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NDP Kinases Suppressed Bax-Dependent Apoptosis in Yeast System

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Many nucleoside diphosphate (NDP) kinases are ubiquitous enzymes responsible for the exchange of γ-phosphates between tri- and diphosphonucleosides. The catalytic reaction follows a ping-pong mechanism in which the enzyme is transiently phosphorylated on a histidine residue conserved in all nucleoside diphosphate kinases. Beside their role in nucleotide synthesis, these enzymes present additional functions, possibly independent of catalysis, in processes such as differentiation, cell growth, tumor progression, metastasis and development. To clone murine nm23-M5, several expressed sequence tags (ESTs) of the GenBank data base, selected according to their homology to nm23-H5 cDNA, reconstituted a complete open reading frame (GenBank AF222750). To test whether murine NDPKs (1, 2, 3, 4, 5, and 6) can inhibit Bax-mediated toxicity in yeast, co-transformation was performed respectively. The yeast *S.cerevisiae* was transformed with a copy expression plasmid containing the histidine selection marker and expressing murine Bax under the control of a galactose-inducible promoter. Several clones were selected and found to be growth inhibited when Bax expression was induced with galactose. A representative clone was transformed again with a copy expression plasmid containing the tryptophane selection marker and expressing either murine Bcl-xL or NDPK under the control of a galactose-inducible promoter. Several subclones of the double-transformants were selected and characterized. The ability of Bcl-xL and NDPKs to suppress Bax-mediated toxicity was determined by growing yeast cells overnight in galactose media and spot-testing on galactose plates starting with an equal number of yeast cells as determined by taking the OD_{600}. Ten-fold serial dilutions were used in the spot-test. Plates were grown at 30° C for 2-3 days. All murine NDPKs suppressed Bax dependent apoptosis. Futher study will be performed whether Bax-toxicity inhibition was caused by NDP kinase activity or additional function.

(Key words) ***Bax, NDPK, apoptosis, yeast, Bcl-xL, nm23-M5***