

Award Number: W81XWH-04-1-0705

TITLE: A Novel Membrane-Permeable, Breast-Targeting, Pro-Apoptotic Peptide for Treatment of Breast Cancer

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REPORT DATE: October 2005

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for Public Release;
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20060524011

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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1. REPORT DATE 01-10-2005		2. REPORT TYPE Annual		3. DATES COVERED 30 Sep 2004 -29 Sep 2005	
4. TITLE AND SUBTITLE A Novel Membrane-Permeable, Breast-Targeting, Pro-Apoptotic Peptide for Treatment of Breast Cancer				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-04-1-0705	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Bin Guo, Ph.D.				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) North Dakota State University Fargo, ND 58105				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. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT In this project, we are trying to create a novel peptide with three properties: membrane permeable, breast targeting, and inducing apoptosis. The peptide is expected to be able to induce apoptosis specifically in breast cancer cells and will be tested as a single therapeutic agent as well as in combination with chemotherapeutic drugs to treat breast cancer. In the first step, we have synthesized the breast-targeting, membrane permeable, pro-apoptotic peptide. The BH3 peptide of Bid (EDIIRNIARHLAQVGDSMDR) has been synthesized with eight d-arginine residues at the N-terminus with a glycine linker residue, followed by a breast-homing sequence (CPGPEGAGC) at the C-terminal. A control peptide with a mutation in the BH3 domain was also synthesized, r8-BH3(L/E)-CPGPEGAGC. Next, we have tested the therapeutic efficacy of the peptide in treatment of breast cancer. The peptide was tested for apoptosis induction first in vitro in cultured breast cancer MCF-7 cells. However, this peptide failed to induce apoptosis in MCF-7 cells at the concentrations being tested. We are in the process to produce an alternative peptide, which has similar design and predicted functions but with stronger apoptosis inducing capability. The new peptide has a hydrocarbon-stapled BH3 helix, and will be tested for its apoptosis-inducing activity.					
15. SUBJECT TERMS Breast cancer, apoptosis, BH3 peptide, breast targeting.					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 5	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

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Introduction

The Bcl-2 family proteins are key regulators of apoptosis. Bid, a pro-apoptotic member of the Bcl-2 family, induces apoptosis through its Bcl-2 homology 3 (BH3) domain. The Bid BH3 peptide is able to induce apoptosis in cancer cells when it is linked to a membrane permeable peptide. A breast-homing peptide, CPGPEGAGC, is shown to specifically target breast tissue. This project aims to design and test a novel therapeutic peptide for breast cancer, which with three properties: membrane permeable, breast targeting, and inducing apoptosis.

The project was initially delayed for about four months because of the difficulties to recruit post-doc associate or graduate students to carry out experiments (the PI is a new investigator, who recently established his own lab in early 2004). Then, after a few months working for the project, the graduate student (Binfeng Xia) left the university to south Carolina. A new graduate student (Tianying Zhu) joined the project recently to continue the experiments. Due to these delays, a request for no-cost extension of one more year was submitted by the PI and approved by the Department of Defense grant office. This is an annual report instead of the final report.

Progress report

In the first step, we have synthesized the breast-targeting, membrane permeable, pro-apoptotic peptide. The BH3 peptide of Bid (EDIIRNIARHLAQVGDSMDR) has been synthesized with eight arginine residues at the N-terminus linked by a glycine linker residue, followed by a breast-homing sequence (CPGPEGAGC) at the C-terminal. A control peptide with a mutation in the BH3 domain was also synthesized, r8-BH3(L/E)-CPGPEGAGC. Next, we have tested the therapeutic efficacy of the peptide in treatment of breast cancer. The peptide was tested for apoptosis induction in cultured breast cancer MCF-7 cells. Cells were treated with various concentrations of peptide ranging from 1 μ M to 100 μ M for 24 hours. Apoptosis induction was detected by TUNEL assay using a GUAVA PCA microcytometer. Unfortunately, this peptide failed to induce apoptosis in MCF-7 cells at these tested concentrations (Figure 1). It's not practical to increase the concentration above 100 μ M, since higher concentration will not be achieved in vivo in mice or in clinic. We are examining whether the peptide has entered the cells by western blotting and confocal microscopy using a polyclonal antibody raised against Bid BH3 domain (Abgent, San Diego, CA). To overcome this problem and improve the apoptosis inducing ability, we have designed an alternative peptide, which has similar design and predicted functions but with stronger apoptosis inducing capability. The new peptide has a hydrocarbon-stapled BH3 helix, produced by ruthenium-catalyzed olefin metathesis (1). This "stapled" BH3 peptide has been shown to have enhanced apoptosis-inducing activity and also is cell permeable. Thus, the eight arginine residues are no longer needed. The breast-homing sequence (CPGPEGAGC) will be added at the C-terminal. This new peptide will then be tested for apoptosis inducing activity.

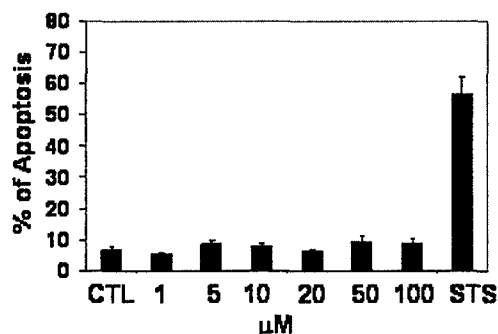


Figure 1. The peptide failed to induce apoptosis in MCF-7 cells. Cells were treated with various concentrations of the peptide for 24 hours. Apoptosis was examined by TUNEL assay. Treatment with 1 μ M Staurosporin (STS) was used as a positive control for apoptosis.

Key research accomplishments

The originally designed BH3 peptides have been synthesized and tested for in vitro apoptosis-inducing activity. The peptide failed to induce apoptosis in breast cancer MCF-7 cells. A new peptide with improved activity has been designed and being produced.

Reportable outcomes

None.

Conclusions

We have synthesized the proposed peptides and tested the peptides in cultured breast cancer cells for induction of apoptosis. However, the peptide has no apoptosis-inducing activity in the current design. New strategy is proposed and being tested to enhance the apoptosis-inducing activity of the peptide.

References

1. Walensky LD, Kung AL, Escher I, Malia TJ, Barbuto S, Wright RD, Wagner G, Verdine GL, Korsmeyer SJ. 2004 Activation of apoptosis in vivo by a hydrocarbon-stapled BH3 helix. *Science*, 305(5689):1466-70.