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BRAIN RESEARCH

Research Report

Bcl-2 overexpression disrupts the morphology of PC12 cells through reduced ERK activation

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ABSTRACT

Bcl-2 has been hypothesized to regulate many cellular functions in addition to its well-characterized role in the prevention of programmed cell death. To understand the role of Bcl-2 in regulating cell morphology and to explore the mechanism of this effect, we examined the effects of Bcl-2 overexpression on the morphology of PC12 cells in culture. We demonstrate that the overexpression of Bcl-2 in PC12 cells results in altered cell morphology and reduced actin expression. Analysis of extracellular signal-regulated kinase (ERK) 1/2 phosphorylation reveals that the morphological changes seen after *bcl-2* transfection are associated with reduced ERK activation. Treatment of control (mock-transfected) PC12 cells with the mitogen-activated ERK-activating kinase (MEK) inhibitor PD98059 converts their flat, process-bearing morphology into the rounded, process-free morphology of *bcl-2*-transfected cells, further confirming the association of ERK activation with altered cell shape. In conclusion, the present study describes a novel function of Bcl-2 in regulating cell shape through reduced ERK activation.

1. Introduction

Changes in cell shape both reflect and determine induction of cell growth, differentiation, and death in response to micro-environmental stimuli. In eukaryotic cells, cell morphology is regulated by the internal organization of molecular cytoskeletal networks, as well as by constraints imposed by cell-to-cell and cell-to-matrix contacts (Fuchs and Karakesisoglou, 2001). Recent studies have provided important new information as to how the actin cytoskeleton contributes to growth control in both normal and transformed cells. In addition to its traditional role as a structural framework, these experiments show that the cytoskeleton plays a critical role in the regulation of various cellular processes linked to transformation including proliferation, contact inhibition, anchorage-independent cell growth, and apoptosis. Disruption of the cytoskeleton is believed to contribute to several aspects of the transformed phenotype, including adhesion-independent cell growth and increased migratory potential (Pawlak and Helfman, 2001). The actin cytoskeleton also affects the organization and function of adhesive structures including the integrins and cadherins (Ben-Ze'ev, 1997; Schoenwaelder and Burridge, 1999).

Bcl-2 is a proto-oncogene that belongs to a family of apoptosis-modulating proteins. In addition to its well-characterized role in the suppression of programmed cell death, Bcl-2 has been associated with cell proliferation, differentiation, mutagenesis, and metastasis (Winter et al., 1998). A

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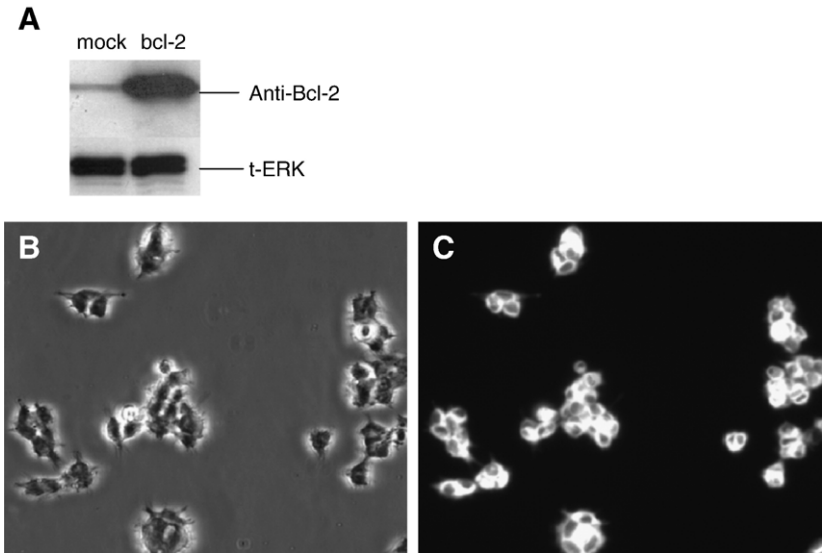

Fig. 1 – Bcl-2 expression in mock- and *bcl-2*-PC12 cells. (A) Western blot showing an increased level of Bcl-2 expression in *bcl-2*-PC12 cells as compared with *mock*-PC12 cells. Total ERK was used as a loading control. (B and C) Phase-contrast micrograph of *bcl-2*-PC12 cells (B) and the same field stained with anti-Bcl-2 antibody (C) showing that virtually all *bcl-2*-PC12 cells express Bcl-2.

recent study by Li et al. revealed that Bcl-2 expression decreases the level of functional E-cadherin, thereby interfering with cell–cell junction formation. Inhibition of cell–cell junction formation decreases cell–cell adhesion, in turn leading to the loss of contact inhibition (Li et al., 2003). However, the mechanisms by which Bcl-2 regulates or modulates these processes are not well understood.

Here, we examine the effects of Bcl-2 expression on cell morphology of undifferentiated PC12 cells. We show that overexpression of Bcl-2 leads to disruption of the actin