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Isoform-specific sub-cellular localization of protein kinase C isoforms in NIH3T3 cells

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Protein kinase C (PKC) is a multi-gene family of enzymes consisting of 10 isoforms. Specific isoforms of PKC play key roles in pathways of signal transduction, growth control and tumorigenesis. Variations in the intracellular localization of individual isoforms are thought to be an important mechanism for isoform specific regulation of enzyme activity and also substrate specificity. To provide a dynamic method for analyzing the localization of specific isoforms of PKC in living cells, we generated fluorescent fusion proteins of PKC α, β1, β2, γ, δ, ε, η, ζ and ι, using the green fluorescent protein (GFP) as a fluorescent marker at the carboxyl termini of these enzymes. Intracellular localization of specific isoforms of PKCs was then examined by fluorescence microscopy after transient transfection of the respective PKC-GFP expression vector into NIH3T3 mouse fibroblasts. The intracellular translocation of specific PKC isoforms induced by TPA was also monitored. We found that specific isoforms of PKC display distinct localization patterns in untreated NIH3T3 cells. For example, PKCα is localized mainly in the cytoplasm and PKC ε is localized mainly in the Golgi apparatus. We also observed that PKCα, β1, β2, γ, δ, ε and η translocate to the plasma membrane within 10 minutes of TPA treatment, while the cellular localization of PKCζ and ι were not affected by TPA. These results suggest that these isoform-specific PKC-GFP fusion proteins may be useful markers for determining the localization of specific isoforms of PKC in various types of living cells, and for elucidating dynamic changes in response to various stimuli, or cell transformation.

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Interleukin-8 reduces human glioma U118MG cell invasion by promoting actin stress fiber formation

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Chemokines have recently been implicated in tumor progression and metastasis. In addition to their chemotactic properties, chemokines and their receptors also play a role in other biological functions relevant to oncogenesis including cell proliferation, protease induction, tumor growth, and angiogenesis. Interleukin-8 (IL-8) is a member of the chemokine family and was identied as a chemotactic factor secreted by activated monocytes and macrophages that promote the directional migration of neutrophils and T lymphocytes. In human glioma, IL-8 is expressed and secreted at high levels both in vitro and in vivo. Recent experiments suggested that it is critical to the gliomagenesis although its role in tumorgenesis is not clear. In this study, we found that IL-8 reduces human glioma U118MG cell invasion in Boyden Chamber System. Unexpectedly IL-8 did not affect expression of tumorgenesis-related genes. The inhibition of human glioma cell invasion by IL-8 involved actin stress fiber formation. IL-8 increased the phosphorylation of FAK, which is known to regulate the focal adhesion and peripheral actin structures. Nuclear translocation of STAT3 known as latent cytoplasmic transcription factor that is activated by many cytokines was also increased by IL-8. Experimental results suggest that IL-8 suppresses invasion of human glioma U118MG cell by promoting actin stress fiber formation via the regulation of FAK and STAT3 activity. [This work was supported by the Korea Science and Engineering Foundation (KOSEF) through the National Research Laboratory Program funded by the Ministry of Science and Technology (project No. M10400000029706J000029710) and the MOHW grant National R&D Program (0420210-3)].

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Interaction of PKC with Ahnak potentiates Raf phosphorylation

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Ahnak is a protein of an exceptionally large size (700kDa) and contains 36 central repeated units (CRUs). We previously reported that CRUs of Ahnak act as scaffolding protein networking PLC-γ and PKC. Here, we demonstrated that four central repeated units (4CRUs) of Ahnak protein bind and activate PKCα in the PS/DG-independent manner. Moreover, treatment of PMA to NIH3T3 cells expressing 4CRUs of Ahnak showed an enhanced c-Raf, MEK, and Erk phosphorylation, compared to parental cells. To evaluate the gain-of-function of Ahnak in cell signaling, we investigated PKC activation and Raf phosphorylation in Ahnak knockout mouse embryonic cells (MEF). Membrane translocation of PKCα and phosphorylation of Raf in Ahnak null MEF were decrease comparing to wild type MEF. Several lines of evidence suggest that PKCα activity regulated by the association with phosphatase 2A (PP2A). Co-immunoprecipitation assay with antibodies against PP2A indicate that the association of PKCα with PP2A was broken off in NIH3T3 cells expressing 4CRUs of Ahnak in response to PMA, leading to enhanced PKCα phosphorylation. These data suggest that 4CRUs of Ahnak potentiate PKC-dependent c-Raf/MEK/Erk cascade through inhibiting interaction of PKC with PP2A.

