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Award Number: DAMD17-01-1-0574

TITLE: Demonstration That a mRNA Binding Protein is Responsible for GADD45 mRNA Destabilization

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REPORT DATE: May 2002

TYPE OF REPORT: Annual

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
	May 2002	Annual (1 May 01 - 30 Apr 02)

4. TITLE AND SUBTITLE

Demonstration That a mRNA Binding Protein is Responsible for GADD45 mRNA Destabilization

5. FUNDING NUNUMBER
DAMD17-01-1-0574

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
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

We are studying the post-transcriptional control of expression of the p53-inducible antiproliferative gene known as Growth Arrest and DNA Damage induced gene 45 (GADD45). Using human breast carcinoma cell lines, we have demonstrated that the half-life of GADD45 mRNA is very responsive to ambient glutamine (GLN) availability. We have cloned the GADD45 cDNA and made several constructs of this cDNA that represent a first step toward making the constructs needed to test the region that is responsible for mRNA destabilization. We have also been developing the transcfection techniques needed to conduct the mRNA stability studies. Unfortunately, the model system, TSE cells, has proven to be relatively difficult to transfect at a reasonable frequency. We are working to improve this frequency by using alternative transfection methods. Due to a shortage of technical help, the aims have not been completed in the first year. We have applied for a one-year no-cost extension to the award period.

14. SUBJECT TERMS
breast cancer, mRMNA binding protein, growth arrest

15. NUMBER OF PAGES
6

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

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INTRODUCTION

We are examining the molecular mechanism by which the expression of an antiproliferative p53 downstream effector gene is post-transcriptionaly controlled in breast cancer cells. The downstream effector gene to be studied is the growth arrest and DNA damage induced gene, GADD45. This gene is transcriptionally activated by wild-type p53; therefore GADD45 expression can be depressed in p53-deficient cells. Employing GADD45 knockout mice, A. J. Fornace and colleagues found that loss of GADD45 expression reproduced a large subset of the effects observed in p53 knockout mice (1). Our ultimate goal is to develop a technique that will upregulate expression of GADD45 in a p53-independent fashion. It is theorized that increasing GADD45 expression in p53-deficient cells will reproduce many of p53's antiproliferative functions. GADD45 expression in breast carcinoma cell lines is tightly controlled by the availability of the amino acid glutamine, primarily through a post-transcriptional mechanism (2). GADD45 mRNA is inherently unstable with a half-life of 30 to 45 minutes. Depriving these cell of media glutamine increased the half-life of GADD45 mRNA by approximately 17-fold. Conversely, repletion of media glutamine caused an immediate and rapid decay of GADD45 mRNA. Thus, this model system can be used to determine the mechanism by which GADD45 gene expression is controlled through mRNA turnover. In analogy to destabilization of AU-rich mRNAs such as c-myc by the AU-rich binding factor AUF-1 (3), it is hypothesized that there exists a mRNA binding protein that binds to GADD45 and causes or initiates its degradation.

BODY

There is very little progress to report at this time. This is due to a shortage of qualified laboratory personnel over this last year. The research technician who was originally to perform the laboratory procedures, Robyn Hassebrook, left the laboratory approximately one year ago. Since then I have conducted a national search for a postdoctoral fellow with no success. A part-time student employee has worked one the project, but progress so far has been very disappointing. For this reason I have requested a no cost extension for the award (see appendix).

We have cloned the GADD45 cDNA and made several constructs of this cDNA that represent a first step toward making the constructs needed to test the region that is responsible for mRNA destabilization. We have also been developing the transcfection techniques needed to conduct the mRNA stability studies. Unfortunately, the model system, TSE cells, has proven to be relatively difficult to transfect at a reasonable frequency. We are working to improve this frequency by using alternative transfection methods.

KEY RESEARCH ACCOMPLISHMENTS:

- Constructed several GADD45 cDNA containing plasmid vectors.
- Evaluated several methods to transfect TSE cells.
- Optimized transfection by cationic lipid method.

REPORTABLE OUTCOMES:

- GADD45 cDNA plasmid constructs

REFERENCES

1. Hollander, M.C. et al. Disruption of gadd45 leads to genomic instability, loss of cellular growth control and radiation-induced carcinogenesis. Proc Amer Assoc Cancer Res 40:413 [abstract #2728], 1999.

2. S.F. Abcouwer, C. Schwarz and R. A. Mequid. Glutamine deprivation induces the expression of GADD45 and GADD153 primarily by mRNA stabilization. J Biol Chem 247:28645-28651, 1999.

3. Wilson, G.M. and G. Brewer. The search for trans-acting factors controlling messenger RNA decay. Prog. Nucleic Acid Res Mol Biol 62:257-91, 1999.

To: Shelley.marken@det.amedd.army.mil
From: Lee Gulbransen
Subject: No Cost Extension
CC: Program_Support_Team 2; Carole.Christian@det.amedd.army.mil; Steve Abcouwer
Date Sent: Thursday, May 16, 2002 8:17 AM

Shelley Marken, Contract Specialist
USAMRAA
Phone: 301-619-2268
Fax: 301-619-3166
Re: No-Cost Extension of Concept Award BC995864 (Award # DAMD17-01-1-0574).

Dear Ms. Marken,

I would like to request a no-cost extension to the duration of the Concept Award BC995864 (Award Number DAMD17-01-1-0574). The new end date would be 4/30/03.

The period of funding for this grant is, of course, one year and a progress report is due on May 31, 2002. Unfortunately, I have very little progress to report at this time. This is due to a shortage of qualified laboratory personnel over this last year. The research technician who was originally to perform the laboratory procedures, Robyn Hassebrook, left the laboratory approximately one year ago. Since then I have conducted a national search for a postdoctoral fellow with no success. A part-time student employee has worked one the project, but progress so far has been very disappointing.

As of this summer, I will have three pre-doctoral students working in the laboratory. I have also recently hired and trained an excellent research technician. Thus, I feel that I will be able to complete the project in the next year if allowed to do so. There is still ample money left in this account to complete the study as proposed during the next year

Your help would be greatly appreciated.

Sincerely,

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