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Award Number: DAMD17-99-1-9511

TITLE: Characterization of a Novel Apoptosis Regulator BI-1

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La Jolla, California 92037

REPORT DATE: July 2000

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 2000	Annual Summary (1 Jul 99 - 30 Jun 00)

4. TITLE AND SUBTITLE
Characterization of a Novel Apoptosis Regulator BI-1

5. FUNDING NUMBERS
DAMD17-99-1-9511

6. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
DISTRIBUTION STATEMENT: Distribution authorized to U.S. Government agencies only (proprietary information, Jul 00). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***
BI-1 is an important cell death regulator that protects cells against Bax-induced cell death. It is expressed in all four prostate cancer lines and overexpresed in two of them, making it an important regulator in prostate cancer progression. I have used genetics, biochemical and cell biology approaches to understand how BI-1 regulate apoptosis and possibly prostate cancer. I have demonstrated the in vivo function of BI-1 homolog in yeast; I have discovered that BI-1 homologs from different species, including plant, fly, yeast and E. coli can function similarly in yeast to protect Bax-induced cell death. I have also mapped important BI-1 domains essential for its function. These provide the basis for understanding the mechanism through which BI-1 regulates apoptosis.

14. SUBJECT TERMS
Prostate Cancer

15. NUMBER OF PAGES
8

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

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Table of Contents

Cover..

SF 298..2

Foreword..3

Table of Contents..4

Introduction..5

Body..5

Key Research Accomplishments..7

Reportable Outcomes...

Conclusions..7

References...

Appendices..

Introduction:
BI-1 is a novel anti-apoptotic regulator identified in our lab through the Bax suppressor screen conducted in yeast. Its anti-apoptotic function in mammalian cells makes it a possible regulator for cancer progression. BI-1 is expressed in all four prostate cancer cell lines tested and highly expressed in two of them, thus makes it a potential target for prostate cancer therapy. We have proposed to use genetics, cell biology and biochemistry approaches to study the structure and function of this novel apoptotic regulator and its potential function in prostate cancers.

Body:

I have focused my first year's training on studying the in vivo function of BI-1 and the structural and functional analysis of BI-1. Several important findings have been made that are described below:

I have successfully identified and demonstrated the function of the potential yeast BI-1 homolog. The yeast BI-1 homolog (yBI-1) was identified through blast search of the yeast genome database against BI-1 sequence. The yBI-1 was not an essential gene and its knock-out yeast strain was therefore obtained and characterized. Because of the protective nature of BI-1, I have tested the function of y-BI-1 against stress-induced cell death. Interestingly, I have found that the surviving ability of yBI-1 deletion strain is much lower compared to wild-type strain, suggesting that yBI-1 is important for protecting yeast cells from heat. Along with this observation, it is recently published that the Arabidopsis BI-1 homolog can be induced by wound and pathogen challenge, further extending that BI-1 is an evolutionarily conserved protein with cell protective function.

More interestingly, overexpression of the yBI-1 gene can suppress Bax-induced cell death in yeast, indicating it is a functional BI-1 homolog. Besides, I have also obtained the putative BI-1 homologs from plants (Arabidopsis, Rice and Tomato), Drosophila and bacteria. Overexpression of all these BI-1 genes rescued the yeast cells from Bax-induced cell death. Therefore, BI-1 appears to be an evolutionarily conserved protective protein present throughout the prokaryotic and eukaryotic kingdom.

Discovery of BI-1 homologs makes it possible to identify potential important BI-1 domains by aligning the homologs and identifying important residues. BI-1 is a membrane protein with six transmembrane domains. Two regions are initially identified through the alignment, with one at the 3' cytosolic end and the other between the last two transmembrane domain. These two domains were mutated and tested for their function in yeast cells. While the one with mutation in the most c-terminal nine amino acids replaced with alanine abolished BI-1 protective function against Bax-induced cell death, the other doesn't show any phenotype. Besides these two mutations, deletion analysis of BI-1 gene was also carried out to identify important domains essential for its function. Thirteen BI-1 deletion mutants were constructed so that each mutant has deletion in each of the TM domain or domain between the two adjacent TM domains. These mutants were then tested for their function in the yeast cells to determine their protective function. All but two of deletion constructs can still rescue yeast from the Bax-induced cell death, and these two deletions are located at the N-terminal cytosolic domain and the region between TM1 and TM2. Thus, it appears that all six TM domains are essential for BI-1 function. Interestingly, deletion or mutation of the most c-terminal nine amino acids into alanine also changed the cellular localization of BI-1 protein, therefore indicating that these nine amino acides are essential for BI-1 function and localization. These mutants will provide us with the necessary tool for dissecting BI-1 structure and function.

Besides working on BI-1 gene, I have screened five more cDNA libraries in order to identify new Bax-suppressors in yeast. These screens have yielded known apoptosis regulator genes such as Bcl-2, Bcl-Xl, Mcl-1, BI-1 and BI-1 homologs, suggesting that the yeast assay is an important system to identify apoptosis regulators. More importantly, I have identified two new genes that can rescue the yeast cells from Bax-induced cell death. I am currently characterizing their functions in mammalian cells. At the meantime, I also started to work on another important gene, BI-2 (BAR), that is identified in the same screen when BI-1 is identified. BAR protects mammalian cells against both the Bax- and Fas- induced cell death, that are the two major cell death pathway. Most interestingly, BAR is overexpressed in many tumor cell lines, thus making it an interesting regulator for tumor progression. Through analysis of these Bax-inhibitor proteins, we will be able to better understand the functions of Bax and its inhibitors.

Work in progress:
In addition to the work described above, some experiments in progress below are described below:

BI-1 homolog in mice has also been identified. Its knockout cell line is identified in the Lexcon database. The Knock-out BI-1 mice has been ordered. Upon receipt, phenotypes will be determined. This will tell us ultimately the in vivo function of BI-1 gene. At the meantime, BI-1 antibody is being raised against a BI-1 peptide. The successful antibody will allow us to test the expression of BI-1 in the prostate cancer cell lines to correlate the BI-1 expression and different prostate cancer specimen.

Expression of the BI-1 homologs will also be conducted in mammalian cells to determine whether they function similarly to the yeast cells. The functions of various BI-1 mutant described above will also be tested in mammalian cells to determine the ultimate important BI-1 domains involved in apoptosis regulation. These study will provide us the basis for understanding BI-1's structure and function and its possible role in prostate cancer progression.

Key research accomplishments:

Demonstrated yeast BI-1 homolog's function in stress-induced cell death.
Demonstrated BI-1 homologs' function in protecting yeast cells against Bax-induced cell death.
Identified several BI-1 domains essential for its protective function.
Discovered two more genes that can protect the yeast against Bax-induced cell death.

Reportable outcomes:
N/A.

Conclusions:
In conclusion, I have made a lot of progress toward understanding the mechanisms by which BI-1 regulate apoptosis: namely, the identification of BI-1 homologs from various species and their similar protective functions; and the identification of BI-1 domains essential for its function. These will provide basis for BI-1's structural and functional analysis. The mouse knock-out study will definitely demonstrate BI-1 in vivo function, while the development will determine whether there are any correlation between BI-1 expression and prostate cancer. These studies will help us better understand BI-1's function and its possible role in prostate cancers.

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