexpression of BCCIP inhibits cell ition, suggesting a potential role of BCCIP cle. To further test this, RNA interference shutdown the expression of BCCIPα and parental HT1080 cell line underwent the radiation. However, cells with BCCIP able to arrest in G_1 phase after the same induction of p21/Cip1 by DNA damage was ls interfered with RNAi. These results BCCIP in DNA damage-induced G_1-S

eration by dibutyryl cyclic AMP in SH- cell line

Yun-Il Lee, So-Young Kim, Yong-Sung mistry and Molecular Biology, College of University, Yongon-Dong 28, Seoul, Republic of

. cAMP signaling pathway can determine oliferation, differentiation, senescence and anism of regulating cell fate by cAMP are or induction of different cellular response MP is poorly understood. Therefore, the ssess the effect of cAMP on cellular e changes in expression and activity of progression in human neuroblastoma SH-

treated with cAMP analogue, dibutyryl thesis and distribution of cells in S phase activity and phosphorylation of vas increased without changes in CDK2 ession of CDK2 related cyclins, cyclin A The expression of p27Kip1 was decreased vas stimulated by treatment with dibutyryl ed cells. When the cells were treated with inhibitor, the degradation of p27kip1 hibited. It suggested that degradation of ia proteosome.

ites cellular proliferation and cell cycle astoma SH-SY5Y cells. It increases CDK2 Rb protein, expression of cyclin A and E protein expression by stimulating the S proteosome.

621.9

Loss of CBF-mediated transcription by deletion of the Cbf-b/Nfy-a gene results in early embryonic lethality, inhibition of cell growth and apoptosis

Anuradha Bhattacharya, Benoit de Crombrugghe, Richard Behringer, sankar Maity. Molecular Genetics, UT MDACC, 1515 Holcombe Blvd., Houston, TX 77030

To understand the physiological function of the mammalian heterotrimeric CCAAT binding factor CBF, in vivo, we have generated a conditional Cbf-b mouse mutant by introducing loxP sites in the murine Cbf-b gene. Controlled expression of cre recombinase deletes the gene, which leads to loss of CBF-mediated transcription, since CBF has no activity in the absence of the CBF-B subunit. Homozygous deletion of Cbf-b in fertilized oocytes causes early mouse embryonic lethality. Similarly, homozygous deletion of Cbf-b in primary cultures of mouse embryonic fibroblast (MEFs) results in inhibition of cell proliferation and later to apoptosis. Cell cycle analysis of the MEFs indicated that Cbf-b null cells show an almost complete block in entry into S-phase and also an arrest in mitosis. An increase in caspase-2 mRNA precedes apoptosis, suggesting that the induction of apoptosis may be caused by activation of caspase-2. Altogether our results demonstrate that: (1) CBF is required for cell cycle progression; (2) CBF is a survival factor in mammalian cells and (3) apoptosis is induced in the absence of CBF-mediated transcription. Ongoing work is testing whether loss of CBF activity inhibits growth of tumors in mice.

621.10

The Role of Endothelial Cells As An Intracellular Mechanism Involved In Antiphospholipid Antibody Upregulation

Monica Charlene Lindsey[1], Sylvia Pierangeli[2]. [1]Biochemistry, Clark Atlanta University, P.O. Box 92637, Atlanta, GA 30314, [2]Microbiology/Biochemistry/Immunology, Morehouse School of Medicine, Atlanta, GA

