ligand-ER[alpha] or ligand-ER[beta] complexes to DNA probes containing GRE (glucocorticoid receptor) was observed.
Source of research support: - DHHS / CDC Grant No. RO4 / CCR 419 466-01.

158.14

Arsenite modulation of the integrity of actin filaments and the increase in cell motility may be mediated through CDC42

Yong Qian[1], Zhuo Zhang[2], Daniel C Flynn[3], Xianglin Shi[2]. [1]PPRB, National Institute for Occupational Safety and Health, 1095 Willaodale Rd, Morgantown, WV 26505, [2]National Institute for Occupational Safety and Health, Morgantown, WV, [3]Cancer Center and Microbiology/Immunology, West Virginia university, Morgantown, WV

Arsenite is a human cancinogen, however, the molecular mechanisms involved are poorly understood. By stimulating human endothelial cells (SVEC4-10) with arsenite, we found that arsenite modulated the integrity of actin filaments, and stimulated the formation of cell motility organelle structures within 5 minutes. Further, we found arsenite increased the cell motility, indicating that structural changes in cell morphology induced by arsenite are relevant to cellular functions. Affinity absorption assays with GST-PAK-PBD demonstrated that arsenite activated small GTPase CDC42 and Rac. We also observed that arsenite induced the formation of hydrogen radicals in SVEC4-10 cells, which are known to exert a regulatory role in arsenite induced effects on SVEC4-10 cells. Taken together, our data demonstrate that arsenite increases cell motility, which may be involved in cancer metastasis.

158.15

Insulin Receptor Substrate-1 (IRS-1) is Localized To and Functional Within the Nuclei of Hepatocytes in the Intact Rat

Philip A. Gruppuso, Joan M. Boylan. Department of Pediatrics, Rhode Island Hospital and Brown University, 593 Eddy Street, Providence, RI 02903

The insulin signaling network involves insulin receptor-dependent phosphorylation of the docking protein, IRS-1. Numerous studies have documented the trafficking of insulin signaling components, including IRS-1, from the cell surface to intracellular, extra-nuclear compartments. During the course of studies aimed at defining the ontogeny of insulin signaling in the rat, we examined the expression of IRS-1. Western immunoblotting showed minimal IRS-1 content in late gestation fetal liver. Immunohistochemical analysis of paraformaldehyde fixed, paraffin-embedded tissue, aimed at corroborating the immunoblotting results, showed intense staining for IRS-1 in the nuclei of mature hepatocytes. Further observations using immunofluorescent staining of liver frozen sections with two antibodies with distinct epitopes confirmed localization of IRS-1 to the nuclear matrix and nucleoli of adult hepatocytes in intact liver. Similar results were found using cultured hepatocytes. Insulin administration to intact animals or cultured hepatocytes did not affect nuclear IRS-1 content. This was confirmed by Western immunoblotting of liver cytosol and nuclear extracts. However, activation of IRS-1-associated phosphatidylinositol-3 kinase (PI3K) derived from nuclear extracts was easily demonstrated. Our results indicate that insulin signaling, which terminates in a spectrum of nuclear events, may originate within the nucleus of normal hepatocytes.

158.16

Signal Transduction by Human CC Chemokine Leukotactin-1 through the Receptor CCR1 in HOS Cells

Hesang Ko[1], In Sik Kim[2], Doe Sun Na[2]. [1]Immunomodulation Research Center, University of Ulsan, 388-1 Poongnap-dong, Songpa-gu, Seoul 138-736 Korea, Republic of, [2]Biochemistry, University of Ulsan, Songpa-gu, Seoul Korea, Republic of

Leukotactin-1 (Lkn-1) is a recently cloned human CC chemokine. We investigated the signal transduction mechanism of Lkn-1 through its receptor CCR1 in human osteogenic sarcoma cells expressing CCR1. Lkn-1-stimulated cells showed elevated phosphorylation of extracellular signal-related kinases (ERK1/2) with a distinct time course. ERK activation was peaked in 30 min and 12 h showing biphasic activation of ERK. Results from cell migration assay showed that the inhibitors of G_i/G_o proteins, phospholipase C (PLC), and protein kinase Cδ (PKCδ) blocked the chemotactic activities of Lkn-1. However, PD98059 did not inhibit the chemotaxis induced by Lkn-1. Activities of PLC and PKCδ were also enhanced by Lkn-1 stimulation. Chemotactic activities of Lkn-1 were inhibited by the treatment of cycloheximide and actinomycin D. Results from gel shift assay showed that the DNA binding activity of NF-κB was enhanced by Lkn-1 stimulation, and the inhibitor of NF-κB inhibited Lkn-1-induced chemotaxis. These results suggest that Lkn-1 transduces the signal through G_i/G_o proteins/PLC/PKCδ/NF-κB and newly synthesized proteins for chemotaxis, while the ERK pathway through G_i/G_o proteins/PLC/PKCδ/ERK plays a role for other cellular events. Supported by the research fund to Immunomodulation Research Center, University of Ulsan from Korea Science and Engineering Foundation (KOSEF).

158.17

Induction of estrogen response element by BCAR3 (breast cancer antiestrogen reistance 3) in a PI3-kinase/Akt-dependent manner.

