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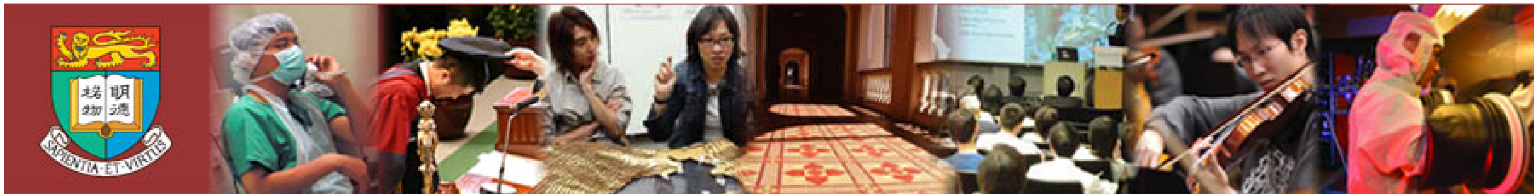

Title	bcl-2 inhibits cytochrome c release during apoptosis in leukemic HL-60 cells
Author(s)	Zhang, Q; Shang, JX; Sheng, HP; Loh, TT
Citation	38th American Society for Cell Biology annual meeting. San Francisco, California, USA. December 12-16, 1998. In Molecular Biology of the Cell, 1998, v. 9 Supp, p. 244A
Issued Date	1998
URL	http://hdl.handle.net/10722/53466
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1413

GROWTH FACTORS PREVENT GLUCOSE-MEDIATED MITOCHONDRIAL DYSFUNCTION IN GLIA J.W. Russell, C.L. Delaney, and E.L. Feldman. Dept. of Neurology, Univ. of Michigan, Ann Arbor, MI 48109

Mitochondrial (Mt) membrane depolarization is an early event in programmed cell death (PCD) leading ultimately to activation of caspases and regulation of Bcl proteins. We have evidence of Schwann cell (SC) apoptosis and Mt disruption in rats made diabetic with streptozotocin for 1 or 12 months. In order to study the role of high glucose and the anti-apoptotic factors NGF and IGF-I in the pathogenesis of SC PCD, we used FACS analysis to study changes in Mt membrane stability and cell death. Using defined serum free conditions, high glucose induces a left shift in the rhodamine 123 peak and increases the % SCs excluding rhodamine (SER) to 31% with 30 mM glucose and to 41% with 150 mM glucose (control, 7.5 mM glucose, 11%). In contrast, both IGF-I and NGF were glial protective, with 100 ng/ml NGF SER-12% and 10 nM IGF-I-15%. In addition, increased PCD with high glucose and serum withdrawal was inhibited by NGF and IGF-I and was glucose concentration dependent. These results suggest that Mt dysfunction is important in initiating PCD in SCs after exposure to apoptotic stressors such as high glucose or serum withdrawal, and can be prevented by NGF or IGF-I. Supported by NIH NS01938 (JWR); NIH NS36778, JDFI and ADA (ELF).

1414

INVESTIGATIONS OF THE ROLE OF THE PROTEIN TRANSLOCATING CHANNELS OF MITOCHONDRIA IN APOPTOSIS. R.C. Murphy[1], A. Moodie[1], E. Schneider[1], C.A. Mannella[1], M.L. Campo[2], and K.W. Kinnally[1]. [1]Mole. Med., Wadsworth Center, Albany, N.Y.[2]Dpto. de Bioquimica, U.de Extremadura, Spain.

There is now compelling evidence that mitochondria are involved in the commitment step of apoptosis and this step is linked to release of cytochrome c from the intermembrane space. Released cytochrome c forms a complex that facilitates caspase activation and progression through apoptosis. We investigated the possible role in these processes of the protein translocation channel, MCC, of the mitochondrial inner membrane. A time line for the onset of apoptotic markers was established for human breast cells (MDA231) treated with teniposide. By patch-clamping mitochondria isolated from these cells at various times after treatment, we found the cyclosporin sensitive-channel MCC was opened in early apoptosis. Other early events include mitochondrial depolarization detected by JC-1 fluorescence and loss of asymmetry of plasma membrane lipid determined by annexin-V binding. These early events were followed by caspase activation. DNA laddering and loss of plasma membrane integrity as indicated by PARP-cleavage, DNA gels, and 7AAD labeling of nuclei, respectively. Overexpression of the anti-apoptotic protein bcl-2 in untreated cells was associated with an increase in mitochondrial membrane potential and a decrease in the detection of MCC activity. Further studies showed bcl-2 overexpression eliminated the calcium-activation of MCC and,hence, suggest a mechanism of action for bcl-2. These findings support a role for opening of the MCC early in the apoptotic cascade. This work was supported by NATO CRG970210 to MLC, ES and KWK, DGICYT B95-0456 to MLC and NSF grant MCB9513439 to KWK.

1415

Localization of the Integrin-Linked Kinase in Mitochondria of Human Rhabdomyosarcoma Cells and Investigation of a Potential Role in Apoptosis. A. Williams, H. Yeger, and G. Hannigan. Dept. of Pediatric Laboratory Medicine, Hospital for Sick Children, and Dept. of Laboratory Medicine and Pathobiology, University of Toronto, Canada.

