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Award Number: DAMD17-00-1-0472

TITLE: Involvement of a Human Endogenous Retrovirus in Breast Cancer

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REPORT DATE: August 2002

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;
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 2002	Annual (1 Aug 01 - 31 Jul 02)

4. TITLE AND SUBTITLE
Involvement of a Human Endogenous Retrovirus in Breast Cancer

5. FUNDING NUMBERS
DAMD17-00-1-0472

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)*
The genomic DNA of a subset of humans contains an endogenous retrovirus closely related to mouse mammary tumor virus (MMTV). Our overall goal is to determine whether the human mammary tumor virus (HMTV) sequences are involved in a subset of human breast cancers. The first specific aim of this proposal is to recruit and clinically characterize cohorts of breast cancer and appropriate control patients. In studies proposed in the second specific aim, we will identify and sequence HMTV nucleic acids in breast cancer tissue, control tissue, and blood of patients from our cohorts. We will also determine the incidence of HMTV in these various control populations, and compare the sequences of several HMTV genes from different individuals to determine the extent of genetic variability. The third specific aim is to construct DNA or cDNA libraries from tissues positive for HMTV proviruses. In studies under specific aim four, we propose to express HMTV proteins in an insect cell system, which allows stable expression of recombinant proteins, and to characterize the immunological reactions of breast cancer patients and controls against HMTV proteins. If a definitive link is established, HMTV will provide a target for vaccine development and breast cancer therapy.

14. SUBJECT TERMS	15. NUMBER OF PAGES
breast cancer, DNA, HMTV	9
	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:

Sequences with very high similarity (>90%) to mouse mammary tumor virus (MMTV) have been amplified by polymerase chain reaction (PCR) from human breast cancer (BC) tissue (Wang *et al., Cancer Research* **55:** 5173-5179, 1995). The authors of this study presented evidence that the genomic DNA of a subset (~38%) of human breast carcinomas, but not normal tissues, contains sequences that are very highly similar to the MMTV envelope gene (*env*). They suggested the existence of a human mammary tumor virus (HMTV) that is spread by the exogenous route of infection (horizontal transmission). We have PCR amplified sequences highly similar (>95%) to the MMTV *env* gene from human genomic DNA samples, including subsets of both BC tissue and nonBC tissues or blood. A ribonuclease protection assay was used to confirm this result using a non-PCR based technique and to determine that the majority of the PCR positive BC tissues, but none of the PCR negative BC tissues, expressed this sequence at the mRNA level. In addition to mice and humans, we amplified sequences from nonBC genomic DNA of a subset of cats and rhesus macaques distinct from, but highly related to, the MMTV *env*. We also amplified from cat DNA a sequence approximately 90% similar to the MMTV group antigen gene (*gag*). Our results differ from those of Wang and coworkers who, with few exceptions, were able to detect MMTV-like sequences only in breast tumors. Our results indicate that vertebrate species other than mice, including some humans, contain an endogenous retrovirus closely related to MMTV. Our overall goal is to determine whether or not HMTV is involved in a subset of human breast cancers. The first specific aim of this proposal is to recruit and clinically characterize cohorts of breast cancer and appropriate control patients. Various tissues will be obtained from subjects in these cohorts and from archival resources. BC tissues will be staged and classified by standard histological techniques. In studies proposed in the second specific aim, we will identify and sequence HMTV nucleic acids in breast cancer tissue, control tissue, and blood of patients from our cohorts. We will also determine the incidence of HMTV in these various control populations, and compare the sequences of several HMTV genes from different individuals to determine the extent of genetic variability. The third specific aim is to construct DNA or cDNA libraries from tissues positive for HMTV proviruses. These libraries will be screened to identify clones representing the entire HMTV genome. The clones will be sequenced to provide further evidence of the relationship of HMTV to other retroviruses. In studies under specific aim four, we propose to express HMTV proteins in an insect cell system, which allows stable expression of recombinant proteins, and to characterize the immunological reactions of breast cancer patients and controls against HMTV proteins. The proposed studies will establish whether or not HMTV is involved in breast cancer. If a definitive link is established, HMTV will provide a target for vaccine development and breast cancer therapy.

BODY:

Task 1. Recruit and clinically characterize cohorts of breast cancer and appropriate control patients, Months 1-6.

a. We have achieved this goal. We have obtained blood and various tissues from about 200 breast cancer and control subjects. BC tissues are being staged and classified by standard histological techniques. In addition to the unblinded sample cohort, a major effort over the last 6 months has been mounting a rigorous blinded "proof-of-concept" trial. We recently completed the preparations for this blinded trial, and have obtained 190 blinded samples, including blood samples from breast cancer patients, healthy controls and a small number of duplicates (10-20).

