

BCL-2/BCL-X_L REGULATES CELL CYCLE THROUGH A NOVEL MECHANISM
IN ADDITION TO CELL SURVIVAL

By

Yelena Melkum Janumyan

Dissertation

Submitted to the Faculty of the
Graduate School of Vanderbilt University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in

Cancer Biology

December, 2005

Nashville, Tennessee

Approved:

Professor Elizabeth Yang

Professor Stephen Brandt

Professor Josiane Eid

Professor William Russell

Professor James Sligh

BCL-2/BCL-X_L REGULATES CELL CYCLE THROUGH A NOVEL MECHANISM
IN ADDITION TO CELL SURVIVAL

YELENA MELKUM JANUMYAN

Dissertation under the direction of Professor Elizabeth Yang

Bcl-2 and Bcl-x_L are anti-apoptotic and inhibit proliferation. During cell cycle arrest, Bcl-2 or Bcl-x_L expression causes elevation of the CDK inhibitor p27, decrease in cell size and RNA content. This enhanced state of G₀ results in delay of G₀-G₁ transition and S phase entry upon cell cycle stimulation. We investigated the mechanism of the Bcl-2/Bcl-x_L cell cycle function and the relationship between apoptosis regulation and cell cycle control. To assess the role of mitochondrial functions in Bcl-2/Bcl-x_L-mediated cell cycle delay, we monitored ATP levels and found that cells expressing Bcl-2 or Bcl-x_L exhibit a delay in peak ATP during cell cycle entry; however, exogenous elevation of ATP did not reverse the delay in cell cycle entry. In mtDNA-free 143B ρ⁰ cells defective in electron transport, Bcl-x_L is still able to delay cell cycle entry but not enhance G₀ arrest. To determine whether enhanced G₀ and subsequent delay in cell cycle are consequences of increased survival, we inhibited cell death by means other than Bcl-2/Bcl-x_L expression. Increasing survival by caspase inhibition partially recapitulated the cell cycle arrest phenotype of Bcl-2/Bcl-x_L, while cells expressing the survival kinase Akt exhibited none of the cell cycle phenotypes of Bcl-2/Bcl-x_L. These data indicate that the

cell cycle delay by Bcl-2/Bcl-x_L is not mediated through the regulation of ATP/ADP exchange, and does not require normal mitochondrial membrane potential. Bcl-2/Bcl-x_L may regulate quiescence partially through the inhibition of caspase-dependent apoptosis, but cell survival is not the sole mechanism of the cell cycle regulation by Bcl-2/Bcl-x_L. In addition to inhibition of mitochondrial apoptosis, mechanisms requiring an intact electron transport chain are necessary for the full cell cycle function of Bcl-2/Bcl-x_L.

Approved _____ Date _____