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Inhibitory effect of thymosin-beta-4 (TB4) to caspase-3 activation in apoptotic tumor cell death

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Thymosin-beta-4 (TB4) as actin-sequestering peptide has been reported to induce tumor metastasis and paclitaxel-resistance. Most significant obstacle to successful therapy is the development of paclitaxel-resistance in tumors. Here, we investigated the inhititory effect of TB4 peptide on tumor cell death by the incubation with paclitaxel. The effect of TB4 peptide was assayed by the measurement of caspase-3 activity, G2/M attest, and Bcl-2 phosphorylation. Cell survival rate was increased and caspase-3 activity was decreased by the treatment with TB4 peptide. In contrast, small interfering RNA (siRNA) of TB4 inhibited cell viability and augmented caspase-3 activity. A significant change in Bcl-2 phosphorylation was detected by TB4 peptide treatment or by the overexpression of TB4 gene in Hela cells. The reduced population in G2/M phase by TB4 peptide treatment was correlated with the decreased expression of cyclin B1. Above data were confirmed in stomach cancer cell lines, SNU 638 (low TB4 level) and SNU 668 (high TB4 level) that was established from clinically isolated tumor cells. In conclusion, soluble TB4 peptide produced in cancer cells could be an obstacle to treat tumor with paclitaxel. Therefore, TB4 could be a novel target to control paclitaxel-resistance.

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Inhibitory effect of obovatal on the migration and invasion of HT1080 cells via the inhibition of MMP-2

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Because the activation of matrix metalloproteinases (MMP) is a key factor in the metastatic process, compounds with the ability to inhibit MMP activity have a potential in the treatment of tumor. Especially MMP-2 can degrade type Ⅳ collagen, one of the major components of the basement membrane, resulting in the promotion of tumor invasion and metastasis. To discovery small molecule for a good MMP-2 inhibitor, we have tested 2000 plant extracts including 150 herbal medicines against MMP-2 enzyme activity. From the screening, obovatal isolated from the extract of the leaves of Magnolia obovata THUNB was a potent inhibitor of MMP-2 enzyme in vitro. To confirm effect of obovatal in cell system, we used human fibrosarcoma cells (HT1080) activated MMP-2. Obovatal inhibited MMP-2 enzyme activity and expression in HT1080 cells. In addition, the compound blocked migration and invasion of the cells. This study demonstrates that obovatal exerts its anticancer effects through blocking migration and invasion by inhibition of MMP-2 expression and activity and also will be a good lead molecule for the development of anti-tumor drug.

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Inhibition of NDRG1 expression induced premature senescence

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NDRG1 cloned as cMyc down regulated and cell differentiation related protein. Recently, we found that the NDRG1protein is a microtubule associated protein that localizes to the centrosome and spindle ester and participates in spindle checkpoint. Here we show the diminished NDRG1 induced premature senescence in HDF. Blocking of endogenous NDRG1 expression by small interfering RNA in HDF cells resulted in growth arrest and flattened morphologic change. Moreover, siRNA introduced cells stained with senescence specific beta galactosidase. However, the expression of NDRG1 had no difference in young or replicative senescent HDF cells. Our findings suggest blocking NDRG1 expression induce senescence phenotype for protecting cells from unfavorable replication even though this gene is not direct involved in aging process