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Regulatory mechanism of ubiquitous protein kinase CK2 by Nopp140

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Human nucleolar phosphoprotein p140 (hNopp140) is a highly-phosphorylated nucleolous protein, which is involved in the biogenesis of nucleolus. We recently observed that it could negatively regulate the catalytic activity of protein kinase CK2, a ubiquitous protein kinase that can phosphorylate various proteins. Highly-phosphorylated hNopp140 binds to the catalytic subunit of CK2 (CK2a), and this binding is prevented by inositol hexakisphosphate (InsP6). The interaction between hNopp140 and CK2a has been quantitatively characterized, and the binding partner of InsP6 was determined. CK2-hNopp140 dependent protein phosphorylation is investigated in cells by 2D-electrophoresis and western analysis using anti-phosphoserine antibody. Decreased phosphorylation level of various CK2 substrate proteins in cells overexpressing hNopp140 indicates that hNopp140 controls constitutive activity of CK2.

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Regulation of transforming growth factor-beta signaling by 14-3-3 sigma-mediated positive feedback

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Transformig growth factor-beta (TGF-β) is a multifunctional cytokine signaling to the nucleus through cell surface transmembrane receptor serine/threonine kinases and cytoplasmic effectors, including Smad proteins. Using a yeast two-hybrid screen, we identified 14-3-3 sigma, a negative regulator of cell cycle, as a TGF-βtype I receptor (TGF-βRI) interacting protein. Immunoprecipitation and confocal microscopic analysis confirmed 14-3-3 sigma association with TGF-βRI and their co-localization. Overexpression of 14-3-3 sigma markdly increased the ability of TGF-β to induce Smad-dependent transcription and cell growth inhibition. Consistently, knockdown of the endogenous 14-3-3 sigma gene with small-interfering RNA (siRNA) substantially suppressed TGF-β-induced Smad3/Smad4 complex formation and up-regulation of TGF-β-induced p15INK4b and p21WAF1 proteins. We also found that 14-3-3 sigma is a Smad3-dependent immediate-early TGF-β target gene. We proposed that 14-3-3 sigma is a novel regulator of TGF-β signaling and may participate in a positive feedback loop to control TGF-β signaling.

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Regulation of mitochondrial fusion and apoptosis by the mitochondrial protein CTMP

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Mitochondria are highly dynamic organelles in eukaryotic cells. Mitochondria undergo continual cycles of fusion and fission, and the balance of these opposing processes regulates mitochondrial morphology. In many cell types mitochondria form tubular structures or networks. But, during apoptosis the mitochondrial network fragments. CTMP (Carboxyl-Terminal Modulator Protein), that is known to negatively regulate the PKB catalytic activity in insulin signaling pathway. CTMP binds specifically to the carboxyl-terminal regulatory domain of PKB at the plasma membrane. Binding of CTMP reduces the activity of PKB by inhibiting phosphorylation on serine 473 and threonine 308. Here, we showed the subcellular localization of CTMP in U2OS cell line. When GFP tagged N terminal of the CTMP that localized cytoplasm and plasma membrane, whereas C terminal tagged construct of the CTMP were localized mitochondrion and cytoplasm. We also confirmed that mitochondrion fusion was mediated by over-expression of CTMP. Our results suggest that CTMP is cytoplasmic and mitochondrial protein. Cytoplasmic form inhibits the PKB activity, and mitochondrial form of CTMP mediates the mitochondrial fusion and resistance to apoptosis.(This work was supported by the SRC/ERC Program (Grant R11-2002-100-02006-0) and the Basic Research Program (R01-2005-000-10240-0, F01-2005-000-10011-0) of the Korea Science & Engineering Foundation)

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Regulation of anti-apoptotic activity of Nkx3.2 by signal transduction pathways