The association of antiphospholipid antibodies with thrombosis in patients with Antiphospholipid Syndrome (APS) is well documented in humans and in animal studies. However, the mechanism(s) by which these antibodies induce thrombosis is the subject of much current study. Previous studies have shown that aPL antibodies upregulate several endothelial cells (ECs) cellular adhesion molecules (CAMs). What is uncertain is the molecular and intracellular events that are induced by aPL interaction with ECs leading to expression of CAM. Although there is a wealth of data suggesting that aPL antibodies activate ECs in vitro and in vivo and they enhance thrombosis, it is unclear what the relative roles are of the ECs adhesion molecules: intracellular cell adhesion molecules-1 (ICAM-1)and vascular cell adhesion monocytes in these processes. We hypothesized that APL antibodies upregulate CAM's on ECs, causing activation. To examine these questions, this investigates the molecular and intracellular events of EC activation mediated by aPL. In this study, we examine the mechanism of aPL by assessing the following areas:

whether aPL-mediated upregulation of CAMs on ECs involves de novo protein synthesis, cytoskeleton movements, and mitogen-activated protein kinase C activation. To achieve these aims, experiments will are conducted through such methods as cell isolation and culture, ELISA CAM expression, western blot, northern blot, and other molecular biology techniques.

621.11

GADD45 is an Oxidative Stress Response Protein

Jacquelyn Jo Bower, Stephen S. Leonard, Yong Qian, Fei Chen, Xianglin Shi. Pathology and Physiology Research Branch, National Institute for Occupational Safety and Health, 1095 Willowdale Road, Morgantown, West Virginia 26505

Cell cycle arrest is a major mechanism for the reparation of DNA damage caused by a variety of insults including UV-induced damage, radiation, and exposure to some transition metals. GADD45 (growth arrest and DNA damage-inducible protein 45) is one of many proteins involved in the regulation of cell cycle arrest. This protein has been shown to interfere with the interaction between the cyclin dependent kinase, cdc2, and Cyclin B1, causing cell cycle arrest at the G2/M phase. In this report we investigated the involvement of reactive oxygen species

(ROS) in Cr(VI)- and As(III)-induced GADD45 expression in the human airway epithelial cell line, BEAS2B. Cr(VI) induces ROS generation as demonstrated by ESR and confocal microscopy using specific fluorescent dyes for superoxide ($O_2^{\cdot-}$) and H_2O_2, the precursor of hydroxyl radical ($^{\cdot}OH$). Western blots indicated an induction of GADD45 by Cr(VI) and As(III), which is correlated with the generation of ROS. Here we report that GADD45 is induced in response to ROS generation, particularly $O_2^{\cdot-}$ and $^{\cdot}OH$. Furthermore, the induction of GADD45 by Cr(VI) was suppressed by the over-expression of superoxide dismutase (SOD) and glutathione peroxidase (GPx). Since the overexpression of SOD and GPx substantially decreased GADD45 induction by Cr(VI), we concluded that GADD45 is an oxidative stress response protein.

621.12

Retinoblastoma dependent and independent functions of epidermal keratin K10 in transgenic mice

Mirentxu Santos[1], Sergio Ruiz[1], Carmen Segrelles[1], Marc Vooijs[2], Hugo Leis[1], Jose Luis Jorcano[1], Jesus M. Paramio[1]. [1]Epithelial Damage, Repair and Tissue Engineering, C.I.E.M.A.T., Avda. Complutense 22, MADRID, MADRID 28040 Spain, [2]Molecular Biology and Pharmacology, Washington University School of Medicine, Saint Louis, MI

Our previous work in vitro and in transgenic mice, shows that overexpression of a differentiation-associated, keratin K10, inhibits keratinocyte proliferation by sequestering signalling molecules (Akt, and an atypical PKC), thereby altering pRb phosphorylation (Paramio et al., Moll.Cell. Biol 21:7449-7459, 2001; Santos et al., J.Biol. Chem. 21:19122-19130, 2002).

To analyze the specific functions and the relationships between K10 and Retinoblastoma (Rb) in epidermis in vivo, we have crossed our transgenic mice in which human K10 is ectopically expressed in the basal layer of epidermis (bK5hK10 mice) with the epidermal-specific Rb knock out mice (Rbf/f K14 cre mice).

Epidermis in K5K10 mice is hypoplasic , highly conificated and BrdU incorporation is decreased while the skin of Rb cre shows moderate hyperplasia and hyperkeratosis, with increased levels of BrdU incorporation.

