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Augmentation of NF-kappaB-mediated antioxidant signaling by Bcl-2: Potential roles of ERK1/2 and Akt/Protein kinase B

Jung Hee Jang and Young Joon Surh

National Research Laboratory, College of Pharmacy, Seoul National University, Korea

Oxidative stress induced by reactive oxygen intermediates often causes cell death via apoptosis, which is regulated by many functional genes and their protein products. The evolutionarily conserved protein Bcl-2 blocks apoptosis induced by a wide array of death signals. Despite extensive research, the molecular milieu that characterizes the anti-apoptotic function of Bcl-2 has not been fully clarified. In this work, we have investigated the role of Bcl-2 in protecting against oxidative death induced by hydrogen peroxide in cultured rat pheochromocytoma PC12 cells. Transfection with the bcl-2 gene rescued PC12 cells from apoptotic death caused by hydrogen peroxide. Addition of NF-κB inhibitors, such as pyrrolidine dithiocarbamate and N-tosyl-L-phenylalanine chloromethyl ketone, to the media aggravated oxidative cell death. PC12 cells overexpressing bcl-2 exhibited relatively high constitutive DNA binding and transcriptional activities of NF-κB compared with the vector-transfected control cells. Western blot analysis and immunocytochemistry revealed that bcl-2-transfected PC12 cells retained a higher level of p65 (the functionally active subunit of NF-κB) in the nucleus compared with vector-transfected controls. In addition, sustained activation of extracellular signal-regulated kinase1/2 and Akt/potien kinase B (upstream of NF-κB) was observed in the bcl-2 overexpressing cells. In contrast, the cytoplasmic inhibitor IκBα was present in lower amounts in the cells overespressing bcl-2. The ectopic expression of bcl-2 increased cellular glutathione and expression of glutamylcysteine ligase expression, which were attenuated by NF-κB inhibitors. These results suggest that NF-κB plays a role in bcl-2-mediated protection against hydrogen peroxide-induced apoptosis in PC12 cells through augmentation of antioxidant capacity.

Keyword : bcl-2, glutamylcysteine ligase, hydrogen peroxide, NF-κB