Jung-Boon Ha[1], Ji-Eun Choi[2], In-Sook Kim[2], Byung Hak Jhun[2]. [1]Pharmacy, College of Pharmacy, Pusan National University, Kumjung-Ku, Jangjeon-Dong, Pusan, Pusan 609-735 Korea, Republic of, [2]College of Pharmacy, Pusan National University, Pusan, Pusan Korea, Republic of

Antiestrogen such as tamoxifen is a drug of choice for the breast cancer treatment. Unfortunately, nearly all patients who initially respond to antiestrogen therapy experience a relapse due to the development of antiestrogen-resistance metastases. One of possible mechanisms for the antiestrogen resistance is overexpression of genes such as EGF receptor, p130Cas and BCAR3. Molecular cloning reveals that BCAR3 contains an SH2 domain, a proline/serine-rich domain and a GDP-exchange factor domain for small GTPases, suggesting its possible role as a signal transducer. In the present study, we found that transient transfection of BCAR3 in immortalized normal human breast MCF-12A cells induces estrogen response element (ERE). Treatment of LY294002, a potent inhibitor of PI3-kinase and cotransfection of dominant-negative Akt completely inhibit the BCAR3-induced ERE activation. Also, active mutants of PI3-kinase and Akt activate ERE induction and co-transfection with BCAR3 synergistically activates ERE induction. Dominant-negative mutant of p85 subunit of PI3-kinase has no inhibitory effect. From these results, we conclude that BCAR3 is involved in the signaling pathway leading to ERE activation and acts through PI3-kinase and Akt.

158.18

PKCδDependent Activation of ERK1/2 in Vascular Smooth Muscle Cells

Roman G Gimnan[1], Harold A Singer[2]. [1]Center for Cardiovascular Sciences, Albany Medical College, 47 New Scotland Ave, albany, New York 12208, [2]Albany Medical College, Albany, NY

It is well established that the ERK signaling cascade can be activated via a PKC-dependent pathway. It has not been firmly established which PKC isoform(s) are involved or how specifically PKC mediates this activation. In a previous study, we found that antisense suppression of PKCa in cultured vascular smooth muscle failed to inhibit Phorbol 12,13-dibutyrate (PDBu) induced ERK1/2 activation. In the present study we found that the PKCd selective inhibitor rottlerin attenuated PDBu induced ERK1/2 activation while Go6976, a reportedly selective PKCa inhibitor, was without effect. The PDBu treatment also resulted in activation of ras but much less as compared to PDGF or EGF treatment. Similarly, infecting VSM cells with adenoviral RasN17, a dominant negative ras construct, only partially inhibited PDBu stimulated ERK1/2 activity. AG1478, an EGF receptor (EGFR) antagonist, and PP2, a selective inhibitor of src like kinases, partially inhibited PDBu stimulated ERK1/2 activity. Furthermore, activation of PYK2, a nonreceptor tyrosine kinase, and EGFR was inhibited upon treatment with rottlerin. Taken together, these data indicate that the PDBu induced ERK activation in VSM cells is mediated by PKCd through at least two distinct pathways; one that is apparently ras-independent and one that is ras-dependent and utilizes tyrosine kinases.

158.19

BRADYKININ B_2 RECEPTOR ACTIVATES ERK IN mIMCD-3 CELLS via EPIDERMAL GROWTH FACTOR RECEPTOR TRANSACTIVATION.

Yurii V. Mukhin[1], Evgeny A. Garnovsky[1], John R. Raymond[2], Maria N. Garnovskaya[1, 2]. [1]Medical University of South Carolina, Charleston, SC, [2]Ralph H. Johnson VA Medical Center, Charleston, SC

Bradykinin (BK) has been implicated in the regulation of renal function. We studied activation of extracellular signal-regulated protein kinase (ERK) by BK in inner medullary collecting duct (mIMCD-3) cells. Exposure of mIMCD-3 cells to BK resulted in concentration-dependent increase in tyrosine phosphorylation of ERK with maximum at 10^{-8}M. The effect of BK was mediated by B_2 receptor. BK-induced ERK activation was Ca^{2+}-calmodulin (CaM) independent. Inhibition of PLC, PKC or PI3-kinase did not alter ERK activation by BK. In contrast, genistein inhibited

igand-ER[alpha] or ligand-ER[beta] complexes to DNA probes containing GRE (glucocorticoid receptor) was observed.
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by the research fund to Immunon
Ulsan from Korea Science and En

158.17

**Induction of estrogen respons
antiestrogen reistance 3) in a PI**

Jung-Boon Ha[1], Ji-Eun Choi
[1]Pharmacy, College of Pharmacy,
Jangjeon-Dong, Pusan, Pusan 6(
Pharmacy, Pusan National Univer

Antiestrogen such as tamoxifen i
treatment. Unfortunately, nearly
antiestrogen therapy experience
antiestrogen-resistance metastases
antiestrogen resistance is overexp
p130Cas and BCAR3. Molecular
SH2 domain, a proline/serine-ric
domain for small GTPases, su
transducer. In the present study,
BCAR3 in immortalized normal
estrogen response element (ERE
inhibitor of PI3-kinase and co
completely inhibit the BCAR3-
mutants of PI3-kinase and Akt act
with BCAR3 synergistically activ
mutant of p85 subunit of PI3-kin
results, we conclude that BCAR
leading to ERE activation and acts

158.18

**PKCδDependent Activation of
Cells**

Roman G Ginnan[1], Harold A
Sciences, Albany Medical Colleg
York 12208, [2]Albany Medical Coll

It is well established that the ERK
PKC-dependent pathway. It has
isoform(s) are involved or how sp

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ABSTRACTS

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SCIENTIFIC AND TECHNICAL CHALLENGES IN THE HUMAN PROTEOME

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