Both the cyclin-dependent kinase inhibitor p27kip1 and ceramide have been implicated in the regulation of apoptosis. Recently, we demonstrated that ceramide induced apoptotic cell death associated with increase in the level of p27kip1 in HL-60 cells. In the present study, we have overexpressed p27kip1 in HL-60 cells to clarify the role of p27kip1 in ceramide-induced cell death. HL-60/p27kip1 cells treated with ceramide have shown marked increase in apoptotic cell death compared to HL-60 cells. However, overexpression of p27kip1 by itself did not induce apoptosis indicating that p27kip1 alone might not be sufficient to induce apoptosis but promotes ceramide-induced apoptosis in HL-60 cells. Overexpression of p27kip1 did not modify the expression of Bcl-2 protein, but increased Bax protein level without ceramide treatment. Furthermore, overexpression of p27kip1 accelerated ceramide-induced cytochrome c release and poly(ADP-ribose) polymerase (PARP) cleavage in HL-60 cells. Ceramide induced PARP cleavage in HL-60/p27kip1 cells at the time which was not seen in HL-60 cells. These findings indicate that p27kip1 promotes ceramide-induced apoptosis through the elevation of Bax expression and activation of caspase with cleavage of the endogenous substrate PARP.

[PC3-4] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

BCL-2 OR BCL-XL ATTENUATES HYDROGEN PEROXIDE- AND BETA-AMYLOID-INDUCED OXIDATIVE PC12 CELL DEATH

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Recent studies have revealed that moderate amounts of intracellular reactive oxygen intermediates (ROIs) can cause cell death via apoptosis while their excessive cellular accumulation leads to necrotic cell death. Cell death is regulated by plenty of functional genes and their protein products. Bcl-2 which is an integral intermitochondrial membrane protein blocks cell death induced by wide variety of toxicants. In the present work, we have investigated a possible protective role of bcl-2 in oxidative death induced by hydrogen peroxide and beta-amyloid in cultured PC12 cells. When PC12 cells were treated with hydrogen peroxide or beta-amyloid, they underwent apoptotic death as determined by morphological features, internucleosomal DNA fragmentation and positive in situ terminal end-labeling (TUNEL staining). Hydrogen peroxide or beta-amyloid caused activation of NF-kappa B, which appears to be mediated via transient induction of mitogen-activated protein kinases (MAPKs). Transfction of PC12 cells with bcl-2 or bcl-XL gene rescued these cells from oxidative death caused by either hydrogen peroxide or beta-amyloid. PC12 cells overexpressing the above anti-apoptotic genes exhibited relatively high constitutive NF-kappa B activation, compared with the vector-transfected control cells. Furthermore, NF-kappa B inhibitors, such as pyrrolidine dithiocarbamate or L-1-tosylamido-2-pentylchloromethyl ketone, sensitized PC12 cells to hydrogen peroxide or beta-amyloid. Western blot analyses revealed that bcl-2 transfected PC12 cells exhibited the higher level of p65, the functionally active subuint of NF-kappa B, in nucleus than did the vector-tranfected controls. In contrast, relatively small amounts of cytoplasmic inhibitor Ikappa B alpha were present in the cells overexpressing bcl-2. These results suggested that the ubiquitous eukaryotic transcriptional factor NF-kappa B plays a role in cell survival against oxidative stress.

[PC3-5] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

Bax is required for ceramide-regulation of cell death

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Bax, a member of Bcl-2 family, has been known to promote apoptotic cell death induced by a wide variety of stimuli. Recently, we have shown that the level of Bax protein was significantly increased during ceramide-induced apoptosis in HL-60 cells. Here we show that Bax translocates from the cytosol to the mitochondria following ceramide treatment. Bax translocation occurred in concert with the release of cytochrome c and poly(ADP-ribose) polymerase. Furthermore, Bax-depleted HL-60 cells generated by using Bax antisense oligonucleotides demonstrated inhibition of cell death induced by ceramide, providing direct evidence that Bax plays a pivotal role in ceramide-induced apoptosis.

[PC3-6] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

Role of Reactive Oxygen Species in Arsenite -induced Tumor Promotion

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Arsenite is a potent carcinogen that may act as a tumor promoter in the carcinogenic process. However, the mechanism of arsenite-induced tumor promotion has not been definitely revealed. Recently, arsenite is believed to be associated with upregulation of growth factor signaling pathway. In the previous study, we reported that reactive oxygen species (ROS) play a key role in cell signaling pathway as an important signaling intermediator. Therefore, we aimed to determine the role of ROS in the signaling pathway activated by arsenite. Arsenite treatment stimulated MAPK and $p70^{S6K}$, which was accompanied with increase in intracellular ROS production. The predominant ROS produced appeared to be hydrogen peroxide (H_2O_2), because the arsenite-induced increase in fluorescence was completely abolished by treatment with catalase. The elimination of H_2O_2 by catalase inhibited the arsenite-induced activation of $p70^{S6K}$, and MAPK indicating possible role of ROS in the arsenite-induced activation of $p70^{S6K}$, and MAPK signaling pathway. Furthermore, activation of $p70^{S6K}$ by arsenite was significantly blocked by specific inhibitors such as rapamycin, wortmannin and LY294002. Taken together, these results suggest that H_2O_2 may be a pivotal mediator in arsenite-induced tumor promotion through the growth factor signaling pathway.

[PC3-7] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

Effects of histone deacetylase inhibitors on angiogenesis

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The reversible acetylation - deacetylation of histones is thought to play a crucial role in transcriptional control in eukaryotic cells. Apicidin was isolated from Fusarium sp. as a potent histone deacetylase (HDAC) inhibitor. We have reported Apicidin and its derivatives have the anti-angiogenic effects on chorioallantoic membrane (CAM) assay. Apicidin and its derivatives suppressed in vitro angiogenesis by interfering tube formation and sprouting of human umbilical vein endotherial cells (HUVECs) and ECV304 cell lines. We examined the effect of Apicidin, Apicidin02 and Apicidin07 on the expression of angiogenic factors such as VEGF, bFGF, TNF-α, angiopoietin-1, angiopoietin-2, angiogenin, TGF-α and TGF-β in cultured HUVEC and ECV304. Reverse transcriptase-polymerase chain reaction indicated that Apicidin and its derivatives modulate the mRNA expression of angiogenic factors in dose-dependent manner.