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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