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## 2. JNK FUNCTIONS AS A STRESS-ACTIVATED AND INTERLEUKIN-3 AGONIST-ACTIVATED BCL2 KINASE

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The addition of interleukin-3 (IL-3) growth factor to factor-dependent cells can stimulate PKC-dependent and PKC-independent Bcl2 phosphorylation at ser70. However, expression of Bcl2 can also prolong cell survival under stress conditions (eg, IL-3 withdrawal, okadaic acid, anisomycin, and chemotherapeutic drug treatment) even in the absence of growth factor. Since phosphorylation of Bcl2 is required for its anti-apoptotic activity,<sup>1</sup> how does Bcl2 become functionally phosphorylated under such stress conditions in the absence of growth factor?

The existence of a stress-activated Bcl2 kinase might explain this phenomenon. We have found that anisomycin, a strong JNK/SAPK activator and protein synthesis inhibitor, can induce Bcl2 phosphorylation at ser70. WT but not phosphorylation-negative S70A mutant

Bcl2 can inhibit anisomycin-induced apoptosis in NSF/N1.H7 cells. JNK can be rapidly activated by the addition of IL-3 to cells, and it can be latently activated following IL-3 withdrawal by an unknown mechanism. Interestingly, we have found that JNK can directly phosphorylate Bcl2 in vitro and can colocalize with Bcl2 in the mitochondrial membrane. Dominant negative (DN) JNK blocks anisomycin-induced and IL-3-stimulated Bcl2 phosphorylation and enhances cell death following anisomycin treatment or IL-3 withdrawal. In addition, okadaic acid (OA), a potent protein phosphatase 1 and 2A inhibitor used as a cell stress treatment, can rapidly activate JNK and ERK1/2 (but not p38) and induce Bcl2 phosphorylation. PD98059, a MEK/MAPK-specific inhibitor, partially inhibits OA-induced Bcl2 phosphorylation and completely blocks OA-induced Bcl2 phospho-

rylation in DN-JNK overexpressing cells. This suggests that OA stimulates Bcl2 phosphorylation via activation of JNK and ERK1/2.

Taken together, these findings uncover a novel role for JNK as both an IL-3-stimulated and stress-activated Bcl2 kinase that can directly phosphorylate Bcl2 and may explain, at least in part, how Bcl2 can maintain functional phosphorylation under stress conditions, even in the absence of growth factor. Thus, stress treatments that may ultimately lead to cell death when the treatment application is prolonged can induce Bcl2 phosphorylation at ser70 likely in an attempt to maintain its function.

### Reference

1. Ito T, Deng X, Carr B, et al. Bcl-2 phosphorylation required for anti-apoptosis function. *J Biol Chem.* 1997;272:11671-11673.