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THE SUPER ANTI-APOPTOTIC FACTOR BCL-XFNK: A NOVEL MUTANT OF RAT BCL-XL WITH A GAIN-OF-FUNCTION PHENOTYPE

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INTRODUCTION. A powerful synthetic anti-apoptotic factor would be useful for future therapeutic applications in medicine. Analysis of the crystal structure of renatured human Bcl-x_L has suggested that it forms an ion channel (1). Additionally, Bcl-x_L has been shown to form an ion channel in synthetic lipid membranes (2). We have independently determined the x-ray structure of native rat Bcl-x_L with a 2.5 Å resolution and found it to have nine intramolecular polar interactions to stabilize the central putative pore-forming domain (α5- α6 helices) (3). We hypothesized that these hydrogen bonds may disturb the anti-apoptotic effect by inhibiting the insertion of the pore-forming domain into membranes. In the present study we designed a mutant Bcl-x_L, named Bcl-xFNK to make the α5- α6 helices more mobile or flexible by disturbing the formation of three hydrogen bonds which stabilize the tip of the putative pore-forming domain.

METHODS. To construct Bcl-xFNK, three amino acid substitutions (Tyr^{22} to Phe [F], Gln^{26} to Asn [N] and Arg^{165} to Lys [K]) were introduced into rat Bcl-x_L by a two-step PCR mutagenesis. Chinese hamster CHO K1 cells were cultured in D-MEM/F-12 medium containing 10 % fetal bovine serum. Human Jurkat cells and IL-3 dependent murine cells, FDC-P1 and BaF/3, were cultured in RPMI 1640 medium containing 10 % FBS. For FDC-P1 and BaF/3 cells, IL-3 was added in the medium. Plasmids were introduced into Jurkat, FDC-P1 and BaF/3 cells by electroporation and into CHO cells using Superfect. Stable transfectants Jurkat cells were treated with various death-inducing stimuli. CHO cell transfectants were deprived of serum. FDC-P1 and BaF/3 transfectant cells were incubated in the absence of IL-3. Surviving cells were counted by the trypan blue exclusion or by the WST-1 assay. BIOTRAK p42/p44 MAP kinase enzyme assay system was used to measure the p42/p44 MAP kinase activity of cells.

RESULTS. When over expressed in Jurkat or CHO cells, Bcl-xFNK was markedly more potent than wild-type Bcl-x_L in prolonging survival against anti-Fas (CH-11), staurosporine, TN-16, camptothecin, hydroxyurea, trichostatin A, hydrogen peroxide, paraquat, a calcium ionophore (A23187), heat treatment and serum deprivation. Interestingly, Bcl-xFNK allowed IL-3-dependent FDC-P1 but not BaF/3 cells to grow without IL-3. In Bcl-xFNK transfectants of FDC-P1 and Jurkat cells, the p42/p44 MAP kinase was activated by 2 to 5 times, but not in BaF/3 and CHO cells. Thus, Bcl-xFNK might acquire a new function to activate the MAP kinase in a cell-type specific manner.

DISCUSSION. These findings of this study suggest that the central α5-α6 pore-forming region of the anti-apoptotic factor Bcl-x_L has a pivotal role in suppressing apoptosis. Bcl-xFNK would be useful for making commercially important anima cell lines robust to culture insults may more effectively prevent cell death in diseases such as ischemia than Bcl-x_L and Bcl-2.

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