Task 2. Identify and sequence of HMTV nucleic acids in breast cancer tissue, control tissue, and blood of patients from our cohorts, Months 3-15.

a. The incidence of HMTV in various breast cancer and control populations is being determined. HMTV env and LTR sequences have been detected in about 85% of BC tissues and in about 10-15% of healthy controls. 50 BC tissues have been examined and about 150 controls have been examined. This task is ongoing.

b. Sequences of HMTV from different individuals are being compared to determine the extent of genetic variability. About 30 different HMTV sequences have been obtained.

c. The level of expression of HMTV gene sequences, relative to that of housekeeping genes, by ribonuclease protection assay will be determined. This task is ongoing.

d. The initial publication based on PCR based molecular epidemiology is being written and submitted.

Task 3. Construct and screen genomic DNA or cDNA libraries from tissues positive for HMTV proviruses, Months 15-24.

a. A DNA library has been constructed in Lambda ZAPII from an HMTV positive tumor.

b. The DNA library is being screened by colony hybridization and PCR to identify clones representing the entire HMTV genome. This task is ongoing.

c. HMTV specific clones will be sequenced to provide further evidence of the relationship of HMTV to other retroviruses and other information. This task is ongoing.

d. Publications based on the complete HMTV sequence will be written and submitted when this task is completed.

Task 4. Characterize immune reactions to HMTV proteins, Months 25-36.

a. HMTV proteins will be expressed in an insect cell system that allows stable expression of recombinant proteins. This task has been initiated.

b. Immunoassays using HMTV recombinant proteins will be developed and validated.

c. Immunological reactions of patients with breast cancer and various control subjects will be characterized.

d. Publications based on results from HMTV serological testing will be written and submitted when this task is completed.

KEY RESEARCH ACCOMPLISHMENTS:

- We have obtained blood and various tissues will be obtained from about 200 breast cancer and control subjects. BC tissues are being staged and classified by standard histological techniques.

- We have obtained 190 blinded samples, including blood samples from breast cancer patients, healthy controls and a small number of duplicates (10-20).

- The incidence of HMTV in various breast cancer and control populations is being determined. HMTV env and LTR sequences have been detected in about 85% of BC tissues and in about 10-15% of healthy controls. 50 BC tissues have been examined and about 150 controls have been examined. This task is ongoing.

- Sequences of HMTV from different individuals are being compared to determine the extent of genetic variability. About 30 different HMTV sequences have been obtained

- A DNA library has been constructed in Lambda ZAPII from an HMTV positive tumor.

- The DNA library is being screened by colony hybridization and PCR to identify clones representing the entire HMTV genome.

- We have initiated studies to express HMTV proteins in an insect cell system that allows stable expression of recombinant proteins. .

REPORTABLE OUTCOMES:

Manuscripts:

Garry, R.F. Human Mammary Tumor Virus: an update. In: Where We Stand with Breast Cancer Research (N.J. Agnantis Ed.) Synedron Press (Athens, Greece), 15-17, 200.

Identification of mouse mammary tumor virus related sequences in the human genome. Soble, S.S., Pei, B., Nangle, S., Costin, J. , Crawford, B.E., Haislip, A.M. and Garry, R.F. in preparation.

Abstracts:

S. Szabo, S., Haislip, A.M., Pai, B. and Garry, R.F. The human contains sequences related to mouse mammary tumor virus Env and LTR. Presented at Tulane Research Day November 2001.

Presentations:

Viral Cause of Human Breast Cancer. Roche Molecular Systems Alameda, California. (March 10th)

Patents and licenses applied for and/or issued: none

Degrees obtained that are supported by this award: none

Development of cell lines, tissue or serum repositories:

We have obtained blood and various tissues from about 400 BC and control subjects.

Infomatics: not applicable.

Funding applied for based on work supported by this award: none

Employment or research opportunities applied for and/or received: none

CONCLUSIONS: We continue to make timely progress on each of the specific tasks outlined in the study. Because of this progress, there are no recommend changes on future work to better address the problem. There remains every indication that current and proposed studies will establish whether or not HMTV is involved in breast cancer. If a definitive link is established, HMTV will provide a target for vaccine development and breast cancer therapy.

REFERENCES: none

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June 14, 2002

CONTINUING REVIEW APPROVAL

This is to certify that the grant, contract or study entitled:

A Human Endogenous Retrovirus Related to MMTV (G0211)

Submitted by: **Robert F. Garry, Ph.D.**

Originally approved on 5/31/01 is an ongoing project that has been re-evaluated in order to provide continuing review.

Re-approval is based on review of the current version of the protocol, available safety data, significant adverse reactions, and updated consent form(s).

HUMAN SUBJECTS – REVIEWED – AT RISK

Continuing approval granted: **6/13/02**
This approval expires on: **6/12/03**

Ina Friedman NP-C, CIM
Ina Friedman, MSN, NP-C, CIM
Chair
Committee on Use of Human Subjects

IF/cl

GENERAL ASSURANCE NUMBER M1260