during aging and down-regulated by CR. Increased CAMs might contribute to the pathophysiological process of vascular aging.

[PC1-28] [04/18/2003 (Fri) 09:30 - 12:30 / Hall P]

Role of hydrogen peroxide in Rac1 mediated activation of p70s6k signaling pathway

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The signal transduction pathway leading to the activation of the p70s6k plays an important role in the progression of cells from G0/G1 to S phase of the cell cycle but remains incompletely characterized. We investigated the role of the Rho family G protein Rac1 in H2O2-mediated p70s6k activation. Transient expression of a dominant negative mutants of the small GTP-binding proteins Rac1 (Rac1N17) and Cdc42 (Cdc42N17) showed reduced levels of slower migration on Western blots of one-dimensional SDS-PAGE in p70s6k and ERK1/2 by PDGF stimulation. Treatment of NIH-3T3 cells with PDGF led to a rapid increase in H2O2, phosphorylation and activation of p70s6k, which were antagonized by the expression of catalase. In an effort to further explore how Rac1 proteins regulate p70s6k activity, we investigated with stable expression of a constant active mutant of Rac1 (Rac1V12) in Rat2 cells, which resulted in a significant increase in intracellular reactive oxygen species (ROS) and S6 kinases (p70s6k and p90rsk) activity. In addition, stable expression of Rac1N17 also inhibited ROS production and PDGF-induced activation of S6 kinases. Rac1V12 transfected Rat2 cells had a considerably faster growth rate and Rac1N17 had a growth-inhibitory effect, compared with control cells transfected with the expression vector alone, indicating that Rac1-induced H2O2 might act as an upstream molecule of p70s6k as well as ERK1/2, p90rsk upstream kinase. Taken together, these results suggest that Rac1 regulates ROS production and leading to p70s6k activation, which have been linked to cell growth.

[PC1-29] [04/18/2003 (Fri) 09:30 - 12:30 / Hall P]

Correlation between signal pathway of chitosan and nitric oxide

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Renal dipeptidase (RDPase, EC 3.4.13.19), an ectoenzyme of renal proximal tubules, is covalently bound to outer leaflet of lipid bilayer via glycosylphosphatidylinositol (GPI)-anchor. Chitin is a major component of the shells of crustacea such as crab, shrimp and crawfish. This study was conducted to examine the effect of chitosan on RDPase release from renal proximal tubules. Nitric oxide (NO), highly reactive free radical, inhibits the release of RDPase from porcine proximal tubules. Porcine proximal tubules were prepared with the protocol of Taub et al (1990) and were treated with L-arginine (0,1,5,10 and 20mM, substrate of NO synthase), and chitosan (0.01, 0.05 and 0.1%) in the presence of L-arginine (10mM) for 20 min at 37℃ followed by centrifugation (18000g, 5min). The activity of released RDPase was assayed according to the fluorometric method of Ito et al (1984). Nitrite was determined spectrophotometrically using the Griess reagent. It was observed that the RDPase release was decreased and NO concentration was increased in a concentration-dependent manner of L-arginine. The decreased RDPase release by L-arginine recovered as a function of chitosan concentration. However, nitrite

concentration was increased in a concentration-dependent manner of chitosan. These results suggest that RDPase release by chitosan may not relate to nitric oxide signal pathway.

[PC1-30] [04/18/2003 (Fri) 09:30 - 12:30 / Hall P]

Roles of Phosphatidylinositol 3-Kinase(PI3K) and Rac1

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Many studies have identified the phosphatidylinositol 3-kinase (PI3K) as a key regulator for various cellular functions including cell survival, growth and motility. We have previously shown that H-ras, but not N-ras, induces invasiveness and motility in human breast epithelial cells (MCF10A), while both H-ras and N-ras induce transformed phenotype. In the present study, we wished to investigate the functional role of PI3K pathway in H-ras-induced invasive phenotype and motility of MCF10A cells. Activation of PI3K in the parental, H-ras- and N-ras MCF10A cells was examined by detecting phosphorylation of Akt, a downstream molecule of PI3K. Marked activation of Akt was detected not only in H-ras MCF10A cells but also in non-invasive/non-motile N-ras MCF10A cells at comparable levels. We then further investigated the functional significance of PI3K activation in invasion and motility by using known PI3K inhibitors, LY294002 and wortmannin. Treatment of LY294002 and wortmannin significantly inhibited invasive phenotype and motility of H-ras MCF10A cells, suggesting that the activation of PI3K pathway is not sufficient, but may be required for H-ras-induced invasion and motility. Prominent downregulation of MMP-2 and MMP-9 were observed in H-ras MCF10A cells treated with LY294002 in a dose-dependent manner. The results provide evidence that PI3K pathway is critical for H-ras-mediated upregulation of MMPs in MCF10A cells, resulting in phenotypic conversion of non-invasive MCF10A cells to an invasive phenotype. In order to study the molecular mechanisms under PI3K effects cell invasion and migration, we investigated activation of ras downstream effector molecules, MAPKs, treated with PI3K inhibitors. Phospharylation of ERK and p38 level is slightly downregulated in H-ras MCF10A cells treated with LY294002. And many studies have identified relation PI3K and Rac with invasion and migration. In order to correlation of PI3K and Rac, we investigated Rac activity in parental, H-ras and N-ras MCF10A cells. Activation of Rac was detected in H-ras MCF10A cells. We then further studied the role of Rac activation in invasion and migration using dominant negative construct of Rac1. H-ras induced invasion and migration was significantly inhibited in DN-Rac1 transfectants. We further investigate the activation of MAPKs in DN-Rac1 transfectants in order to study the molecular mechanisms under Rac effects cell invasion and migration.

[PC1-31] [04/18/2003 (Fri) 09:30 - 12:30 / Hall P]

Involvement of MAPKs in GDNF-induced Proliferation and Migration in Hs683 Glioma Cells

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Glial cell-derived neurotrophic factor (GDNF) is a potent neurotrophic factor that enhances survival of midbrain doparminergic neuron. GDNF and its receptors are widely distributed in brain and are believed to be involved in the control of neuron survival and differentiation. GDNF