The dramatic decrease in epidermal proliferation observed in K5K10 mice, is restored in the Rb f/f K14 cre bK5hK10 mice where a great hyperplasia,hyperkeratosis and elevated levels of BrdU incorporation are observed.

These results suggest that the control of epithelial proliferation in the skin in vivo, elicited by K10, needs Rb functional.

621.13

Ethanol causes p53-dependent cell cycle arrest and apoptosis of SK-N-SH neuroblastoma cells.

Yongil Kwon, Myungae Bae, Wonho Kim, Byoungjoon Song. LMBB/NIAAA/NIH, Park 5 Bldg, room 425, 12420 Parklawn drive, Rockville, Maryland 20892-8110

Chronic alcohol drinking can damage many organs including liver, pancreas, and brain. However, the mechanism of cell or organ damage is still poorly understood. Therefore, we hypothesized that changes in the early signaling cascades are critically important in ethanol-mediated cell death. In this study, we investigate the role of the MAP kinases during ethanol-induced damage to SK-N-SH neuroblastoma cells. Ethanol caused time- and dose-dependent cell death in SK-N-SH cells. Ethanol increased c-Jun N-terminal protein kinase (JNK) activity in a time- and concentration dependent manner. Within 15 min after ethanol exposure, JNK activity increased and the elevated JNK activity persisted until 16 h after exposure to ethanol. In contrast, p38 kinase activity was transiently increased between 15 min and 4 h after ethanol treatment before it returned to control level. The activation of JNK and p38 kinase was important in cell death since treatment with a respective inhibitor of JNK or p38 kinase significantly reduced the rate of ethanol-induced cell death. Ethanol-induced cell death was accompanied by increased cytochrome C release and caspase 3 activity observed at 12 h in a successive manner. In contrast, the level of anti-apoptotic Bcl-2 protein did not change. In addition to JNK activation, ethanol also increased phosphorylation of p53, which led to accumulation of p53 protein at 1 h after ethanol exposure. The p53 activation was followed by increase in p21 tumor suppressor protein accompanied by a gradual decrease in phospho-Rb protein. Taken together, these results strongly indicate that ethanol causes apoptosis of SK-N-SH neuroblastoma cells by stimulating p53-related cell cycle arrest and apoptosis possibly mediated through activation of the JNK-related cell death pathway.

COMPLEX BIOLOGICAL OXIDATIONS (622.1-622.11)

622.1

Cloning and expression of ribonucleotide reductase from *Archaeoglobus fulgidus*

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Ribonucleotide reductases (RNRs) catalyze the conversion of nucleotides to deoxynucleotides in all organisms thus playing a central role in DNA synthesis and repair. RNRs are important targets in biotechnology because drugs that inhibit these enzymes would find ready use as anticancer agents. Although all RNRs catalyze the same reaction, their essential metal cofactors have not been evolutionarily conserved. There are currently three classes of RNRs that are distinguished by their enzymatic cofactors. Class I contains a diferric cluster and an observable tyrosyl radical, Class II contains coenzyme B12 (adenosylcobalamin; AdoCbl), and Class III contains a FeS cluster and an observable glycyl radical. We have identified the *Archaeoglobus fulgidus* gene encoding the coenzyme B12 dependent ribonucleotide reductase (afRNR) protein by sequence homology, and this gene has been amplified and cloned. The gene was sequenced and expression of recombinant (His)6-tagged RNR protein was confirmed by SDS-PAGE and N-terminal sequencing. Progress is being made in the biochemical characterization of this enzyme.

