AD_____

Award Number: W81XWH-04-1-0084

TITLE: Identification of a Protein for Prostate-Specific Infection

PRINCIPAL INVESTIGATOR: Shen Pang, Ph.D.

CONTRACTING ORGANIZATION: The University of California
Los Angeles, California 90024

REPORT DATE: December 2004

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
	December 2004	Annual Summary (1 Dec 03-30 Nov 04)

4. TITLE AND SUBTITLE
Identification of a Protein for Prostate-Specific Infection

5. FUNDING NUMBERS
W81XWH-04-1-0084

6. AUTHOR(S)
Shen Pang, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The University of California
Los Angeles, California 90024

E-Mail: SHENP@DENTNET.DENT.UCLA.EDU

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)

In this proposal, we will identify and clone a protein that can be used to generate infection-specific gene therapy vector. We expect that using this protein to modify various gene therapy vectors, we can specifically deliver cytotoxic genes into prostate cancer cells using systemic treatment, and eventually eradicate metastatic prostate cancer cells in patients.

During the first year, we have generated the cDNA expression library (Task 1). We have picked up clones to check the percentage of clones that contain cDNA inserts and the sizes of the cDNA inserts. We found that approximately 70% of our clones containing cDNA inserts. Most of the inserts are between 1.0 to 2.0 kb. We have screened approximately 1,200 colonies, fulfill the requirement as our SOW stated and we expected by screening more cDNA clones, we will be able to identify the genes related to tissue-specific delivery of lentiviral genes into prostate cells.

14. SUBJECT TERMS
No subject terms provided.

15. NUMBER OF PAGES
5

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

Table of Contents

Title: Identification of a Protein for Prostate-Specific Infection
PI: Shen Pang
Start date: Dec. 1, 2003
Grant number: W81XWH-04

Introduction:

Using gene therapy vectors to eradicate prostate cells is extensively studied. It is expected that if we can use a target-specific gene therapy to deliver cytotoxic genes into metastatic prostate cancer cells in patients, we can eradicate these malignant cells and cure the patients. To find an approach to generate infection-specific vectors is critical for target-specific cancer cell eradication. Because viral vectors infect cells by the binding of their surface proteins to their receptors on the target cells, it is expected that infection-specific vectors can be generated by modifying their surface proteins with proteins that can specifically bind to target cells. In our Preliminary Studies, we found that the lentiviral vector (an HIV-based retroviral vector) generated from a cell line derived from human oral tissue (HOT) can specifically infect LNCaP prostate cancer cells. Virus generated from other cell lines has much lower infectivity to LNCaP cells (200 to 1200-fold lower). These results strongly suggest that the HOT cells express a very specific membrane protein that can be picked by lentiviral vectors to modify their envelope. With such protein on its envelope, the viral vectors are able to specifically infect LNCaP cells.

If this is the case, we expect that a protein for generating infection-specific vector can be obtained by screen the cDNA library of the HOT cell line.

Body:

The approved SOW is listed below.

Task 1. To prepare cDNA library in eukaryotic gene expression vectors (months 1-8).

Task 2. To perform first-round screening to identify the cDNA clone groups that contain the cDNA clones encoding the protein responsible for tissue-specific infection. (months 9-20)

Task 3. To perform the second round of screening to identify the individual clones that contain the cDNA encoding the protein responsible for tissue-specific infection (months 21-24)

Task 4. To sequence the identified gene (months 25-26)

Task 5. To use sequence analysis to characterize the identified gene (months 27-28)

Task 6. To generate deletions of the identified gene and to use them to confirm the functional domains of the identified gene (months 31-36)

To follow the schedule, we should complete Task 1 and a part of Task 2.

Progress of our research:

During the first year, we have generated the cDNA expression library (Task 1). We have picked up clones to check the percentage of clones that contain cDNA inserts and the sizes of the cDNA inserts. We found that approximately 70% of our clones containing cDNA inserts. Most of the inserts are between 1.0 to 2.0 kb (Fig. 1). These sizes and percentage of our cDNA library fulfill our expectation. The total colonies in our cDNA library were approximately 1×10^5. We expected that the amount of the expected mRNA expressed in the HOT cell line was between 0.01 to 0.1% of the total mRNA. The minimal requirement of cDNA clone number is 1,000 to 10,000. Therefore, we believe the cDNA library that we

generated is qualified for gene screening.

We have screened approximately 1,200 colonies (Partial Task 2). We will screen more in the second year. In our proposal, we plan to screen 6,000 to 12,000 colonies. We expected that by screening more colonies, we will obtain the expected gene.

We make progress following our SOW.

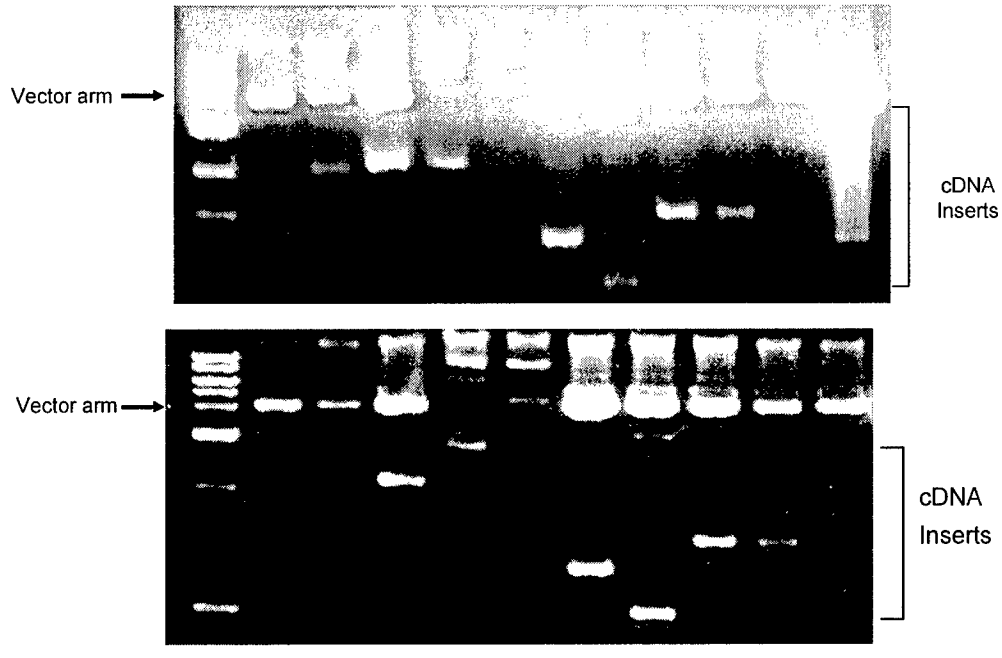

Fig. 1. Checking cDNA inserts of the cDNA library. The eukaryotic cDNA expression cloning kit was purchased from the Invitrogen. The cDNA inserts can be released by digestion of Kpn I restriction endonuclease. As the figure shown, more than 70% clones have cDNA inserts with the sizes of greater than 1 kb.

Key Research accomplishments:

Because we have not identified the genes responsible for tissue-specific infection of LNCaP cells, currently, we have no very exciting result for publication.

Reportable Outcomes:

Currently, none.

Conclusions:

We have constructed a cDNA library containing approximately 105 cDNA clones. We expect that the genes we are seeking are in the cDNA pool. We have screened 1,200 cDNA clones and we believe that by screening another 6,000 to 12,000 cDNA clones, we should be able to identify some genes responsible for tissue-specific gene delivery.