Kang KeonWook, Lee SeungJin°, Park JeongWeon, SangGeon Kim

서울대학교 약학대학

Expression of phase II detoxifying genes is regulated by NF-E2-related factor 2 (Nrf2)-mediated antioxidant response element (ARE) activation. Phosphatidylinositol 3-kinase (PI3-kinase) plays an essential role in ARE-mediated rGSTA2 induction by oxidative stress and controls microfilaments and translocation of actin-associated proteins. This study was designed to investigate the PI3-kinase-mediated nuclear translocation of Nrf2 and the interaction of Nrf2 with actin. Pretreatment of the cells with PI3-kinase inhibitors (wortmannin/LY294002) prevented nuclear translocation of Nrf2 by tert-Butylhydroquinone (*t*-BHQ). *t*-BHQ relocalized Nrf2 in concert with changes in actin microfilament architecture, as visualized by confocal microscopy. Furthermore, *t*-BHQ increased the level of nuclear actin, co-immunoprecipitated with Nrf2, which returned to that of control by pretreatment with PI3-kinase inhibitors. Cytochalasin B, an actin disruptor, alone stimulated actin-mediated nuclear translocation of Nrf2 and induced rGSTA2. These results were blocked by phalloidin that stabilizes actin filaments. Subcellular fractionation and immunoblot analyses allowed us to detect both 57 kDa and 100 kDa Nrf2. Immunoprecipitation assays showed that the 100 kDa protein comprised both Nrf2 and actin. This study demonstrates that the PI3-kinase regulates rearrangement of actin in response to oxidative stress and that depolymerization of actin causes a complex of Nrf2 to translocate into nucleus.

[PA1-4] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

Hydrogen Peroxide Activates ERK in Cultured Feline Ileal Smooth Muscle Cells

Song HyunJu°, Lee TaiSang, Jeong JiHoon, Park JoonHong, Choi TaeSik, Lee DooWon, Sohn UyDong

Department of Pharmacology, College of Pharmacy, Chung Ang University, Seoul, Korea

H_2O_2 has been shown to act as a signaling molecule involved in many cellular functions such as oxidant-induced stress, apoptosis, proliferation. In this study, we investigated the action mechanisms of H_2O_2 on activation of Extracellular Signal-Regulated Protein Kinase(ERK) in cultured feline ileal smooth muscle cells(ISMC). Western blot analysis done with phospho-specific MAP kinases antibodies demonstrated that potent activation of ERK and moderate activation of SAPK/JNK occurred within 30 min of H_2O_2 treatment. However, p38 MAP kinase was not activated by H_2O_2. The activation of ERK by H_2O_2 was reduced by MEK inhibitor PD98059, removal of extracellular Ca^{2+}, depletion of the intracellular Ca^{2+} pool by thapsigargin, or pretreatment of ISMC with the calmodulin antagonist W-7. In addition, H_2O_2-induced ERK activation was attenuated by a tyrosine kinase inhibitor genistein, but not by downregulation of protein kinase C(PKC) with phorbol-12-myristate-13-acetate(PMA) or by a PKC inhibitor GF109203X. Further, ERK activation by H_2O_2 was blocked by pretreatment with either *N*-acetyl-cysteine, *o*-phenanthroline, or mannitol. Taken together, these data show the factors controlling MAPK activation by H_2O_2 in intestinal smooth muscle cells and suggest that ERK plays a critical role in the oxidant cell injury induced by H_2O_2.

[PA1-5] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

Arachidonic Acid Liberated through Activation of $iPLA_2$ Mediates the Production of Reactive Oxygen Species and Apoptosis Induced by N-Ethylmaleimide in HepG2 Human Hepatoma Cellls

Lee YongSoo°

College of Pharmacy, Duksung Women's University, Seoul 132-714, Korea

We have previously reported that activation of K^+-Cl^--cotransport (KCC) by N-ethylmaleimide (NEM) induces apoptosis through generation of reactive oxygen species (ROS) in HepG2 human hepatoblastoma cells. In this study we investigated the possible role of phospholipase A_2 (PLA_2)-arachidonic acid (AA) signals in the mechanism of the NEM actions. In these experiments we used arachidonyl trifluoromethylketone ($AACOCF_3$), bromoenol lactone (BEL) and *p*-bromophenacyl bromide (BPB) as inhibitors of the calcium-dependent cytosolic PLA_2 ($cPLA_2$), the calcium-independent PLA_2 ($iPLA_2$) and the secretory PLA_2 ($sPLA_2$), respectively. BEL significantly inhibited the NEM-induced KCC activation, ROS production and apoptosis, whereas $AACOCF_3$ and BPB did not. NEM increased AA liberation in a dose-dependent manner, which was markedly prevented only by BEL. The NEM-induced actions (KCC activation, ROS generation and apoptosis) were not significantly altered by treatment with indomethacin and nordihydroguaiaretic acid (NDGA), selective inhibitors of cyclooxygenase (COX) and lipoxygenase (LOX), respectively. Treatment with AA or 5, 8, 11, 14-eicosatetraynoic acid (ETYA), a non-metabolizable analogue of AA, markedly activated the KCC, produced ROS and induced apoptosis. Collectively, these results suggest that AA liberated through activation of $iPLA_2$ may mediate the NEM-induced ROS generation, KCC activation, and apoptosis induction in HepG2 cells.

[PA1-6] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

Inhibitory effect of 2-amino-3-ethoxycarbonyl-1-methyl pyrolo (3,2-b) naphtho-4,9-dione on tumor cell invasion in human fibrosarcoma cells by downregulating matrix metalloproteinase-2 and 9

Hyen Joo Park°, Hye Jin Hwang, Hyun-Jung Lee, Myung-Eun Suh, Sang Kook Lee

College of Pharmacy, Ewha Womans University, Seoul 120-750, South Korea,

Matrix metalloproteinases (MMPs) play an important role in tumor invasion and metastasis by matrix degradation. To analyze the effect of 2-amino-3-ethoxycarbonyl-1-methyl pyrolo (3,2-b) naphtho-4,9-dione (compound 1) on the invasion or metastasis of cancer cells the expression of matrix metalloproteases (MMPs) was investigateded in human fibrosarcoma HT1080 cells by RT-PCR or gelatin zymographic methods. As a result, compound 1 decreased the expression of MMP-2 and 9, but increased the expression of a tissue inhibitor of metalloproteinase-1 (TIMP-1). In addition, compound 1 inhibited cancer cell migration and colony formation. These results suggest that compound 1 might be inhibiting tumor cell invasion and metastasis by suppression of MMPs and elevation of TIMP-1 production in tumor cells, and thus compound 1 could be a lead candidate for developing anti-metastatic or anti-invasive agent against cancer cell growth. (This work was supported in part by Korea research FoundationGrant, KRF-2001-005-F00023).

[PA1-7] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

High-Throughput Screening for Novel Inhibitors of Protein-Tyrosine Phosphatase-1B

Lee Inki°, Son MiWon, Jung MiYoung, Shin ChangYell, Kim DongSung, Kim SoonHoe, Yoo MooHi, Kim WonBae

Research Laboratories, Dong-A Pharm. Co. Ltd. 47-5, Sanggal, Giheung, Yongin, Gyeonggi 449-900, Korea

Protein-tyrosine phosphatases (PTPs) constitute a family of receptor-like and cytoplasmic enzymes, which catalyze the dephosphorylation of phosphotyrosine residues in a variety of receptors and