Integrin-Linked Kinase (ILK) is a protein ser/thr kinase that was identified by means of its interaction with the cytoplasmic domain of the $\beta 1$ integrin subunit. ILK is implicated in the regulation of $\beta 1$ integrin signalling, which in turn regulates cell growth, differentiation and survival. Although $p59^{ILK}$ localizes to the plasma membrane via interaction with integrins, our immunocytochemical studies suggest that a significant proportion localizes in the mitochondria of rhabdomyosarcoma and osteosarcoma cells. This mitochondrial localization indicates a possible role for ILK in the regulation of cellular processes independent of a direct association with integrin subunits. In particular, this localization suggests a role for ILK in the regulation of anoikis, a cell-adhesion-linked form of apoptosis. The distribution of mitochondrial $p59^{ILK}$ is currently being further investigated, by immunocytochemistry and transfection of fluorescently-labelled $p59^{ILK}$ (using GFP) into sarcoma cells. Double-labelled studies and confocal microscopy will be used to co-localize $p59^{ILK}$ with apoptosis-regulating proteins resident in the mitochondrial membrane. Coimmunoprecipitation and *in vitro* binding assays are aimed at identifying interactions between $p59^{ILK}$ and the bcl-2 family of apoptotic-regulatory proteins, and preliminary *in vitro* studies suggest a strong association between $p59^{ILK}$ and bcl-2.

1416

THE EXPRESSION OF THE PROTO-ONCOGENE BCL-2 IN CEM CELLS TREATED WITH THE ANTI-HIV DRUG DDC. ((A.N. Stevenson, M. Davis, and L. D. Taylor)) Department of Biology, Morgan State University, Baltimore, MD 21251.

Apoptosis, the morphological changes associated with programmed cell death involves cell shrinkage and DNA fragmentation. 2',3'-Dideoxycytidine is a nucleoside analog and reverse transcriptase inhibitor approved to treat HIV/AIDS. Data from this laboratory indicate that ddC is cytotoxic, targets mitochondria and may induce apoptosis in human leukemic cells. The bcl-2 gene encodes for an inner mitochondrial membrane protein that inhibits apoptosis. The present study investigates changes in bcl-2 expression in ddC treated CEM cells. 2×10^5 cells/ml cultured in complete RPMI 1640 were exposed to ddC (10-30 ug/ml) and maintained for three days at 37°C in a humidified CO_2 incubator. On day 3, the cells were counted, fixed with 1% paraformaldehyde and permeabilized with 0.05% Triton X-100. Cells were immunostained with rabbit anti-human bcl-2 antibody, goat anti-rabbit-FITC antibody and a CytoFluor II microplate reader was used to detect fluorescence. A five-fold decrease in bcl-2 expression was observed for ddC treated cells and we concluded that drug-induced apoptosis occurred. Research support-MBRS 1S06GM1A15197-01A1, RIMI 2P20RR-011606-02.

1417

Bcl-2 Inhibits Cytochrome C Release During Apoptosis In Leukemic HL-60 Cells Q. H. Zhang, J. X. Zhang, H. P.Sheng, & T. T. Loh. Department of Physiology, Faculty of Medicine, The University of Hong Kong, Hong Kong.

Recent studies have demonstrated that a variety of apoptotic stimuli can cause the release of cytochrome c from the mitochondria to the cytosol. Cytochrome c, in turn, activates the cleavage of caspases-3 which then initiates the final process of apoptosis. In this study, we investigated whether cytochrome c redistribution was a universal phenomenon during apoptosis, and the effect of Bcl-2 on the release of cytochrome c. Apoptosis in both control HL-60/neo cells and transfected HL-60/Bcl-2 cells were studied qualitatively and quantitatively by DNA fragmentation and flow cytometry analysis, and the redistribution of cytochrome c by western blot. Our results showed that cytochrome c was not released from the mitochondria in thapsigargin (TG)-induced apoptosis in HL-60/neo cells, and that this TG-induced apoptosis could be blocked by overexpression of Bcl-2. However, other agents such as C_2-ceramide, PKC inhibitors (STS, sphingosine, H7) and campothecin induced apoptosis via cytochrome c release and cpp32 activation. Overexpression of Bcl-2 inhibited the release of cytochrome c and apoptosis by these agents. Caspase 1 inhibitor (Ac-YVAD-cmk) partially blocked C_2-ceramide induced cpp32 cleavage and then apoptosis, but failed to prevent the release of cytochrome c induced by C_2-ceramide. These results suggest that the cytochrome c release from mitochondria may only be one of the pathways involved in the process of apoptosis, and that it acts upstream of cpp32. However, this apoptosis via cytochrome c release can be blocked by the overexpression of Bcl-2.

1418

Bcl-2 increases E-cadherin mediated cell adhesion prior to the onset of apoptosis D.W. Andrews, B. Leber, W. Zhu. Departments of Biochemistry and Medicine, McMaster University, Ontario, Canada.

In epithelia one of the earliest events in apoptosis is a profound reduction of cell-cell and cell-substrate adhesion. Using confocal microscopy we demonstrate that down-regulation of E-cadherin is well underway two hours after the addition of thapsigargin to MCF-7 cells. Loss of E-cadherin from the cell surface is not blocked by the addition of caspase inhibitors and precedes detectable activation of caspases, release of cytochrome C from mitochondria and mitochondrial permeability transition. Subsequent to the activation of caspases, E-cadherin is further modified by a process blocked by zVAD-fmk and YVAD-cho but only slightly reduced by DEVD-cho. The modified cadherins show reduced affinity for catenins. Expression in MCF-7 cells of Bcl-2, but not inactive Bcl-2 mutants, leads to an up-regulation of cadherin interaction with catenins and to increased cell adhesion prior to an apoptotic stimulus. Control experiments demonstrated that localization but not expression of catenins is altered by the initiation of apoptosis and by the expression of Bcl-2. Thus, one function of Bcl-2 in non-apoptotic cells is the regulation of cell adhesion. By increasing adhesion prior to the onset of apoptosis, Bcl-2 off-sets the loss in E-cadherin mediated cell adhesion that results from apoptosis induced by thapsigargin.

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