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Nkx3.2, a member of the NK class of homeoproteins, is initially expressed in chondrogenic precursor cells, and later, during cartilage maturation, its expression is restricted to proliferating chondrocytes. We have previously demonstrated that Nkx3.2 enhances cell survival by constitutively activating the NF-kB pathway in proliferating chondrocytes. In this work, we have further characterized the anti-apoptotic activity of Nkx3.2. To investigate which signaling pathways are associated with anti-apoptotic function of Nkx3.2 in chondrocytes, the effects of chemical inhibitors against various signaling pathways were tested. As we have previously shown, chondrocyte apoptosis induced by NF-kB inhibitors, such as BAY 11-7085, was efficiently protected by stable overexpression of Nkx3.2, and this protection was significantly attenuated by LY294002, a specific chemical inhibitor against the PI3K pathway. Co-IP experiments using several PI3K signal transduction constructs revealed that PI3K pathway is required for its interaction with NF-kB/IkB-a. In addition, 4x-kappaB reporter gene assays demonstrated that PI3K signal pathway is required for Nkx3.2 to modulate transcriptional functionality of NF-kB in vivo. These results suggest that the PI3K pathway is required to be functional for Nkx3.2 to exert its anti-apoptotic activity in chondrocytes.

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Radiotherapeutic effects in p53-null human lung cancer H1299 cells using 3-dimensional culture system.

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The mutation of p53 gene has been found in approximately 90 % of lung cancer although p53 gene has been known as tumor suppressor gene in human. Recently radiotherapy generally has been used for treatment to patients with non-small cell lung cancer (NSCLS). A number of studies has reported that the chemotherapeutic mechanism of ionizing radiation (IR) is to generate DNA damage and induce cancer cell death using the monolayer culture system. However, many cancer cells has been demonstrated to lose some of their morphologyical characters when grown in vitro as monolayer or suspension cultures. In this study, we employed the H1299 cell lines, a broadly used cancer cell lines induced from non-small cell lung cancer (NSCLC) lacking the expression of p53 tumor suppressor and investigate the sensitivity of ionizing radiation (IR) to H1299 cells forming in vivo mimic spheroids by thermo-reversible gelation polymer. H1299 was successfully grown to 3D spheroids similar to in vivo system. In addition, 3D spheroid cultured H1299 had the increased sensitivity to IR dependent on growing time suggesting that 3-dimensional cell culture systems might be better reflect in vivo behavior than the in vitvo behavior of most cell types. We expected that 3D culture system might to be the model which more closing the real lung cancer environment than monolayer culture system and useful in the study for clinical mechanism of radiotherapy.

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Quercetin sensitizes human hepatoma cells to TRAIL-induced apoptosis by up-regulation of DR5 and down-regulation of c-FLIP

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Quercetin (3',3',4',5,7-pentahydroxyflavone), a flavonoid found in many fruits and vegetables, is known to have anti-cancer effects. Here, we show that treatment with subtoxic doses of quercetin in combination with TRAIL significantly induce rapid apoptosis in TRAIL-resistant hepatoma cells. While the proteolytic processing of procaspase-3 by TRAIL was partially blocked in various hepatoma cells, treatment with quercetin efficiently recovered TRAIL-induced activation of caspases. Neither TNF-α- nor Fas-mediated apoptosis was sensitized in hepatoma cells by cotreatment with quercetin, suggesting that quercetin can selectively sensitize hepatoma cells to TRAIL-induced apoptosis, but not to apoptosis mediated by other death receptors. We found that quercetin treatment significantly up-regulated mRNA and protein levels of DR5, a death receptor of TRAIL. The quercetin-enhanced TRAIL-induced apoptosis was significantly reduced by administration of a blocking antibody or small interfering RNAs for DR5, indicating its critical role in this cell death. Furthermore, treatment with quercetin significantly decreased the protein levels of cellular FLICE-inhibitory protein (c-FLIP), an inhibitor of death receptor-mediated apoptosis. Overexpression of c-FLIP attenuated quercetin-stimulated TRAIL-induced apoptosis, suggesting its involvement in TRAIL-resistance of hepatoma cells. Collectively, quercetin triggers and amplifies TRAIL-induced apoptotic signaling pathway via up-regulation of DR5 and down-regulation of c-FLIP in TRAIL-resistant hepatoma cells. Thus, combined treatment with TRAIL and quercetin may provide an attractive strategy for safely treating resistant hepatomas.