UNCLASSIFIED

AD NUMBER

ADB264450

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies only; Proprietary Information; Jul 2000. Other requests shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, MD 21702-5012

AUTHORITY

USAMRMC ltr, 26 Nov 2002

THIS PAGE IS UNCLASSIFIED

AD_____

Award Number: DAMD17-99-1-9511

TITLE: Characterization of a Novel Apoptosis Regulator BI-1

PRINCIPAL INVESTIGATOR: Ning Ke, Ph.D.

CONTRACTING ORGANIZATION: The Burnham Institute 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

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.

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20010322 158

NOTICE

USING GOVERNMENT DRAWINGS, SPECIFICATIONS, OR OTHER DATA INCLUDED IN THIS DOCUMENT FOR ANY PURPOSE OTHER ANY GOVERNMENT PROCUREMENT DOES NOT IN WAY THAN THE U.S. GOVERNMENT. THE FACT THAT THE OBLIGATE THE DRAWINGS, FORMULATED OR SUPPLIED GOVERNMENT DATA DOES NOT LICENSE THE SPECIFICATIONS, OR OTHER HOLDER OR ANY OTHER PERSON OR CORPORATION; OR CONVEY ANY RIGHTS OR PERMISSION TO MANUFACTURE, USE, OR SELL ANY PATENTED INVENTION THAT MAY RELATE TO THEM.

LIMITED RIGHTS LEGEND

Award Number: DAMD17-99-1-9511 Organization: The Burnham Institute Location of Limited Rights Data (Pages):

Those portions of the technical data contained in this report marked as limited rights data shall not, without the written permission of the above contractor, be (a) released or disclosed outside the government, (b) used by the Government for manufacture or, in the case of computer software documentation, for preparing the same or similar computer software, or (c) used by a party other than the Government, except that the Government may release or disclose technical data to persons outside the Government, or permit the use of technical data by such persons, if (i) such release, disclosure, or use is necessary for emergency repair or overhaul or (ii) is a release or disclosure of technical data by, a foreign government that is in the interest of the Government and is required for evaluational or informational purposes, provided in either case that such release, disclosure or use is made subject to a prohibition that the person to whom the data is released or disclosed may not further use, release or disclose such data, and the contractor or subcontractor or subcontractor asserting the restriction is notified of such release, disclosure or use. This legend, together with the indications of the portions of this data which are subject to such limitations, shall be included on any reproduction hereof which includes any part of the portions subject to such limitations.

THIS TECHNICAL REPORT HAS BEEN REVIEWED AND IS APPROVED FOR PUBLICATION.

Mminghe Charan Minm or/15/07

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 074-0188		
Public reporting burden for this collection of in maintaining the data needed, and completing a including suggestions for reducing this burden VA 22202-4302, and to the Office of Manage	formation is estimated to average 1 hour per resp nd reviewing this collection of information. Senc to Washington Headquarters Services, Directorat ement and Budget, Paperwork Reduction Project	onse, including the time for review comments regarding this burden e for Information Operations and F 0704-0188), Washington, DC 205	ving instructions, sea estimate or any othe Reports, 1215 Jeffer 503	arching existing data sources, gathering and r aspect of this collection of information, son Davis Highway, Suite 1204, Arlington,		
1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE July 2000	3. REPORT TYPE AND Annual Summary	DATES COVERED (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) Ning Ke, Ph.D.		· · · · · · · · · · · · · · · · · · ·				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The Burnham Institute			8. PERFORMING ORGANIZATION REPORT NUMBER			
La Jolla, California 92037						
E-MAIL: <u>nke@burnham-inst.org</u>		S)				
U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				AGENCY REPORT NUMBER		
11. SUPPLEMENTARY NOTES						
12a. DISTRIBUTION / AVAILABILITY STATEMENT DISTRIBUTION STATEMENT: Distribution authorized to U.S. Government agencies only (proprietary infor Jul 00). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Comm 504 Scott Street, Fort Detrick, Maryland 21702-5012.				mation, and,		
13. ABSTRACT (Maximum 200 Wo BI-1 is an important of death. It is expressed making it an important biochemical and cell b possibly prostate cano yeast; I have discover yeast and E. coli can have also mapped impor basis for understandin	rds) ell death regulator that d in all four prostate regulator in prostate iology approaches to un er. I have demonstrate ed that BI-1 homologs f function similarly in y tant BI-1 domains essen g the mechanism through	at protects cells cancer lines and cancer progress aderstand how BI- ed the in vivo fu from different sp yeast to protect atial for its fur h which BI-1 regu	s against d overexpr ion. I ha -1 regulat unction of pecies, in Bax-induc nction. T ulates apo	Bax-induced cell resed in two of them, we used genetics, a apoptosis and BI-1 homolog in cluding plant, fly, red cell death. I These provide the optosis.		
14. SUBJECT TERMS				15. NUMBER OF PAGES		
Flostate cancer			-	16. PRICE CODE		
17. SECURITY CLASSIFICATION	18. SECURITY CLASSIFICATION	19. SECURITY CLASSIFICATION		20. LIMITATION OF ABSTRACT		
Unclassified	Unclassified	Unclassified		Unlimited		
NSN 7540-01-280-5500			Star Presc 298-'	ndard Form 298 (Rev. 2-89) ribed by ANSI Std. 239-18 102		
n Şi Şi Şi Şi Şi						

.

and the strength of the streng

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

 $\underline{N/A}$ Where copyrighted material is quoted, permission has been obtained to use such material.

 $\underline{N/A}$ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

 $\underline{N/A}$ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

<u>N/A</u> In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

N/A For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

 $\underline{N/A}$ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

 $\underline{N/A}$ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

 $\underline{N/A}$ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

8/1/00

PI - Signature

Date

Table of Contents

Cover
SF 2982
Foreword
Table of Contents4
Introduction5
Body5
Key Research Accomplishments7
Reportable Outcomes
Conclusions7
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.

<u>Reportable outcomes:</u>

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.



DEPARTMENT OF THE ARMY

US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND 504 SCOTT STREET FORT DETRICK, MARYLAND 21702-5012

REPLY TO ATTENTION OF:

MCMR-RMI-S (70-1y)

26 Nov 02

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for this Command. Request the limited distribution statement for the enclosed accession numbers be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Kristin Morrow at DSN 343-7327 or by e-mail at Kristin.Morrow@det.amedd.army.mil.

FOR THE COMMANDER:

Μ. Depu Child of Staff for f

In

ormation Management

Encl

ADB263708				
ADB257291				
ADB262612				
ADB266082				
ADB282187				
ADB263424				
ADB267958				
ADB282194				
ADB261109				
ADB274630				
ADB244697	-			
ADB282244		•		
ADB265964				
ADB248605				
ADB278762				
ADB264450				
ADB279621'				
ADB261475				
ADB279568				
ADB262568-	•			
ADB266387				
ADB279633				
ADB266646				
ADB258871.				
ADB266038				
ADB258945 -				
ADB278624.				
