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Award Number: DAMD17-99-1-9428

TITLE: Apoptosis Based Gene Therapy of Breast Cancer

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REPORT DATE: August 2001

TYPE OF REPORT: Annual Summary

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	August 2001	Annual Summary (1 Aug 00 - 31 Jul 01)

4. TITLE AND SUBTITLE
Apoptosis Based Gene Therapy of Breast Cancer

5. FUNDING NUMBERS
DAMD17-99-1-9428

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)*

This project is an attempt to develop a gene therapy model for the treatment of metastatic breast cancer. Our goal is to develop adenoviral vectors that deliver a cytocidal gene selectively to breast cancer cells. We have employed recombinant molecules of caspase-3, a distal effector protein in the apoptotic cascade. Recombinant caspase-3 molecule, unlike the parent molecule, is constitutively active; expression of it in breast cancer cells rapidly induces apoptosis. As expression of caspase-3 in non-malignant cells could be toxic, we are attempting to limit expression to cancer cells by employing breast and tumor specific promoters. We have demonstrated that the promoter of the Hexokinase-II gene is tumor specific and the mammoglobin promoter is expressed only in breast cells. In order to tightly control expression to cancer cells we are attempting to employ the CRE / Lox system. One adenovirus would express the CRE gene under the control of the Hexokinase-II promoter. Another adenovirus would express an inactivated recombinant caspase-3 under control of the mammoglobin promoter. Only the co-infection of these two viruses in cells in which both of these promoters are active, i.e. breast cancer cells, would permit the expression of caspase-3. These viruses are presently being constructed.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Breast Cancer, gene therapy, apoptosis	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

Conventional therapy for patients with metastatic breast cancer is unsuccessful as despite therapy with hormonal agents and chemotherapy virtually all patients die of their disease. This project is an attempt to develop a model for gene therapy of breast cancer that acts by selectively inducing apoptosis in breast cancer cells. Caspase–3, (c3), is a distal effector protein in the cascade that induces apoptosis. Expression of the c3 gene product is regulated by post translational cleavage and reassembly of the two fragments into the active molecule. Our proposal is to develop a recombinant c3 that is constitutively active (1). This recombinant c3 activates the apoptosis cascade and rapidly induces cell death. As recombinant c3 activates apoptosis far "downstream" it bypasses the mechanisms of resistance to chemotherapy and other cytotoxic agents that most cancer cells have developed by the time they have developed metastatic potential. Therefore this represents a powerful cytotoxic agent for gene therapy of cancer.

One potential problem with the use of c3 as the "killing gene" is that expression in any normal cell would also be toxic. Therefore the expression of c3 must be very tightly regulated and exclusively limited to malignant cells. In order to accomplish this we intend to employ the following strategy. It has two essential parts. The first part is the use of two separate adenoviral vectors, (with different promoters); both adenoviral vectors must infect the target cell. The second regulatory step is the use of the CRE / Lox system(2). These two together will induce tumor targeted recombinant c3 expression and apoptosis.

One vector will induce the expression of the CRE gene under the control of the Hexokinase II promoter. We have previously shown that the Hexokinase II promoter is expressed in malignant breast and lung tumor cells but not in normal lung, breast and liver cells (3). Therefore only malignant cells will have functional CRE recombinase.

The gene expressed by the second adenoviral vector will be regulated by the promoter of the mammoglobin gene which is expressed solely in the breast (4). This vector codes for a construct that consists of 2 lox sites that flank "junk" DNA that includes a stop codon; downstream of this is the recombinant c3 that is constitutively active. This vector, by itself will have no functional effect on breast or any other cells, as the gene it delivers is inactive without the co-expression of CRE by the first vector. When a permissive cell is infected by both of these viruses the junk DNA is cut out, expression of constitutively active caspase-3 occurs, and apoptosis should be induced. This double system will tightly regulate recombinant c3 expression to only malignant cells.

Body

Progress to date:

Task 1: Expression of recombinant Caspase-3 induces apoptosis in Breast Cells

As stated in last year's report, we have shown that reverse caspase-3, a recombinant c3 molecule that has been engineered to have the p11 fragment 5' of the p20 fragment, no longer requires post translational cleavage and is constitutively active. As demonstrated last year recombinant c3 induces apoptosis in 5 different breast cancer cell lines. We are using this for our present viral constructs.

Task 2: Selectivity of our Site Specific Promoters

This task has as well been accomplished. As stated in last years report, we have shown that the Hexokinase II promoter can induce high level expression in malignant cells but is poorly expressed in normal cells. Similarly, the mammoglobin promoter is selectively expressed in breast cells, benign or malignant.

Task 3: Generation of Adenoviral vectors

This year's work has been particularly frustrating. We have worked hard to clone the required plasmids necessary for vector production. Several constructs were made that appeared to be the required plasmids, but then were later determined to be the wrong constructs. This has caused a great deal of lost time.

The problems we have had relate to technical problems with the cloning. We have tried a variety of cloning techniques to create the adenoviral transfer plasmids. These are multistep cloning exercises. One problem is that the fragments to be cloned are fairly large. For example the Hexokinase II promoter itself is almost 4 kB. All of them are less than the approximately 10 kB allowable size for inserts in the second generation adenovectors that we have been employing.

A second problem relates to the fact that shortly after this year began my technician left my lab and I have been unable to find a suitable replacement. Therefore we are still in the phase of transfer plasmid production, and are not even ready to create the first viral vectors. As such we have no significant new data to deliver in this report.

A third issue relates to my move from Virginia Commonwealth University to the Lahey Clinic in Burlington, Mass, this summer. This has profoundly changed the direction of my work. These gene therapy experiments have been terminated as of August 2001. A revised statement for the continuation of this research award has been submitted.

Key Research Accomplishments

- Demonstrated that recombinant revCaspase-3 is constitutively active, and induces apoptosis.

- Demonstrated that the mammoglobin and Hexokinase II promoters are regulatory elements that can control gene expression in breast cancer cells.

Reportable outcomes

None this year

Conclusions

See above

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Appendices

none