622.2

Engineering Terpene Synthases for Novel Product Specificity

Bryan Thomas Greenhagen[1], Yuxin Zhao[2], Paul O'Maille[3], Joe Noel[3], Robert M Coates[2], Joe Chappell[1]. [1]University of Kentucky, N221W Ag. Sci. Ctr. N, Lexington, KY 40546, [2]University of Illinois, Urbana, IL, [3]Salk Insitiute, La Jolla, CA

Terpene cyclases catalyze the strereospecific conversion of simple linear isoprenoid polymers into cyclic terpenes. Engineered chimeras of closely related terpene cyclases (5-epi-aristolochene synthase (EAS) and premnaspirodiene synthase (HPS)) which catalyze reaction pathways that differ only in a penultimate carbon bond migration and terminal proton elimination were subjected to a deductive analysis for structure-function relationships. We developed contact maps based on a well resolved substrate analog positioned within the active site of crystallized EAS enabling us to make mechanistic inferences about residues which might control the regiospecificity proton elimination. A double mutant, EAS-T402S/V516I, catalyzed formation of 4-epieremophilene, the double bond regioisomer of the native EAS product as the dominant reaction product. These mutations essentially reposition a methyl group in the second tier of residues surrounding the active site. Significantly, this new enzyme, 4-epieremophilene synthase, retains wild type cyclase kinetic parameters, a factor of great importance in mechanistic interpretation of the current results and in the practical application of new terpene cyclases.

622.3

Rat cyt19 encodes arsenic methyltransferase and reductase functions

Stephen Brian Waters[1], Miroslav Styblo[2], David Thomas[3]. [1]University of North Carolina-Chapel Hill, Manning Drive, Chapel Hill, North Carolina 27599, [2]Pediatrics, University of North Carolina-Chapel Hill, Chapel Hill, North Carolina, [3]National Health and Environmental Effects Research Laboratory, US Environmental Protection Agency, Research Triangle Park, North Carolina

In many species, biomethylation of inorganic arsenic (iAs) yields methyl- and dimethylarsenic. This enzymatically-catalyzed process involves the reduction of arsenic from the pentavalent to trivalent

Program # 621.11

GADD45 is an Oxidative Stress Response Protein

Jacquelyn Jo Bower, Stephen S. Leonard, Yong Qian, Fei Chen, Xianglin Shi. Pathology and Physiology Research Branch, National Institute for Occupational Safety and Health, 1095 Willowdale Road, Morgantown, West Virginia 26505

Cell cycle arrest is a major mechanism for the reparation of DNA damage caused by a variety of insults including UV-induced damage, radiation, and exposure to some transition metals. GADD45 (growth arrest and DNA damage-inducible protein 45) is one of many proteins involved in the regulation of cell cycle arrest. This protein has been shown to interfere with the interaction between the cyclin dependent kinase, cdc2, and Cyclin B1, causing cell cycle arrest at the G2/M phase. In this report we investigated the involvement of reactive oxygen species (ROS) in Cr(VI)- and As(III)-induced GADD45 expression in the human airway epithelial cell line, BEAS2B. Cr(VI) induces ROS generation as demonstrated by ESR and confocal microscopy using specific fluorescent dyes for superoxide ($O_2^{\cdot-}$) and H_2O_2, the precursor of hydroxyl radical ($^{\cdot}OH$). Western blots indicated an induction of GADD45 by Cr(VI) and As(III), which is correlated with the generation of ROS. Here we report that GADD45 is induced in response to ROS generation, particularly $O_2^{\cdot-}$ and $^{\cdot}OH$. Furthermore, the induction of GADD45 by Cr(VI) was suppressed by the over-expression of superoxide dismutase (SOD) and glutathione peroxidase (GPx). Since the overexpression of SOD and GPx substantially decreased GADD45 induction by Cr(VI), we concluded that GADD45 is an oxidative stress response protein.

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PART II

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American Society for Pharmacology and Experimental Therapeutics
American Society for Investigative Pathology
American Society for Nutritional Sciences
American Association of Anatomists

American Federation for Medical Research
The American Society for Clinical Nutrition
Latin-American Association of Physiological Sciences
The Biomedical Engineering Society
Cajal Club
The Histochemical Society
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The Microcirculatory Society
North American Vascular Biology Organization
Society for Experimental Biology and Medicine
Society for International Nutrition Research

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ABSTRACTS

(continued from Part I)

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APRIL 13, 2003

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American Association of Anatomists

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SUNDAY AFTERNOON
APRIL 13, 2003

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